Aus dem Institut für Tierhygiene und Öffentliches Veterinärwesen

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Untersuchungen zur Antibiotikaresistenz und Virulenz von *Aliarcobacter cryaerophilus*- und *Aliarcobacter butzleri*-Isolaten von Wassergeflügel aus Thüringen

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Für meine Familie

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Abkürzungsverzeichnis

A.	*Aliarcobacter*
ABC	ATP-binding cassette
AB-Zusatz	Antibiotika-Zusatz
AFLP	amplifizierter Fragmentlängenpolymorphismus
ANI	Average Nucleotide Identity (durchschnittliche Nukleotid-Identität)
Asp	Asparaginsäure
*bla*3	Gen für die OXA-15-β-Laktamase (OXA-15-like beta-lactamase gene)
*bes*A	siehe *iro*E
bzw.	beziehungsweise
*cad*F	Gen für das Fibronektin-bindende Protein CadF
*cat*3	Gen für die Chloramphenicol-Acetyltransferase 3
*cdi*A	siehe *hec*A
*cfr*B	Gen für einen Colicin-I-Rezeptor
*cia*B	Gen für das Invasionsprotein CiaB
*cir*A1	siehe *irg*A
*cir*A3	siehe *cfr*B
*cj*1349	Gen für das Fibronektin-bindende Protein Cj1349
*cme*ABC	Multidrug efflux system
*cpn*60	Gen für das Chaperonin 60
DDH	DNA-DNA-Hybridisierung
*deg*P	Gen für die periplasmatische Serin-Endoprotease DegP
DNA	Desoxyribonukleinsäure
EP	Effluxpumpe
ERIC-PCR	enterobakterielle repetitive intergenische Konsensus-Polymerase-Kettenreaktion

E-Test	Epsilometertest
FISH	Fluoreszenz-*in-situ*-Hybridisierung
fsr	Gen für das Fosmidomycin-Resistenz-Protein
*fla*A	Gen für die große Flagellin-Untereinheit
*fla*B	Gen für die kleine Flagellin-Untereinheit
*flh*A	Gen für das Flagellin-Biosyntheseprotein
gv.	Genomovar
*gyr*A	Gen für die DNA-Gyrase Subunit A
hag	Gen für das Flagellin
*hec*A	Gen für die 16S rRNA Endonuclease CdiA
*hec*B	Gen für das Hämolysin-Transportprotein ShlB
*htr*A	siehe *deg*P
Ile	Isoleucin
*irg*A	Gen für einen Colicin-I-Rezeptor
*iro*E	Gen für die Ferri-Bacillibactin-Esterase BesA
LPS	Lipopolysaccharid
MALDI-TOF MS	Matrix-unterstützte Laser-Desorptions-Ionisations-Massenspektrometrie
MFS	major facilitator superfamily
MLST	Multilocus-Sequenztyp bzw. Multilocus-Sequenztypisierung
*mur*J	siehe *mvi*N
m-PCR	multiplex Polymerase-Kettenreaktion
*mvi*N	Gen für die Lipid II Flippase MurJ
*opr*F2	siehe *cad*F
PCR	Polymerase-Kettenreaktion (polymerase chain reaction)
PFGE	Pulsfeld-Gelelektrophorese

*pld*A	Gen für die Phospholipase A (PldA)
QRDR	Quinolone resistance-determinig region (Chinolon-Resistenz bestimmende Region)
RAPD-PCR	zufällig amplifizierte polymorphe DNA-PCR
RFLP	Restriktionsfragmentlängenpolymorphismus
RND	resistance-nodulation-division
*rpl*D	Gen für das 50S ribosomale Protein L4
*rpl*V	Gen für das 50S ribosomale Protein L22
rRNA	ribosomale Ribonukleinsäure
*shl*B	siehe *hec*B
SMR	small multidrug resistance
SNP	Single Nucleotide Polymorphism (Einzelnukleotid-Polymorphismus)
spp.	species pluralis
ST	Sequenztyp
subsp.	Subspezies
Tab.	Tabelle
*tet*A	Gen für das Tetracyclin-Resistenzprotein der Klasse C (tetracycline resistance protein, class C)
tet(O)	Gen für das ribosomales Schutzprotein Tet(O)
Thr	Threonin
*tly*A	Gen für das Hämolysin TlyA
Tyr	Tyrosin
z. B.	zum Beispiel
16S rRNA	16S ribosomale Ribonukleinsäure (S steht für Svedberg-Einheit)
23S rRNA	23S ribosomale Ribonukleinsäure (S steht für Svedberg-Einheit)

1 Einleitung

Aliarcobacter cryaerophilus und *Aliarcobacter butzleri* gelten weltweit als lebensmittelbedingte und zoonotische Krankheitserreger (ICMSF 2002), welche zusammen mit sieben weiteren Spezies zur Gattung *Aliarcobacter* in die Familie der *Arcobacteraceae* gehören (WAITE et al. 2017, OREN et al. 2020b). Aufgrund der morphologischen sowie biochemischen Ähnlichkeit zu den thermophilen *Campylobacter* spp. und dem Fehlen standardisierter Identifizierungs- und Untersuchungsmethoden, werden *Aliarcobacter* spp. häufig falsch als *Campylobacter* spp. identifiziert. In der Folge führt dies zu einer Unterschätzung der tatsächlichen Prävalenz von *Aliarcobacter* spp. (COLLADO et al. 2011, HÄNEL et al. 2016).

Aliarcobacter spp. wurden bisher aus einer Vielzahl an Habitaten isoliert. Durch ihr vermehrtes Vorkommen in Lebensmitteln, insbesondere Geflügelfleisch, erlangten die beiden Spezies *Aliarcobacter cryaerophilus* und *Aliarcobacter butzleri* im letzten Jahrzehnt eine erhöhte Aufmerksamkeit (HÄNEL et al. 2016). Beide *Aliarcobacter*-Spezies werden überwiegend über kontaminierte Lebensmittel oder Trinkwasser übertragen und lösen in Tieren neben gastrointestinalen Erkrankungen auch Mastitiden, Reproduktionsstörungen sowie Aborte aus. Beim Menschen führt eine *Aliarcobacter*-Infektion zu einer akuten selbstlimitierenden Enteritis, die zwar einer *Campylobacter*-Infektion ähnelt, aber mit wässriger statt blutiger Diarrhö einhergeht (RAMEES et al. 2017), sodass eine antimikrobielle Therapie in der Regel nicht notwendig ist. Vor dem Hintergrund der nahen Verwandtschaft zu den thermophilen *Campylobacter* spp., deren antimikrobielle Resistenz in den letzten Jahren deutlich zugenommen hat, ist die Identifizierung und Bewertung des phänotypischen Resistenzmusters sowie der zugrunde liegenden genetischen Resistenzmechanismen der *Aliarcobacter* spp. von zentraler Bedeutung. Ebenso wichtig wie die Ermittlung der Resistenz, ist die Identifizierung von Virulenzgenen. Denn sie sind für die Expression von wirtschädigenden Proteinen und/oder Faktoren verantwortlich und bestimmen so die Entstehung und Ausprägung einer Infektion.

Ziel dieser Arbeit war deshalb die Bestimmung der phänotypischen und genotypischen Antibiotikaresistenz sowie die Ermittlung des Virulenzprofiles von *Aliarcobacter cryaerophilus*- und *Aliarcobacter butzleri*-Stämmen, welche zuvor aus Kotproben von Wassergeflügel aus Thüringen isoliert worden waren.

2 Literaturübersicht

2.1 *Aliarcobacter*

2.1.1 Taxonomie

Die Gattung *Arcobacter* wurde 1991 eingeführt, um zwei aerotolerante *Campylobacter*-Spezies (*Campylobacter cryaerophila*, heute: *Aliarcobacter cryaerophilus*; *Campylobacter nitrofigilis*, heute: *Arcobacter nitrofigilis*) neu zu klassifizieren (VANDAMME et al. 1991). Diese neue Gattung wurde aufgrund ihrer Ähnlichkeit zur Gattung *Campylobacter* in die Familie der *Campylobacteraceae* eingeordnet (VANDAMME et al. 1991). Diese Familie gehört zusammen mit den *Helicobacteraceae* in die Ordnung der *Campylobacterales* in der Klasse der *Epsilonproteobakterien*, welche dem Stamm der *Proteobakterien* zugeordnet ist. Die Gattung *Arcobacter* wurde 1992 und 2005 um jeweils zwei weitere Spezies erweitert (*Arcobacter butzleri* und *Arcobacter skirrowii* sowie *Arcobacter cibarius* und *Arcobacter halophilus*) (VANDAMME et al. 1992, DONACHIE et al. 2005, HOUF et al. 2005).

Kurze Zeit nachdem die Gattung *Arcobacter* eingeführt wurde, gliederten KIEHLBAUCH et al. (1991) und VANDAMME et al. (1992) die Spezies *Arcobacter cryaerophilus* aufgrund des unterschiedlichen Restriktionsfragmentlängenpolymorphismus (RFLP) der 16S- und 23S-rRNA-Gene, des Gesamtzellprotein- und Fettsäuregehalts in zwei Untergruppen - Untergruppe 1A und Untergruppe 1B. In nachfolgenden Studien wurde diese Unterteilung mehrfach diskutiert (VANDAMME et al. 2005, DEBRUYNE et al. 2008), woraufhin 2010 die Taxonomie der *Arcobacter cryaerophilus*-Untergruppen mit Hilfe des amplifizierten Fragmentlängenpolymorphismus (AFLP) und der phylogenetischen Analyse des *cpn*60-Genes erneut untersucht wurde (DEBRUYNE et al. 2010). Die Untersuchung ergab, dass trotz der komplexen Taxonomie von *Arcobacter cryaerophilus* diese Untergruppierung aufgegeben werden sollte. Des Weiteren wurde vorgeschlagen, den *Arcobacter cryaerophilus* Typ-Stamm LMG 24291 der Untergruppe 1A durch den Stamm LMG 10829 (ein Vertreter der Untergruppe 1B) zu ersetzen, da LMG 24291 weniger verbreitet und daher kein typischer Vertreter der Spezies *Arcobacter cryaerophilus* ist (DEBRUYNE et al. 2010, COLLADO et al. 2011). Dieses Ergebnis wird durch eine kürzlich veröffentlichte Studie unterstützt, in der eine Unterteilung von *Arcobacter cryaerophilus* in vier Genomovare (Cluster I - IV) vorgeschlagen wurde (PÉREZ-CATALUÑA et al. 2018a). Aufgrund ihrer Untersuchungen ordneten PÉREZ-CATALUÑA et al. (2018a) die Stämme LMG 10829 und LMG 24291 dem Cluster I (*Arcobacter cryaerophilus gv. pseudocryaerophilus*) bzw. dem Cluster III (*Arcobacter cryaerophilus gv. cryaerophilus*) zu. Erwähnenswert ist, dass trotz des Vorschlages von DEBRUYNE et al. (2010) und der eben genannten Studie sowie zusätzlichen Untersuchungen an der Ernennung von LMG 24291 zum Typ-Stamm der Spezies *Arcobacter cryaerophilus* festgehalten wurde (PÉREZ-CATALUÑA et al. 2018a, PÉREZ-CATALUÑA et al. 2018b, 2019).

Im Jahre 2017 wurde von WAITE et al. (2017) nach der Analyse der 16S rRNA-Gene und der 23S rRNA-Gene sowie der Untersuchung von 120 Einzelkopie-Markerproteinen eine neue phylogenetische Ordnung

vorgeschlagen. Dabei sollte die Gattung *Arcobacter* auf die Ebene einer eigenen Familie, der *Arcobacteraceae*, gehoben werden und die Ordnung *Campylobacterales* dem neuen Stamm *Campylobacterota* in der neuen Klasse *Campylobacteria* zugeordnet werden (WAITE et al. 2017, 2018). Seit Mai 2020 gehört die Gattung *Arcobacter* offiziell zur Familie der *Arcobacteraceae* in der Ordnung der *Campylobacterales* (OREN et al. 2020b). Allerdings wurde die Gattung bereits 2018 aufgrund neuer taxonomischer und phylogenetischer Untersuchungen durch folgende sechs separate Gattungen ersetzt: *Arcobacter*, *Aliarcobacter*, *Pseudarcobacter*, *Halarcobacter*, *Malaciobacter* und *Poseidonibacter* (PÉREZ-CATALUÑA et al. 2018b, 2019). Die bisher bekannten 29 *Arcobacter* spp. verteilen sich auf die sechs verschiedenen Gattungen (siehe Tab. 1).

Gattung	Spezies
Arcobacter	*Arcobacter nitrofigilis* *Arcobacter caeni* *Arcobacter lacus*
Aliarcobacter	*Aliarcobacter butzleri* *Aliarcobacter cibarius* *Aliarcobacter cryaerophilus* *Aliarcobacter faecis* *Aliarcobacter lanthieri* *Aliarcobacter skirrowii* *Aliarcobacter thereius* *Aliarcobacter trophiarum*
Pseudarcobacter	*Pseudarcobacter aquimarinus* *Pseudarcobacter aticola* *Pseudarcobacter cloacae* *Pseudarcobacter defluvii* *Pseudarcobacter ellisii* *Pseudarcobacter suis* *Pseudarcobacter venerupis*
Halarcobacter	*Halarcobacter anaerophilus* *Halarcobacter bivalviorum* *Halarcobacter ebronensis*
Malaciobacter	*Malaciobacter canalis* *Malaciobacter halophilus* *Malaciobacter marinus* *Malaciobacter molluscorum* *Malaciobacter mytili* *Malaciobacter pacificus*
Poseidonibacter	*Poseidonibacter antarcticus* *Poseidonibacter lekithochrous*

Tab. 1: Die verschiedenen Gattungen der Familie der *Arcobacteraceae* mit den dazugehörigen Spezies

Demzufolge umfasst die Gattung *Aliarcobacter* (*A.*) derzeit acht Spezies: *A. butzleri*, *A. cryaerophilus*, *A. cibarius*, *A. faecis*, *A. thereius*, *A. skirrowii*, *A. trophiarum* und *A. lanthieri* (früher bekannt als *Arcobacter butzleri*, *Arcobacter cryaerophilus*, *Arcobacter cibarius*, *Arcobacter faecis*, *Arcobacter thereius*, *Arcobacter skirrowii*, *Arcobacter trophiarum* und *Arcobacter lanthieri*) (PÉREZ-CATALUÑA et al. 2019, OREN et al. 2020a).

2.1.2 Morphologie, Wachstumsbedingungen und biochemisches Verhalten

Aliarcobacter spp. sind gramnegative, nicht sporenbildende, s-förmig gekrümmte Stäbchen, die etwa 0,2 bis 0,5 µm breit und 1 bis 3 µm lang sind. Aufgrund einer polaren Geißel ist das Bakterium beweglich, wobei sie aber auf Agarplatten kein Schwarmverhalten zeigen (PÉREZ-CATALUÑA et al. 2019). Auf Blut-basierenden Nährmedien stellen sich *Aliarcobacter* spp. als kleine runde, weiße, grau-weiße oder gräuliche Kolonien mit einem Durchmesser von 2 bis 4 mm dar (COLLINS et al. 1996).

Im Gegensatz zu *Campylobacter* spp. wachsen *Aliarcobacter* spp. sowohl unter aeroben als auch mikroaerophilen Bedingungen und haben zudem eine große Temperaturspanne (15 – 42°C) (LEHNER et al. 2005, HO et al. 2006a). Das Wachstumsoptimum liegt bei circa 30°C unter mikroaerophilen Bedingungen (DEBRUYNE et al. 2008, HÄNEL et al. 2016).

Laut PÉREZ-CATALUÑA et al. (2019) weist die Gattung *Aliarcobacter* folgendes biochemisches Profil auf: Oxidase-positiv, Katalase-positiv, Indol-positiv, positiv in Bezug auf die Nitratreduktion sowie Urease-negativ. Da keine Fermentation von Kohlenhydraten stattfindet, wird die Gattung als chemoorganotroph bezeichnet. Ferner werden keine fluoreszierende Pigmente produziert (PÉREZ-CATALUÑA et al. 2019).

2.1.3 Vorkommen

Aliarcobacter spp. wurden bisher aus verschiedenen Habitaten isoliert. Dazu gehören unter anderem Lebensmittel tierischen Ursprungs (z. B. Geflügel-, Rind- und Schweinefleisch, Milchprodukte), aber auch Meeresfrüchte, Gemüse, Trinkwasser, Abwässer sowie Oberflächen in lebensmittelverarbeitenden Betrieben (COLLADO et al. 2011, HAUSDORF et al. 2013, FERREIRA et al. 2017, MILLAR et al. 2017, PÉREZ-CATALUÑA et al. 2018a, PÉREZ-CATALUÑA et al. 2018b, ON et al. 2019, PARISI et al. 2019). Außerdem konnten *Aliarcobacter* spp. in humanen Stuhlproben sowie in Kotproben von erkrankten als auch gesunden Tieren nachgewiesen werden (VAN DRIESSCHE et al. 2003, VAN DRIESSCHE et al. 2004, VAN DEN ABEELE et al. 2014, RAMEES et al. 2017, BRÜCKNER et al. 2020). Eine Langzeitstudie in Belgien zeigte, dass *A. butzleri* und *A. cryaerophilus* die viert- bzw. siebthäufigsten *Campylobacter*-ähnlichen Organismen sind, die aus menschlichen Stuhlproben isoliert wurden (VANDENBERG et al. 2004). Weitere Studien ergaben, dass *A. butzleri* am häufigsten in Stuhlproben von Menschen vorkommt, die an Diarrhö leiden, gefolgt von *A. cryaerophilus* und *A. skirrowii* (TAYLOR et al. 1991, SAMIE et al. 2007, JIANG et al. 2010, COLLADO et al. 2011).

Aus abortierten Feten und Plazenten von Rindern, Schweinen und Schafen sowie aus der Milch von Kühen mit Mastitiden wurden *Aliarcobacter* spp. ebenso isoliert, wie aus dem Verdauungstrakt, aus Präputialspülungen und Vaginalabstrichen gesunder Tiere (DE OLIVERIA et al. 1999, ON et al. 2003, VAN DRIESSCHE et al. 2003, HO et al. 2006a, ATABAY et al. 2008, COLLADO et al. 2011, RAMEES et al. 2017, MILLER et al. 2018).

Von allen zur Gewinnung von Lebensmitteln dienenden Tieren gilt Geflügel als Hauptreservoir, da *Aliarcobacter* spp. zur Kommensalflora des Darmtraktes des Geflügels gehören (LEHNER et al. 2005, HO et al. 2008, RAMEES et al. 2017, CHIEFFI et al. 2020). Aufgrund der Gefahr einer Kontamination während des Schlachtprozesses stellt Geflügelfleisch die Hauptinfektionsquelle für den Menschen dar (ATABAY et al. 2008, COLLADO et al. 2011).

2.1.4 Übertragung

Der Verzehr von *Aliarcobacter*-kontaminierten, rohen bzw. nicht ausreichend erhitzten tierischen bzw. pflanzlichen Lebensmitteln sowie Trinkwasser gilt als der wesentliche Übertragungsweg auf den Menschen (COLLADO et al. 2011, HAUSDORF et al. 2013, GIACOMETTI et al. 2015, FERREIRA et al. 2016, RAMEES et al. 2017). Der enge Kontakt mit Haustieren, vor allem zu Hunden und Katzen, stellt einen weiteren Übertragungsweg dar (FERA et al. 2009).

Bei Tieren wurden sowohl die horizontale (Aufnahme kontaminierter Futtermittel bzw. kontaminierten Wassers) als auch die vertikale Übertragung (intrauterine Übertragung vom Muttertier auf die Feten) beschrieben (HO et al. 2006a, HO et al. 2006b, COLLADO et al. 2011, RAMEES et al. 2017).

2.1.5 Erkrankungen

Die folgenden vier *Aliarcobacter*-Spezies sind bislang mit Erkrankungen bei Mensch und Tier assoziiert worden: *A. butzleri*, *A. cryaerophilus*, *A. thereius* und *A. skirrowii* (HO et al. 2006a, COLLADO et al. 2011, FERREIRA et al. 2016, PÉREZ-CATALUÑA et al. 2018a). Beim Menschen können diese Bakterien eine akute selbstlimitierende Enteritis verursachen, die mit wässrigem Durchfall und gelegentlich Übelkeit, Abdominalschmerzen sowie Fieber einhergeht (COLLADO et al. 2011, RAMEES et al. 2017). Die Ursache der Diarrhö bei *A. butzleri*-Infektionen ist bereits bekannt: Veränderungen in den Tight-Junction-Proteinen und die Induktion der epithelialen Apoptose bewirken eine Dysfunktion der epithelialen Barriere im Darmtrakt (BÜCKER et al. 2009).

In seltenen Fällen können auch schwere Erkrankungen, z. B. Bakteriämie oder Septikämie, auftreten (HSUEH et al. 1997, WOO et al. 2001, LAU et al. 2002, COLLADO et al. 2011).

Aliarcobacter spp. werden bei Tieren mit dem Auftreten von Aborten, Reproduktionsstörungen, Mastitiden und gastrointestinalen Erkrankungen in Zusammenhang gebracht (COLLADO et al. 2011, RAMEES et al. 2017). Interessanterweise zeigen infizierte Tiere häufig keine spezifisch klinische Symptomatik (HO et al. 2006a, HÄNEL et al. 2016). *A. butzleri* wird häufig mit Enteritis und Diarrhö bei Schweinen, Rindern und Pferden assoziiert, während *A. skirrowii* als Auslöser von Diarrhö und hämorrhagischer Kolitis bei Schafen und Rindern angesehen wird (VANDAMME et al. 1992, HO et al. 2006a, RAMEES et al. 2017). Im Gegensatz dazu verursacht *A. cryaerophilus* vorrangig Aborte und Reproduktionsstörungen, insbesondere bei Schweinen (DE OLIVEIRA et al. 1997, RAMEES et al. 2017).

2.1.6 Diagnostik

2.1.6.1 Allgemeines

Eine standardisierte Methode zur Identifizierung von *Aliarcobacter* spp. steht aktuell nicht zur Verfügung. Aufgrund der verschiedenen Anzuchtmethoden und molekularbiologischer Nachweisverfahren, variiert die Nachweisrate zum Teil stark, sodass bis heute keine eindeutigen Daten zur weltweiten Prävalenz von *Aliarcobacter* spp. vorliegen (FALLAS-PADILLA et al. 2014, HÄNEL et al. 2016, RAMEES et al. 2017). Da *Aliarcobacter* spp. ein ähnliches biochemisches Reaktionsmuster wie *Campylobacter* spp. aufzeigen, ist die Identifizierung und Differenzierung durch biochemische Testsysteme (z. B. API® Campy) schwierig und kann zu Missidentifizierungen führen (ATABAY et al. 1998, GONZALEZ et al. 2006). Zusätzlich wird die Differenzierung innerhalb der Gattung *Aliarcobacter* durch den variablen Ausgang einzelner biochemischer Reaktionen erschwert (DEBRUYNE et al. 2008, COLLADO et al. 2011). Deshalb ist die Anzucht und Isolierung von *Aliarcobacter* spp. bis heute der Gold-Standard (RAMEES et al. 2017).

2.1.6.2 Anzucht und Isolierung

Verschiedene Methoden zur Anzucht und Isolierung wurden von ELLIS et al. (1977), COLLINS et al. (1996), DE BOER et al. (1996), ATABAY et al. (1997), HOUF et al. (2001), FERA et al. (2010), MORITA et al. (2004) und JOHNSON et al. (1999) beschrieben. Allerdings wurden bislang nur wenige vergleichende Studien durchgeführt, sodass kein Konsens darüber besteht, welche Methode für die Anzucht und Isolierung von *Aliarcobacter* spp. am besten geeignet ist (COLLADO et al. 2011, RAMEES et al. 2017, CHIEFFI et al. 2020). Beim Vergleich der verschiedenen Methoden bemerkte COLLADO et al. (2011), dass allen Methoden der Anreicherungsschritt mit einer Bouillon mit Antibiotikazusatz (AB-Zusatz), welcher in der Regel 48 Stunden dauert, gemein war. Obwohl HOUF et al. (2002) und HO et al. (2006b) über eine Abnahme der Spezies-Diversität bei der Verwendung des Anreicherungsschrittes berichteten, basieren die derzeitigen Anzucht-Protokolle auf einer 48 Stunden umfassenden Anreicherung (Bouillon mit AB-Zusatz), einer anschließenden passiven Filtration und der Übertragung der Bouillon auf eine Nähragarplatte (mit oder ohne AB-Zusatz), welche 48 bis 72 Stunden bebrütet wird (COLLADO et al. 2011, CHIEFFI et al. 2020).

Für eine erfolgreiche Kultivierung von *Aliarcobacter* spp. spielt die Bebrütungsatmosphäre eine wichtige Rolle. Im Gegensatz zu *Campylobacter* spp. können *Aliarcobacter* spp. sowohl unter aeroben als auch unter mikroaerophilen Bedingungen wachsen (LEHNER et al. 2005). Deshalb wird für die exakte Differenzierung der *Aliarcobacter* spp. von den *Campylobacter* spp. die Anzucht unter aeroben Bedingungen bei 15°C , 25°C und 37°C empfohlen (COLLADO et al. 2011). Andere Autoren befürworten dagegen die Anzucht unter mikroaerophilen Bedingungen (DEBRUYNE et al. 2008, HÄNEL et al. 2016), obgleich der Nutzen bzw. die Notwendigkeit dieser Anzuchtbedingung laut COLLADO et al. (2011) unzureichend erforscht ist.

2.1.6.3 Molekulare Identifizierung

Eine Unterscheidung zwischen den verschiedenen *Aliarcobacter*-Spezies und auch zwischen *Aliarcobacter* spp. und *Campylobacter* spp. ist wegen der phänotypischen und biochemischen Ähnlichkeit schwierig (DOUIDAH et al. 2010, COLLADO et al. 2011). Aus diesem Grund werden gegenwärtig diverse molekulare Methoden zur Differenzierung angewandt. Dazu zählen verschiedene Polymerase-Kettenreaktionen (PCR), RFLP, Matrix-unterstützte Laser-Desorptions-Ionisations-Massenspektrometrie (MALDI-TOF MS) sowie Fluoreszenz-in-situ-Hybridisierung (FISH) (COLLADO et al. 2011, RAMEES et al. 2017, CHIEFFI et al. 2020). Die am häufigsten verwendete PCR, ist die von HOUF et al. (2000) entwickelte Spezies-spezifische multiplex-PCR (m-PCR), obwohl in einer vergleichenden Studie herausgefunden wurde, dass diese m-PCR zum Teil die falschen *Aliarcobacter*-Spezies identifiziert (LEVICAN et al. 2013). Für eine schnelle und sichere Identifizierung hat sich die Verwendung der MALDI-TOF MS bewährt (ALISPAHIC et al. 2010, RAMEES et al. 2017).

2.1.6.4 Genotypisierung

Die Genotypisierung von *Aliarcobacter* spp. dient der Unterscheidung verschiedener *Aliarcobacter*-Stämme einer Spezies, wodurch zum einen Übertragungswege sowie Ausbrüche nachvollziehbar und zum anderen Untersuchungen zur genetischen Diversität einzelner *Aliarcobacter*-Spezies unterstützt werden (COLLADO et al. 2011, RAMEES et al. 2017). Die bisher angewandten Methoden umfassen: die enterobakterielle repetitive intergenische Konsensus-PCR (ERIC-PCR), die zufällig amplifizierte polymorphe DNA-PCR (RAPD-PCR), der AFLP und die Pulsfeld-Gelelektrophorese (PFGE) (HUME et al. 2001, HOUF et al. 2002, ON et al. 2003, RAMEES et al. 2017, CHIEFFI et al. 2020). Die Zuordnung der *Aliarcobacter* spp. zu verschiedenen Sequenztypen (ST) wurde 2009 durch die Entwicklung des Multilocus Sequenztyp (MLST)-Schemas und dessen Online-Datenbank (https://pubmlst.org/arcobacter/) möglich (MILLER et al. 2009).

2.1.7 Antimikrobielle Resistenz

2.1.7.1 Phänotyp

Die Bestimmung der phänotypischen Antibiotikaresistenz erfolgte bislang vorrangig für *A. butzleri* und in einigen Fällen auch für *A. cryaerophilus* und *A. skirrowii* (COLLADO et al. 2011, HÄNEL et al. 2018, FERREIRA et al. 2019). Anhand mehrerer Studien wurde festgestellt, dass die Mehrzahl der *Aliarcobacter* spp. empfindlich gegenüber Gentamicin, Ampicillin, Amoxcillin und Tetracyclinen (FERA et al. 2003, ABAY et al. 2012, SHAH et al. 2013, ÜNVER et al. 2013, VAN DEN ABEELE et al. 2016, FERREIRA et al. 2019), aber sehr häufig resistent gegenüber Azithromycin, Ciprofloxacin, Vancomycin und Cefotaxim sind (COLLADO et al. 2011, SHAH et al. 2013, ÜNVER et al. 2013, RAMEES et al. 2017, FERREIRA et al. 2019) .

Erwähnenswert ist, dass bis heute weder ein standardisiertes Protokoll noch spezifische Grenzwerte für die antimikrobielle Empfindlichkeit von *Aliarcobacter* spp. vorliegen (COLLADO et al. 2011, CHIEFFI et al. 2020). Dadurch ist die Vergleichbarkeit der bisherigen Studien zur phänotypischen Resistenzbestimmung stark eingeschränkt.

2.1.7.2 Genotyp

Über die für die Antibiotikaresistenz verantwortlichen Resistenzgene in *Aliarcobacter* spp. ist derzeit nur wenig bekannt. Die in der Literatur beschriebenen Resistenzmechanismen werden vorrangig im Chromosom vermutet, da bislang in keinem, der in *Aliarcobacter* spp. vorhandenen und beschriebenen Plasmiden ein Resistenzgene nachgewiesen werden konnte (ABDELBAQI et al. 2007, MILLER et al. 2007, DOUIDAH et al. 2014, FERREIRA et al. 2016).

Eine mögliche Ursache der Ciprofloxacin-Resistenz in *A. butzleri* wurde 2007 erstmalig beschrieben. Laut ABDELBAQI et al. (2007) ist eine Punktmutation in der Chinolon-Resistenz-bestimmenden Region (quinolone resistance-determining region; QRDR) an Position 85 des *gyr*A-Genes (Thr-85-Ile) ursächlich für die Resistenz gegenüber Ciprofloxacin. Der in diesem Zusammenhang stattfindende Basenaustausch von Cytosin zu Thymin führt zum Aminosäureaustausch von Threonin zu Isoleucin und beeinflusst so die Fluorchinolon-Bindung in der QRDR. Durch die Identifizierung einer Punktmutation an Position 89 des *gyr*A-Genes (Asp-89-Tyr), konnte 2018 eine weitere Ursache für die Ciprofloxacin-Resistenz in *A. butzleri* nachgewiesen werden (FERREIRA et al. 2018). Hierbei erfolgt ein Basenaustausch von Guanin zu Thymin, in dessen Folge die Aminosäure Asparaginsäure mit der Aminosäure Tyrosin ersetzt wird (FERREIRA et al. 2018).

Die Etablierung der Gesamtgenom-Sequenzierung und die ansteigende Zahl von *Aliarcobacter*-Genomsequenzen ermöglichte in den letzten zwei Jahrzehnten unter Zuhilfenahme der Bioinformatik die Identifizierung und Charakterisierung vorhandener potentieller Resistenzgene (MILLER et al. 2007, FANELLI et al. 2019, FANELLI et al. 2020, ISIDRO et al. 2020). Im Jahr 2007 konnten MILLER et al. (2007) durch die Sequenzierung des kompletten *A. butzleri*-Genomes RM4018 zum ersten Mal den Zusammenhang zwischen der An- bzw. Abwesenheit eines potentiellen Resistenzgenes und der zuvor untersuchten phänotypischen Antibiotika-Empfindlichkeit aufzeigen. So steht beispielsweise die Resistenz gegenüber Chloramphenicol mit dem Vorhandensein des *cat*3-Genes in Verbindung (MILLER et al. 2007). Laut kürzlich veröffentlichter Studien wurden weitere potentielle Resistenzgene identifiziert, wie z. B. das *fsr*-Gen (kodiert das Fosmidomycin-Resistenz-Protein), das *tet*A-Gen (kodiert das Tetracyclin-Resistenz-Protein der Klasse C) und das *bla*3-Gen (kodiert die OXA-15-β-Lactamase) (FANELLI et al. 2019, FANELLI et al. 2020, ISIDRO et al. 2020).

2.1.8 Virulenz und Virulenzgene

Über die Virulenz und potentiell vorhandene Virulenzgene in *Aliarcobacter* spp. ist geringfügig mehr bekannt als über die antimikrobiellen Resistenzgene. In mehreren *in-vitro*-Untersuchungen wurden dieser Bakterien-Gattung adhäsive, invasive und zytotoxische Fähigkeiten attestiert (MUSMANNO et al. 1997, JOHNSON et al. 2002, CARBONE et al. 2003, HO et al. 2007, COLLADO et al. 2011). Die Fähigkeit der *in-vitro*-Invasion von Zelllinien wurde jedoch vorwiegend für *A. cryaerophilus* demonstriert (FERNÁNDEZ et al. 1995, HO et al. 2007). In der Studie von FERNÁNDEZ et al. (1995) wurde beobachtet, dass die untersuchten *A. cryaerophilus*-Stämme eine Ansammlung von Flüssigkeit und Elektrolyten in den Ileumschlingen von Rattendärmen

induzierten und sich enterotoxisch sowie invasiv gegenüber Hep-2-Zellen verhielten. Eine andere Studie zeigte, dass *A. butzleri* möglicherweise die Spezies mit der stärksten Virulenz unter den *Aliarcobacter* spp. ist (WESLEY et al. 1996).

Bis zur Veröffentlichung der kompletten Genomsequenz von *A. butzleri* RM4018 war nicht bekannt, welche Gene in die Virulenzmechanismen der *Aliarcobacter* spp. involviert sind. Wie bei den Untersuchungen zu den Resistenzgenen, waren MILLER et al. (2007) hier ebenso Vorreiter und identifizierte in *A. butzleri* RM4018 zehn mutmaßliche Virulenzgene, die denen vom *Campylobacter jejuni* stark ähnelten. Darunter waren unter anderem Gene für die Fibronektin-bindenden Proteine CadF und Cj1349 (*cad*F und *cj*1349), das Invasionsprotein CiaB (*cia*B), den Virulenzfaktor MviN (*mvi*N), die Phospholipase A (*pld*A) und das Hämolysin TlyA (*tly*A) (MILLER et al. 2007). Mit Hilfe der Bioinformatik und einer höheren Anzahl an verfügbaren Gesamt-Genomsequenzen konnten in den letzten Jahren weitere potentielle Virulenzgene, wie z. B. Gene für die Biosynthese des Flagellums und Gene für ein komplettes Chemotaxis-System, identifiziert werden (ROVETTO et al. 2017, FANELLI et al. 2019, FANELLI et al. 2020, ISIDRO et al. 2020).

3 Publikationen

3.1 Stellungnahme zum Eigenanteil an den Arbeiten zur Publikation 1

“Genomic Analysis and Antimicrobial Resistance of *Aliarcobacter cryaerophilus* Strains From German Water Poultry“

Müller E., Hotzel H., Ahlers C., Hänel I., Tomaso H. and Abdel-Glil M.Y. (2020) Genomic Analysis and Antimicrobial Resistance of *Aliarcobacter cryaerophilus* Strains From German Water Poultry. Front. Microbiol. 11:1549.

An der Konzeption des Themas waren mein Betreuer PD Dr. Herbert Tomaso und Dr. Mostafa Y. Abdel-Glil maßgeblich beteiligt.

Der Manuskriptentwurf (inklusive Literaturrecherche) wurde eigenständig verfasst.

Die im Material- und Methodenteil erwähnten Untersuchungen hinsichtlich DNA-Extraktion sowie Plasmid-Detektion habe ich selbstständig durchgeführt. Bei der Antibiotika-Empfindlichkeitstestung wurde ich von Frau Sandra Thierbach unterstützt. Die bioinformatischen Analysen erfolgten in Zusammenarbeit mit Dr. Mostafa Y. Abdel-Glil.

Die Auswertung und Bewertung der Daten im Ergebnisteil erfolgten ebenfalls selbstständig. Hinsichtlich der Taxonomie, der Phylogenie sowie der genetischen Beschreibung der zirkularisierten Genome erfolgte die Auswertung und Beschreibung in Zusammenarbeit mit Dr. Mostafa Y. Abdel-Glil.

Bei Revision des Manuskripts erhielt ich Hilfe von den anderen Co-Autoren.

Die zur Publikation gehörenden Supplemente befinden sich im Anhang.

3.1.1 Publikation 1

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Genomic Analysis and Antimicrobial Resistance of *Aliarcobacter cryaerophilus* Strains From German Water Poultry

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Aliarcobacter cryaerophilus (formerly *Arcobacter cryaerophilus*) is a globally emerging foodborne and zoonotic pathogen. However, little is known about the species' genomic features and diversity, antibiotic resistance and virulence. In this study, 27 *A. cryaerophilus* strains from water poultry in Thuringia, Germany, were investigated using whole-genome sequencing. Four of these strains were sequenced using long- and short-read sequencing methods to obtain circularized genomes. The German strains belong to the *A. cryaerophilus* cluster I. Cluster I genomes exhibited a high degree of genetic diversity in which variable sites comprised 9.1% of the core genome. The German strains formed three subgroups that contained 2, 6, and 9 strains, respectively. The genomic analysis of cluster I revealed variable presence of mobile elements and that 65% of the strains lack CRISPR systems. The four circularized genomes carried a ~2 Mbp chromosome and a single megaplasmid (size 98.1–154.5 Kbp). The chromosome was densely packed with coding sequences (~92%) and showed inversions and shifts in the gene blocks between different strains. Antimicrobial resistance was assessed using a gradient strip diffusion method and showed that all 27 strains were resistant to cefotaxime and susceptible to erythromycin, gentamicin, and ampicillin. Sixteen strains were also resistant to ciprofloxacin, whereas 23 were resistant to streptomycin. The genetic prediction of antibiotic resistance identified numerous efflux pumps similar to those found in *A. butzleri*. All strains harbored two beta-lactamase genes which may explain the cefotaxime resistance. A correlation between the *gyr*A point mutation (Thr-85-Ile) and ciprofloxacin resistance was partially discovered in 15 out of 16 strains. *In silico* virulence profiling showed a wide range of virulence factors including a full chemotaxis system and most of the flagellar genes. In contrast to *A. butzleri*, no urease cluster was found. This study provides new insights into the genomic variability of *A. cryaerophilus* strains of cluster I. The different genetic makeup of these strains may contribute to the virulence of strains and the severity of the infections in humans.

Keywords: ***Aliarcobacter***, ***Arcobacter***, **antibiotic susceptibility, whole-genome sequencing, antimicrobial resistance, virulence, plasmids**

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INTRODUCTION

Aliarcobacter (*A.*) *cryaerophilus* (formerly *Arcobacter cryaerophilus*) is a Gram-negative, curved motile rod that grows between 15°C and 42°C. Strains of *A. cryaerophilus* belong to cluster "1a" of the genus *Aliarcobacter* according to a recent taxonomic classification (Pérez-Cataluña et al., 2018b). *A. cryaerophilus* exhibits a high degree of heterogeneity and has been divided into two subgroups (1A and 1B) based on restriction fragment length polymorphisms of the rRNA genes, whole-cell proteins and fatty acid content (Kiehlbauch et al., 1991; Vandamme et al., 1992). However, this subgrouping was not supported using the amplified fragment length polymorphism method and the sequence analysis of the *hsp*60 gene (Debruyne et al., 2010). Recently, Pérez-Cataluña et al. (2018a) proposed the subdivision of *A. cryaerophilus* into four clusters (also called genomovars) based on whole-genome sequence analyses. The *in silico* calculation of average nucleotide identity and digital DNA-DNA hybridization indicated that these four clusters should not be assigned to a single species. *A. cryaerophilus* represents a species complex in which the four clusters represent four different species (Pérez-Cataluña et al., 2018a).

A. cryaerophilus, as well as other related species, namely *A. butzleri*, *A. skirrowii*, and *A. thereius*, have been associated with diseases in humans and animals (Ho et al., 2006; Collado and Figueras, 2011; Ferreira et al., 2016; Pérez-Cataluña et al., 2018a). In humans, the bacteria can cause self-limiting acute enteritis with watery diarrhea, fever and abdominal pain. A long-term study done by Vandenberg et al. (2004) showed that *A. cryaerophilus* was the seventh most common *Campylobacter*-like organism isolated from human feces. In rare cases, *A. cryaerophilus* can cause severe illnesses e.g., bacteremia (Hsueh et al., 1997). The International Commission of Microbiological Specifications for Food (ICMSF) has classified *Aliarcobacter* as a serious threat to human health in 2002 (ICMSF, 2002). Since then, *Aliarcobacter* spp. have been identified as emerging foodborne and zoonotic pathogens around the globe (Collado and Figueras, 2011; Ramees et al., 2017). In animals, *A. cryaerophilus* has been isolated from aborted fetuses and placentas of bovine, porcine, and ovine origin as well as from milk of cows with mastitis, but also from the digestive tract, feces, preputial washings, and vaginal swabs of healthy animals (Ho et al., 2006; Collado and Figueras, 2011; Ramees et al., 2017; Miller et al., 2018). Furthermore, *A. cryaerophilus* has been associated with abortion and other reproductive disorders in sows (Ramees et al., 2017).

Aliarcobacter cryaerophilus is present in food of animal origin such as poultry meat, dairy products and seafood as well as in drinking water and sewage (Ho et al., 2006; Millar and Raghavan, 2017; Pérez-Cataluña et al., 2018a; On et al., 2019). *Aliarcobacter* spp. are commensals in the intestinal microbiota of poultry, which can contaminate carcasses during the slaughtering process (Ho et al., 2008). Therefore poultry is a natural reservoir and acts as a major source of infection for humans (Atabay et al., 2008; Collado and Figueras, 2011). Consumption of contaminated water or food is considered as the main route of transmission to humans, while contact with companion animals is also a possible way of transmission (Ferreira et al., 2016; Ramees et al., 2017). In animals, the possibility of venereal transmission is also described as strains of *A. butzleri* and *A. cryaerophilus* have been found in the preputial washings of bulls (Ho et al., 2006).

Previous studies showed that *A. cryaerophilus* has fewer virulence-associated genes than *A. butzleri* (Ferreira et al., 2016; Brückner et al., 2020). However, limited information is currently available regarding virulence and antimicrobial susceptibility of this species (Ramees et al., 2017). Very little is known about the antimicrobial resistance and their mechanisms in *A. cryaerophilus*. Described resistance determinants are mostly located chromosomal, and no antimicrobial resistance genes have been identified on plasmids, yet. Until now, only a few studies have reported the presence of plasmids in the genus *Aliarcobacter* (Harrass et al., 1998; Douidah et al., 2014; On et al., 2019).

Here, we describe the genetic diversity and antibiotic susceptibility of 27 *A. cryaerophilus* strains isolated from seven water poultry farms in Thuringia, Germany. Furthermore, we complemented these data with 17 *A. cryaerophilus* genomes from the NCBI database and described genomic features as well as virulence-associated and antibiotic resistance genes for cluster I of the *A. cryaerophilus* complex.

MATERIALS AND METHODS

Bacterial Strains, Culturing and Identification

In 2016 and 2017, 165 fecal samples were collected from clinically healthy animals from seven water poultry farms in Thuringia, Germany. In detail, 100 fecal samples were collected in 2016 from four water poultry farms from 50 geese (*Anser anser*), 20 Muscovy ducks (*Cairina moschata*), 20 Pekin ducks (*Anas platyrhynchos domesticus*), and ten mulard ducks (*Cairina moschata* × *Anas platyrhynchos domesticus*). In 2017, 65 fecal samples were collected from 35 geese, 15 Muscovy ducks, ten Pekin ducks and five mulard ducks from five water poultry farms. A veterinarian gathered the fecal samples with the permission of the animal owners.

For this study, no ethical review process was required, as it was no experiment with animals as defined by the German Animal Protection Law (Tierschutzgesetz) and the Animal Welfare Laboratory Animal Regulation (Tierschutz-Versuchstierordnung).

The *Aliarcobacter* isolates were cultivated in *Arcobacter* broth (Oxoid GmbH, Wesel, Germany). The broth was supplemented with three antibiotics (cefoperazone, amphotericin, and teicoplanin (CAT), Oxoid GmbH). After 48 h of incubation at 30° C under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2), the broth was spread with a 10 µL inoculation loop on plates (Mueller-Hinton agar/CAT/5% defibrinated bovine blood, Sifin GmbH, Berlin, Germany) and incubated for 24–48 h at 30° C under microaerophilic conditions. Suspicious colonies were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using IVD Bacterial Test Standard and Biotyper 3.1 software (both Bruker Daltonik GmbH, Bremen, Germany) as described before (El-Ashker et al.,

2015; Hänel et al., 2018). Species identification was also done with a multiplex PCR assay (Houf et al., 2000) and sequencing of the PCR products. The DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was determined by using the gradient strip diffusion method (*E*-Test™, bioMérieux, Nürtingen, Germany) following the manufacturer's instructions. Briefly, the *Aliarcobacter* strains were incubated on Mueller-Hinton agar plates (Sifin GmbH) for 48 h at 30°C under microaerophilic conditions. The colony material was put into five mL *Arcobacter* broth (Oxoid) and incubated at 30°C under microaerophilic conditions for 48 h. Then, the optical density of the broth was adjusted to 0.08 ± 0.02 at λ = 588 nm. Next, 750 μL of the broth was spread on each Mueller-Hinton agar plate (Sifin GmbH), and the antibiotic gradient strips were placed on the plates. The following antibiotics were used for testing: erythromycin (0,015–256 μg/mL, MA0108F, Oxoid GmbH), ciprofloxacin (0,002–32 μg/mL, MA0104F, Oxoid GmbH), streptomycin (0,064–1024 μg/mL, 526800, bioMérieux), gentamicin (0,06–1024 μg/mL, MA0117F, Oxoid GmbH), tetracycline (0,015–256 μg/mL, MA0105F, Oxoid GmbH), doxycycline (0,016–256 μg/mL, 412328, bioMérieux), ampicillin (0,016–256 μg/mL, 412253, bioMérieux) and cefotaxime (0,002–32 μg/mL, 412281, bioMérieux). The minimum inhibitory concentration was determined after 48 h of incubation at 30°C under microaerophilic conditions. The *A. cryaerophilus* type strain DSM 7289 was used as a control. Cut-off values for *Campylobacter* spp. provided by EUCAST (2019) were used for erythromycin, ciprofloxacin, doxycycline, and tetracycline. For gentamicin, ampicillin and cefotaxime we used the breakpoints for *Enterobacterales* from EUCAST (2019). For streptomycin, the cut-off values for *Campylobacter* spp. provided in the EFSA Journal were used (European Food Safety Authority et al., 2019). The bacterial strains were classified as sensitive (S) or resistant (R).

DNA Extraction and Whole-Genome Sequencing

The DNA extraction was performed for 27 *A. cryaerophilus* isolates. Colony material of one to two *Aliarcobacter* culture plates was needed to obtain sufficient material for DNA preparation. The plates were washed with two milliliters of phosphate-buffered saline (PBS) and the liquid was collected in a 2-mL tube. The tubes were centrifuged for 15 min at 5,400 rpm, then the supernatant was discarded. The remaining content was washed at least twice with PBS until the supernatant was clear. The resulting pellet was further processed for DNA recovery using the QIAGEN Genomic-tip 20/G (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. The concentration of the double-stranded DNA (dsDNA) was examined with Qubit 3 Fluorometer using the Qubit™ dsDNA HS Assay Kit (both Invitrogen™, Thermo Fisher Scientific, Berlin, Germany). The Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA, United States) was used to generate a paired-end sequencing library according to the manufacturer's instructions. Whole-genome sequencing was done with an Illumina MiSeq platform generating reads of 300 bp in length (Illumina Inc.).

For plasmid DNA extraction, the QIAGEN Plasmid Mini Kit (Qiagen GmbH) was used according to the manufacturer's instructions. The obtained plasmid DNA was dissolved in 10 μL Tris-hydroxymethyl-aminomethane (TRIS) buffer (10 mM, pH 8.3) and visualized using 1% agarose gel electrophoresis. As a size marker, 5 μL of the λDNA/*Hin*dIII Digest (Jena Bioscience GmbH, Jena, Germany) was used.

The *A. cryaerophilus* isolates in which a plasmid was detected and another strain from our sample collection (total = 4) were further investigated using the Oxford Nanopore Technology (ONT) MinION. For this purpose DNA was purified with the QIAGEN Genomic-tip 100/G (Qiagen GmbH). Sequencing libraries for ONT MinION was prepared using the ONT 1D Ligation Sequencing Kit (SQK-LSK109) with the Native Barcoding Expansion Kit (EXP-NBD104) as recommended by the manufacturer.

Bioinformatics Analyses

Raw data from the Illumina MiSeq sequencer were assembled using shovill v1.04 [1] with options for trimming and filtering enabled (–trim, –minlen 500, –mincov 3). For the ONT data, the raw FAST5 files were processed using Guppy_basecaller v3.4.1 with high-accuracy models (dna_r9.4.1_450bps_hac) for base calling, followed by Guppy_barcoder v3.4.1 for demultiplexing. Long-read only assembly was performed using Flye v2.6 (Kolmogorov et al., 2019). Assembly polishing was performed with several rounds of Racon v1.4.3 (Vaser et al., 2017) and Medaka v0.10.0[2]. Pilon v1.23 (Walker et al., 2014) was used to correct the assembled data from ONT with Illumina reads using standard settings.

For genome annotation, the software Prokka v1.14.5 was used in default settings (Seemann, 2014). Prediction of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) was done using the CRISPR Recognition Tool in the Geneious prime® 2019.2.3 software (Kearse et al., 2012). A search for Insertion sequences (IS) and genomic islands (GI) was done using ISEscan v1.5.4 (Xie and Tang, 2017) and Islandviewer4 (Bertelli et al., 2017), respectively. Prophages were predicted using prophage_hunter (Song et al., 2019).

The 16S rRNA genes were extracted using barrnap v0.9[3] and aligned using mafft v7.307 (Katoh et al., 2002). The program Mega X (Kumar et al., 2018) was used for the phylogenetic analysis of the 16S rRNA sequences. The Average Nucleotide Identity (ANI) was calculated using pyani v0.2.3 (module ANIm) (Pritchard et al., 2016). *In silico* DNA-DNA hybridization (DDH) was done using Genome-to-Genome Distance Calculator software (Meier-Kolthoff et al., 2013). Multilocus sequence

[1] https://github.com/tseemann/shovill
[2] https://github.com/nanoporetech/medaka
[3] https://github.com/tseemann/barrnap

typing (MLST) was done using the mlst tool v2.15.2[4] and the PubMLST database (Jolley and Maiden, 2010) with default settings. Core genome-based phylogeny was performed using Parsnp v1.2 within Harvest suite with default parameters (Treangen et al., 2014). Genome comparison was carried out using progressiveMauve (Darling et al., 2010).

Public antimicrobial resistance (AMR) databases were searched for resistance-associated genes using ABRicate v0.8.10[5] which uses the BLASTN algorithm to search AMR databases e.g., ResFinder, CARD, ARG-ANNOT, and NCBI [PRJNA313047] (Zankari et al., 2012; Gupta et al., 2014; Jia et al., 2017; Feldgarden et al., 2019).

For further investigations, the virulence and antimicrobial resistance determinants from the corresponding *A. butzleri* strains described by Isidro et al. (2020) were extracted with Geneious Prime® 2019.2.3 (Kearse et al., 2012). All extracted genes were grouped in a custom database and searched within the genomes. For that, a pangenome was constructed for all strains using Roary v3.12.0 (options -i 90 -s) (Page et al., 2015). Then, the sequences of the pangenome were BLASTed against the custom database from Isidro et al. (2020) using BLASTP (Altschul et al., 1990) with the following thresholds: coverage 70% and *E*-value 1–20e. BLAST hits with more than 40% identity at the protein level were reported (Pearson, 2013).

Furthermore, the virulence-associated genes that were first found in a plasmid from an *A. cryaerophilus* isolate from a New Zealand mussel (On et al., 2019), were excised using Geneious Prime® 2019.2.3 (Kearse et al., 2012) and put together into a custom database within ABRicate. The sequences used in this study were screened for those genes with a detection value of more than 30% coverage and 85% identity.

RESULTS AND DISCUSSION

Bacterial Strains and Whole-Genome Sequencing

Out of 165 fecal samples, 14 were positive for *A. cryaerophilus*, nine in 2016 and five in 2017. These were obtained from ten geese, two Pekin ducks, one Muscovy duck, and one mulard duck (**Table 1**). Due to the different morphology of *A. cryaerophilus* on the culture plates, one to five single colonies were picked and processed separately. In total, 27 *A. cryaerophilus* strains were recovered. MALDI-TOF MS and PCR identified these isolates as *Aliarcobacter cryaerophilus*.

In the present study, whole-genome sequencing of 27 *A. cryaerophilus* strains was performed. The Illumina sequencing yielded an average number of 0.9 million reads per strain and an average depth of coverage 86.4X. High-quality genome assemblies were obtained except for three genomes. Those showed high contig numbers and low N50 values (**Table 2**). For the other 24 strains, an average N50 value of 213.3 Kbp and an average of 36 contigs per strain was calculated (**Table 2**).

Taxonomic Classification of *A. cryaerophilus* From Germany

The 27 strains sequenced in this study were taxonomically classified as *A. cryaerophilus* at species level using the 16S rRNA gene (**Figure 1**). The 16S rRNA genes (1,520 bp) extracted from all strains were 99% identical to the 16S rRNA gene from the reference genome ATCC 43158^T (accession: NZ_CP032823.1), representing the type strain of *A. cryaerophilus*. Based on 16S rRNA gene analysis, the closest related species was *A. trophiarum*, with 98.6% identity. Additionally, ANI was calculated between each genome pair based on the whole-genome sequences. Results showed that the 27 German strains were highly similar (>95%) to cluster I genomes, exhibiting an average pairwise ANI of 98.1% (range 96.6–99.00%). The *in silico* DDH was additionally ascertained, showing DDH values higher than 70% when comparing the German strains to the cluster I reference genome LMG 10229^T (accession: GCF_002993045.1) (**Supplementary Table S2**). These DDH values dropped to less than 70% when the German strains were compared to the reference strains from clusters II (LMG 9065^T; accession: GCF_002993025.1), III (LMG 24291^T; accession: GCF_002992955.1) and IV (LMG 10210; accession: GCF_002992935.1) (**Supplementary Table S2**). Based on these results, we concluded that the investigated *A. cryaerophilus* strains from Germany belong to cluster I, also named *A. cryaerophilus gv. pseudocryaerophilus* based on the updated taxonomy proposal (Pérez-Cataluña et al., 2018a,b).

Currently, 24 *A. cryaerophilus* genomes are available at the NCBI GenBank database (Benson et al., 2013)[6]. The NCBI genomes represent strains from cluster I to IV and were collected from different hosts in different countries. Of those, 17 strains were assigned to Cluster I comprising one circularized genome (ATCC 49615; accession: NZ_CP032825.1) (Miller et al., 2018) and 16 fragmented draft genomes (average N50: 256 Kbp; average contig number: 80) (**Supplementary Table S1**). These strains were reported in different host species including humans ($n = 4$), pigs ($n = 2$), cattle ($n = 1$), shellfish ($n = 2$), goose ($n = 1$), dog ($n = 1$), water ($n = 2$), and wastewater ($n = 4$) (**Table 1** and **Figure 2**). The strains span a period between 1987 and 2016 and were isolated from different countries (United States = 2, Canada = 8, Switzerland = 1, Belgium = 3, Ireland = 1, and New Zealand = 2).

A High Genetic Diversity Between *A. cryaerophilus* From Germany Despite Limited Sources of Strain Isolation

In order to investigate phylogenetic relatedness of cluster I genomes of *A. cryaerophilus* ($n = 44$; 27 sequenced herein and 17 available at the NCBI), a core genome of 906.9 Kbp was identified. Variable sites in this cluster comprise 9.1% (82,531 SNPs) of the estimated core genome. The size of the core genomes was reduced to 579.6 Kbp when all genomes from the four clusters ($n = 52$) were taken into consideration. Of these, 11.5% (67,232 SNPs) comprise variable sites. **Figure 2A** visualizes the four

[4]https://github.com/tseemann/mlst

[5]https://github.com/tseemann/abricate

[6]https://www.ncbi.nlm.nih.gov/genome/browse/#!/prokaryotes/11530/

TABLE 1 | The metadata of 44 *A. cryaerophilus* strains of cluster I used in this study.

WGS	Strain	BioProject	Submitter	Isolation source	Year of isolation	Geographic location	Farm Nr. – Sample Nr.*
SRR11794137	16CS0366-1-AR-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Tanna	A – 1
SRR11794136	16CS0366-1-AR-2	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Tanna	A – 1
SRR11794125	16CS0366-1-AR-3	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Tanna	A – 1
SRR11794117	16CS0366-1-AR-4	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Tanna	A – 1
SRR11794116	16CS0369-1-AR-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Tanna	A – 2
SRR11794115	16CS0369-1-AR-4	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Tanna	A – 2
SRR11794114	16CS0814-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Uhlstädt-Kirchhasel	B – 3
SRR11794113	16CS0830-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Tanna	A – 4
SRR11794112	16CS0847-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Schorba	C – 5
SRR11794111	16CS0847-2	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Schorba	C – 5
SRR11794135	16CS0847-4	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Schorba	C – 5
SRR11794134	16CS0847-5	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Schorba	C – 5
SRR11794133	16CS0847-6	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Schorba	C – 5
SRR11794132	16CS1043-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (pekin duck)	2016	Germany: Tanna	A – 6
SRR11794131	16CS1285-3	PRJNA632720	Friedrich-Loeffler-Institut	feces (mulard duck)	2016	Germany: Kyffhäuserland, Seega	D – 7
SRR11794130	16CS1285-4	PRJNA632720	Friedrich-Loeffler-Institut	feces (mulard duck)	2016	Germany: Kyffhäuserland, Seega	D – 7
SRR11794129	16CS1290-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Kyffhäuserland, Seega	D – 8
SRR11794128	16CS1292-3	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Kyffhäuserland, Seega	D – 9
SRR11794127	16CS1292-4	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2016	Germany: Kyffhäuserland, Seega	D – 9
SRR11794126	17CS0830-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2017	Germany: Schorba	C – 10
SRR11794124	17CS0996-A	PRJNA632720	Friedrich-Loeffler-Institut	feces (muscovy duck)	2017	Germany: Remda-Teichel	E – 11
SRR11794123	17CS0996-B	PRJNA632720	Friedrich-Loeffler-Institut	feces (muscovy duck)	2017	Germany: Remda-Teichel	E – 11
SRR11794122	17CS1055-A	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2017	Germany: Grabfeld, Wolfmannshausen	F – 12
SRR11794121	17CS1055-B	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2017	Germany: Grabfeld, Wolfmannshausen	F – 12
SRR11794120	17CS1061	PRJNA632720	Friedrich-Loeffler-Institut	feces (goose)	2017	Germany: Freienbessingen	G – 13
SRR11794119	17CS1201-1	PRJNA632720	Friedrich-Loeffler-Institut	feces (pekin duck)	2017	Germany: Tanna	A – 14
SRR11794118	17CS1201-2	PRJNA632720	Friedrich-Loeffler-Institut	feces (pekin duck)	2017	Germany: Tanna	A – 14
NZ_CP032825.1	ATCC 49615	PRJNA66819	USDA, ARS, WRRC	human blood	-	United States	–
GCF_002080085.1	AZT-1	PRJNA302819	Portland State University	wastewater	2013	United States: Tucson, Arizona	–
GCF_001572865.1	L397	PRJNA307600	Agriculture and Agri-Food Canada	wastewater	2008	Canada: Lethbridge, Alberta	–
GCF_001572855.1	L398	PRJNA307600	Agriculture and Agri-Food Canada	water	2008	Canada: Oldman River, Alberta	–
GCF_001573015.1	L399	PRJNA307600	Agriculture and Agri-Food Canada	wastewater	2008	Canada: Lethbridge, Alberta	–
GCF_001573005.1	L400	PRJNA307600	Agriculture and Agri-Food Canada	wastewater	2008	Canada: Lethbridge, Alberta	–
GCF_001572845.1	L401	PRJNA307600	Agriculture and Agri-Food Canada	feces (goose)	2009	Canada: Levit, Alberta	–
GCF_001572875.1	L406	PRJNA307600	Agriculture and Agri-Food Canada	water	2008	Canada: Indian Farm Creek, Alberta	–
GCF_006503595.1	123	PRJNA294644	Ghent University	feces (dog)	2006	Belgium	–
GCF_006503545.1	151	PRJNA294645	Ghent University	feces (human)	2005	Switzerland	–
GCF_006503605.1	382	PRJNA294646	Ghent University	feces (human)	2008	Belgium	–
GCF_006503615.1	938	PRJNA308312	Ghent University	feces (human)	2013	Belgium	–
GCF_006508135.1	LMG 10228	PRJNA294642	Ghent University	tissue of aborted porcine fetus	1987	Canada	–
GCF_002993045.1	LMG 10229	PRJNA369468	Universitat Rovira i Virgili	aborted porcine fetus	1990	Canada	–
GCF_002993065.1	LMG 9861	PRJNA369468	Universitat Rovira i Virgili	peritoneum of aborted bovine fetus	1990	Ireland	–
GCF_008086605.1	G18RTA	PRJNA431460	Lincoln University	shellfish (*Perna canaliculus*)	2016	New Zealand: Canterbury	–
GCF_008086685.1	M830A	PRJNA431460	Lincoln University	shellfish (*Perna canaliculus*)	2016	New Zealand: Canterbury	–

TABLE 2 | Sequencing, assembly statistics and annotation of 27 German *A. cryaerophilus* strains.

Strain	Sequencing statistics					Assembly statistics			Annotation		
	Sequencing platform	Total number of reads (× 1000)	Total number of sequences (Mbp)	Average read length (bp)	Coverage depth (*X*)	Genome size	N. contigs	N50	Total CDS	rRNA	tRNA
16CS0369-1-AR-4	Illumina MiSeq+	1088.5	247.5	227 (35–301)	121	Chromosome: 2.02 Mbp	–	–	2047	15	49
	ONT MinION	110.0	1,590.7	14,459.9 (33–285,487)	240 (chromosome) 87 (plasmid)	Plasmid: 154.49 Kbp	–	–	135		2
16CS0830-1	Illumina MiSeq +	913.7	200.8	219 (35–301)	98	Chromosome: 2.05 Mbp	–	–	2056	15	49
	ONT MinION	96.5	1,597.8	16,557.5 (27–247,704)	239 (chromosome) 105 (plasmid)	Plasmid: 128.99 Kbp	–	–	129	–	–
16CS1285-4	Illumina MiSeq +	273.5	56.4	206 (35–301)	27	Chromosome: 2.13 Mbp	–	–	2215	15	50
	ONT MinION	231.1	1,646.1	712.1 (56–206,180)	229 (chromosome) 147 (plasmid)	Plasmid: 98.09 Kbp	–	–	125	–	–
16CS1292-4	Illumina MiSeq+	889.9	200.3	225 (35–301)	98	Chromosome: 2.02 Mbp	–	–	2044	15	50
	ONT MinION	150.7	2,223.4	14,758.7 (37–223,549)	241 (chromosome) 123 (plasmid)	Plasmid: 137.35 Kbp	–	–	135	–	–
16CS0366-1-AR-1	Illumina MiSeq	504.9	118.7	235 (35–301)	58	2.15 Mbp	31	327,568	2,168	3	43
16CS0366-1-AR-2	Illumina MiSeq	734.6	167.5	228 (35–301)	82	2.15 Mbp	32	327,568	2,170	3	43
16CS0366-1-AR-3	Illumina MiSeq	481.2	115.9	240 (35–301)	56	2.15 Mbp	35	210,780	2,167	3	43
16CS0366-1-AR-4	Illumina MiSeq	735.1	167.0	227 (35–301)	81	2.14 Mbp	31	231,260	2,165	3	42
16CS0369-1-AR-1	Illumina MiSeq	667.9	151.4	226 (35–301)	74	2.15 Mbp	31	327,568	2,167	3	43
16CS0814-1	Illumina MiSeq	1,954.9	277.9	142 (35–301)	136	2.06 Mbp	39	125,155	2,092	3	41
17CS0830-1	Illumina MiSeq	443.6	112.6	253 (35–301)	55	2.09 Mbp	38	185,374	2,108	3	41
16CS0847-1	Illumina MiSeq	438.4	107.2	244 (35–301)	52	2.2 Mbp	114	40,445	2,228	3	42
16CS0847-2	Illumina MiSeq	679.3	156.7	230 (35–301)	76	2.11 Mbp	40	130,189	2,141	3	42

(Continued)

TABLE 2 | Continued

Strain	Sequencing statistics					Assembly statistics			Annotation		
	Sequencing platform	Total number of reads (× 1000)	Total number of sequences (Mbp)	Average read length (bp)	Coverage depth (*X*)	Genome size	N. contigs	N50	Total CDS	rRNA	tRNA
16CS0847-4	Illumina MiSeq	1,362.4	279.8	205 (35–301)	137	2.11 Mbp	43	113,538	2,139	3	42
16CS0847-5	Illumina MiSeq	429.1	60.4	140 (35–301)	29	2.08 Mbp	179	24,590	2,087	3	42
16CS0847-6	Illumina MiSeq	667.6	155.1	232 (35–301)	76	2.11 Mbp	41	119,731	2,138	3	42
16CS1043-1	Illumina MiSeq	2,241.7	300.7	134 (35–301)	147	2.09 Mbp	56	124,529	2,099	3	42
16CS1285-3	Illumina MiSeq	577.0	134.5	233 (35–301)	65	2.35 Mbp	58	117,068	2,354	3	40
16CS1290-1	Illumina MiSeq	552.5	139.4	252 (35–301)	68	2.11 Mbp	38	174,358	2,141	3	42
16CS1292-3	Illumina MiSeq	1,453.5	290.9	200 (35–301)	142	2.14 Mbp	63	134,716	2,164	3	43
17CS0996-A	Illumina MiSeq	738.8	183.8	248 (35–301)	90	2.02 Mbp	18	371,261	2,035	3	41
17CS0996-B	Illumina MiSeq	937.6	211.2	225 (35–301)	103	2.02 Mbp	16	371,261	2,033	3	41
17CS1055-A	Illumina MiSeq	1,203.4	269.2	223 (35–301)	131	2.16 Mbp	27	178,188	2,189	3	41
17CS1055-B	Illumina MiSeq	852.5	199.6	234 (35–301)	97	2.12 Mbp	17	326,991	2,153	3	41
17CS1061	Illumina MiSeq	750.8	169.3	225 (35–301)	82	1.96 Mbp	30	223,789	1,987	3	41
17CS1201-1	Illumina MiSeq	466.9	47.3	101 (35–301)	23	2 Mbp	540	5,487	1,926	3	30
17CS1201-2	Illumina MiSeq	1,191.5	261.6	219 (35–301)	128	2.19 Mbp	36	144,802	2,208	3	41

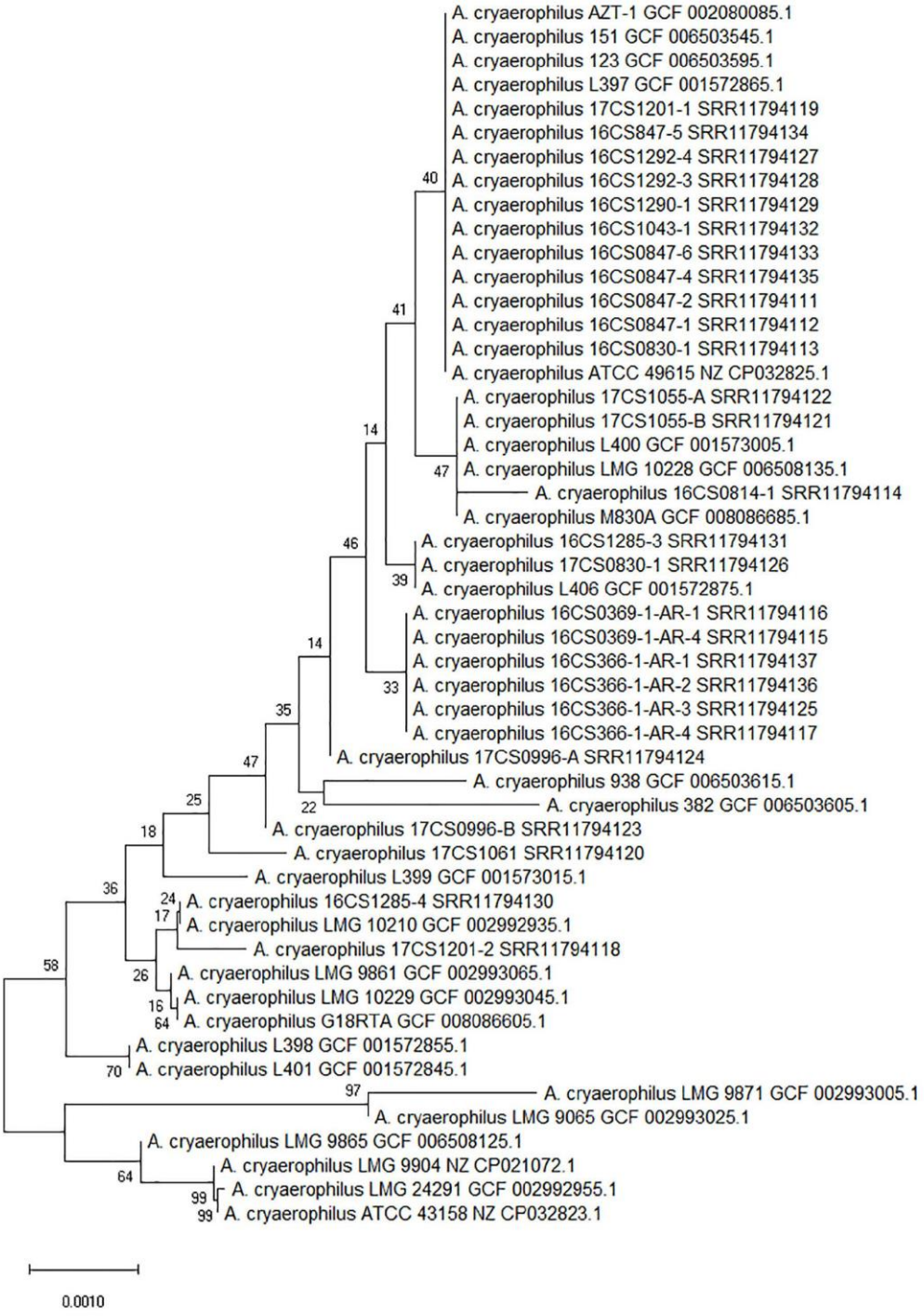

FIGURE 1 | Phylogenetic tree constructed with 51 *A. cryaerophilus* strains based on the extracted 16S rRNA genes (1,520 bp). Numbers at tree branches denote the bootstrap value. Scale bar indicates the base substitution per site.

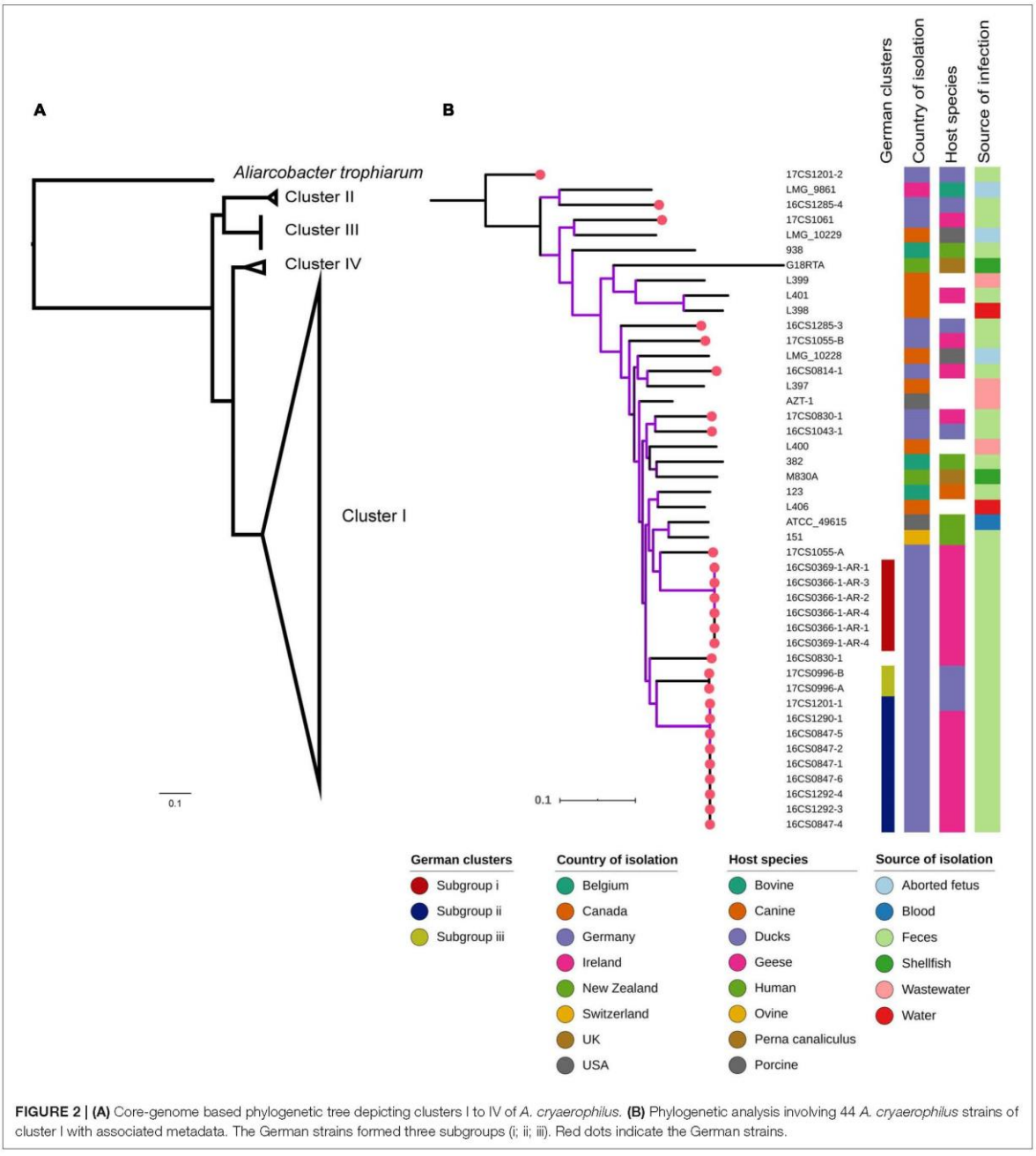

FIGURE 2 | (A) Core-genome based phylogenetic tree depicting clusters I to IV of *A. cryaerophilus*. **(B)** Phylogenetic analysis involving 44 *A. cryaerophilus* strains of cluster I with associated metadata. The German strains formed three subgroups (i; ii; iii). Red dots indicate the German strains.

main clusters of *A. cryaerophilus*, with most of the tree branches presenting high bootstraps values.

Based on the core genome analysis, the 27 isolates from German waterfowls (which represent seven farms in seven different places in Thuringia, see **Table 1**) were assigned to cluster I. In this cluster, the mean pairwise genetic distance between the German strains was 10,773 SNPs. However, three subgroups (i, ii, and iii) including 17 strains could be depicted (**Figure 2B**). These were mostly the strains that had been isolated from a single sample from the same farm. The subgroup i includes six strains retrieved from two fecal samples from ducks that were collected from a farm in Tanna in 2016. Two additional fecal samples were

collected at the same time from the same farm, but the strains recovered were distant by more than 10,000 SNPs to subgroup i, and to each other by 10,862 SNPs. The same farm had been sampled once more in 2017, and from this additional sample, two strains were recovered. They were ∼20,000 SNPs distant from each other. In fact, one of these two strains, namely 17CS1201-1, grouped with strains from different localities in subgroup ii, with only six to seven SNP differences. The observation that strains recovered from the same sample can be highly divergent was also found in 1) two strains from Grabfeld, Wolfmannshausen isolated in 2017, in which 11,903 SNPs were detected; and 2) two strains from Kyffhaeuserland, Seega isolated in 2016, with 20,531 SNPs difference. Subgroup ii surprisingly included strains ($n = 9$) that were recovered from fecal samples collected from three farms in three different cities, five from Schorba (one sample in 2016 from a goose), three from Kyffhaeuserland, Seega (two samples in 2016 from geese) and one from Tanna (one sample in 2016 from a duck). Subgroup iii included two strains recovered from one sample from a duck farm in Remda-Teichel in 2017.

Additionally, MLST was performed based on the whole-genome sequences. All 44 genomes of cluster I were assigned to new sequence types (ST) (**Supplementary Table S3**). The presence of paralogs for *glyA* (i.e., multiple copies in the same genome) precluded the proper designation of STs for some of the genomes ($n = 3$). The *glyA* paralogs were identical in two genomes [17CS1055-B and L401(accession: GCF_001572845.1)] but showed variants in one genome (ATCC 49165). Isidro et al. (2020) also observed this for the *glyA* gene in strains of the species *A. butzleri*. Thus, this specific locus may not be suitable for MLST typing because it may lead to an incorrect allele calling as it has been reported for *Acinetobacter baumanii* (Gaiarsa et al., 2019). Further, we observed the absence of two MLST loci in three genomes, L397 (accession: GCF_001572865.1) lacked *glyA*, while 938 (accession: GCF_006503615.1) and AZT-1 (accession: GCF_002080085.1) both missed the *gltA* locus. It has to be noted that the quality of the assembly may influence the detection of loci. Therefore, the absence of these loci could not be confirmed. The PubMLST database (pubmlst.org, accessed on 11.02.2020) lists sequence data of 118 *A. cryaerophilus* isolates from 11 different countries, with no MLST data currently available from Germany. Those 118 strains were typed into 99 STs indicating a high genetic diversity as explained before for the core genome analysis.

These results indicate a high degree of genetic diversity among *A. cryaerophilus* strains. This was observed although the collection of the 27 strains investigated herein was restricted to one federal state (Thuringia, Germany), a particular host (water poultry) and a short study period (2 years). The strain diversity within this species is independent of the host species, as similar isolates were detected in geese and ducks (e.g., as observed in subgroup ii). This was also reflected by the global phylogeny of cluster I, in which no major clade could be identified based on the host species. Similarly, the phylogenetic analysis did not support distinct clustering based on the geographical or ecological niche of the strains. Nonetheless, it was striking that highly similar strains exist in different farms located in different places, a finding that indicates a possible epidemiological connection between these farms which might be a common source of animals. The farmer informed us that a single company in Germany supplies most of the geese-fattening farms farms in Thuringia with young animals. Muscovy ducks and mulard ducks are bred and imported from France. They are coming to Germany from a rearing farm that raises them until they are about 3 weeks old. This distributor, in turn, sells the raised animals to the fattening farms.

Genomic Description of Cluster I *A. cryaerophilus* Strains Employing Circularized Genomes

As mentioned above, the German *A. cryaerophilus* strains belong to cluster I. To investigate the genomic features of this cluster, we focused on closing four selected genomes of *A. cryaerophilus*, as only a single genome (ATCC 49615) from this cluster had been circularized. The strains were selected based on their plasmid content as determined using a conventional plasmid detection kit (see section "Materials and Methods"). We sequenced four *A. cryaerophilus* strains additionally with the ONT sequencing method. The genome assemblies were polished with Illumina reads to improve sequence accuracy (see section "Materials and Methods"). The four genomes were composed of a single chromosome and a single megaplasmid. The size of the megaplasmids ranged from 98.1 to 154.5 Kbp with a GC content between 24.8 and 25.7% (**Table 2**). These plasmids carried 122–145 coding sequences, 70% thereof were hypothetical proteins.

In the five circularized genomes (four sequenced herein and one public available), the chromosome structure was found to be consistent in terms of length, GC content, RNA genes and coding capacity. The chromosome was approximately 2 Mbp long (range: 2.02–2.14 Mbp) and had a GC content of 27.5% (range: 27.51–27.68%). Each chromosome carried five rRNA operons comprising three successive genes (16S, 23S, and 5S rRNA genes); and 50 tRNA genes (range: 49–51). A striking feature was that the chromosome was densely packed with coding sequences, with an average of 2,110 CDS (range: 2,055–2,243) that represent 91.5–93.4% of the chromosome size. This was nearly similar to *Campylobacter jejuni*, in which 94.3% of the genome code for proteins and was reported to be the densest bacterial genome reported to date (Parkhill et al., 2000). Additionally, the alignment of the chromosome from our four strains together with strain ATCC 49615 identified a considerable degree of synteny between the strains (**Figure 3**). The order of gene blocks (locally collinear blocks; LCB) was similar in two strains (16CS0369-1-AR-4 and 16CS0830-1) while an inversion of a single LCB was observed around the terminus of replication in the strains 16CS01292-4 and ATCC 49615. The strain 16CS1285-4 was found to have undergone several rearrangement events of the chromosomal LCBs.

Mobile element proteins (IS, GI, and prophages) were found to constitute a small part of the genome except for one strain (16CS1285-4; **Supplementary Table S4**). Interestingly, this strain showed several rearrangements in the homolog gene blocks. In the strains ATCC 49615, 16CS0369-1-AR-4, 16CS0830-1, 16CS1292-4 and 16CS1285-4 we detected 2, 8, 13, 14, and 63 IS

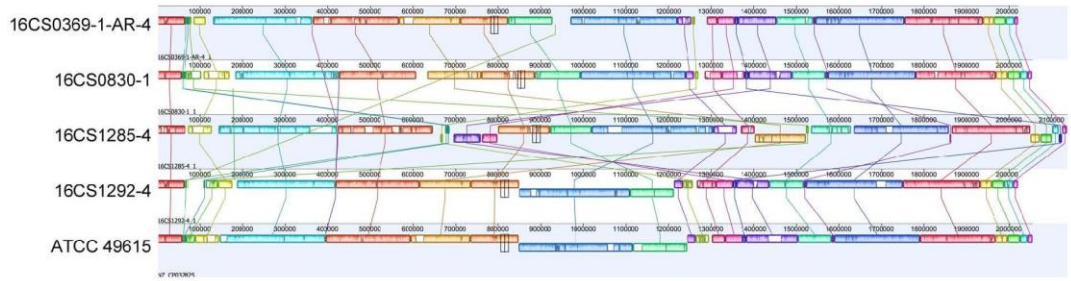

FIGURE 3 | Alignment of the chromosomes from our four circularized strains together with strain ATCC 49615. The order of gene blocks was similar in 16CS0369-1-AR-4 and 16CS0830-1. In the strains 16CS01292-4 and ATCC 49615 was an inversion of a single LCB observed around the terminus of replication. Strain 16CS1285-4 had undergone several rearrangements of the chromosomal LCBs.

elements, respectively, as well as 6, 4, 8, 6, 15 GIs, respectively. One prophage was found in three strains (16CS0369-1-AR-4, 16CS0830-1 and 16CS1285-4). Only two strains (16CS0830-1, 16CS0369-1-AR-4) carried CRISPR elements (**Supplementary Table S4**). Additionally, an average of 300 repeats of size >1,000 bp were detected in each chromosome. These results were also similar in the remaining fragmented genomes of cluster I (n = 39). However, mobile elements usually exist in multiple copies in the genome and therefore an underestimation of their occurrence in the fragmented genomes may occur. This is because Illumina sequencing is not compatible with repeats (Torresen et al., 2019). In general, the fragmented genomes (n = 39) carried an average of 9 (range 1–30) insertion sequences and five (range 1–10) genomic islands. Prophages and CRISPR elements were detected only in 24 and 13 genomes, respectively.

Genetic Prediction of Antibiotic Resistance and Concordance With the Resistance Phenotype

The 27 *A. cryaerophilus* strains from Germany were susceptible to erythromycin, gentamicin and ampicillin, but were resistant to cefotaxime (**Table 3**). Strains 16CS1292-3 and 16CS1292-4 were resistant to tetracycline, while the strains 16CS0336-1-AR-1, 16CS0366-1-AR-2, 16CS0366-1-AR-4, and 16CS0814-1 were resistant to doxycycline. These results are in line with studies already conducted, although in these studies resistance to ampicillin has been described controversially (Ünver et al., 2013; Ferreira et al., 2016, 2019; Van den Abeele et al., 2016; Pérez-Cataluña et al., 2017). Additionally, 23 isolates showed resistance to streptomycin, while 16 strains were resistant to ciprofloxacin. This result is also largely consistent with previous studies (Van den Abeele et al., 2016; Pérez-Cataluña et al., 2017; Ferreira et al., 2019). However, previous studies used the disk diffusion method to determine antimicrobial susceptibility. Therefore, our results can be compared with those of earlier studies to a limited extent. Van den Abeele et al. (2016) compared both, the disk diffusion test and the gradient strip diffusion method. They concluded that the gradient strip method should be preferred over the disk diffusion test and that the agreement of both methods stands at 60%. This strongly argues for the need of a standardized method for measuring the antimicrobial susceptibility of *Aliarcobacter* spp. and for the evaluation of the results.

Utilizing the genomes of cluster I the genetic prediction of antimicrobial resistance genes was done using the custom database created by Isidro et al. (2020). Out of 19 efflux pump (EP) systems which have been detected in *A. butzleri* genomes, 16 were found in the *A. cryaerophilus* genomes belonging to cluster I (n = 44) (**Figure 4**). The three missing efflux pump systems are EP9, EP11, and EP19. Six EP systems were present in all genomes: (a) EP2 and EP12 [both belong to the major facilitator superfamily (MFS)]; (b) EP5 and EP6 [both belong to the ATP-binding cassette (ABC) superfamily]; (c) EP7 [belongs to the resistance-nodulation-division (RND) family]; and (d) EP8 [belongs to the small multidrug resistance (SMR) family]. The remaining ten EP systems belong to the RND, ABC and MFS families and were present at least in one strain. Those findings showed that *A. cryaerophilus* harbors all major families of efflux transporters that are present in prokaryotes apart from the multidrug and toxic efflux (MATE) family (Webber and Piddock, 2003). Since the protein size of regulator TetR (RM4018p_22360) from EP16 is supposed to correlate with the erythromycin resistance, Isidro et al. (2020) hypothesized that truncating mutations in TetR lead to an overexpression of EP16 and thus to increased erythromycin excretion and ultimately cause resistance or tolerance to this antibiotic. The regulator TetR was not present in our 27 German *A. cryaerophilus* strains, which were all susceptible to erythromycin. EP3, a member of the ABC family, might also be involved in erythromycin resistance as it contains *mac*A and *mac*B genes that encode macrolide export proteins (Fanelli et al., 2019). Although both genes were found in all German strains, the strains were susceptible to erythromycin.

A complete type I secretion system (T1SS) was not detected in any of the 44 *A. cryaerophilus* genomes of cluster I. Apart from the fact that only six instead of seven genes were detected (DNA-binding response regulator gene (RM4018p_10330) was missing), these genes were not present in all strains. 16CS1285-3 was the only genome that carried five T1SS genes.

The search for other antimicrobial resistance determinants revealed that all 44 *A. cryaerophilus* strains harbor two beta-lactamase genes (*bla*1, RM4018p_05810; *bla*2, RM4018p_13040),

TABLE 3 | Antimicrobial susceptibility of 27 *A. cryaerophilus* isolates.

Isolates	ERY		CIP		DC		TC		GEN		STX		AMP		CTX	
	mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		mg/L	
16CS0366-1-AR-1	4	S	32	R	3	R	2	S	2	S	8	R	6	S	>32	R
16CS0366-1-AR-2	4	S	32	R	3	R	2	S	2	S	8	R	5	S	>32	R
16CS0366-1-AR-3	2	S	32	R	2	S	2	S	2	S	8	R	5	S	>32	R
16CS0366-1-AR-4	4	S	32	R	3	R	2	S	2	S	8	R	5	S	>32	R
16CS0369-1-AR-1	4	S	32	R	2	S	2	S	2	S	8	R	6	S	>32	R
16CS0369-1-AR-4	2	S	32	R	2	S	2	S	2	S	8	R	4	S	>32	R
16CS0814-1	3	S	0,12	S	4	R	1	S	1,5	S	4	S	3	S	>32	R
16CS0830-1	1	S	32	R	1	S	0,38	S	0,75	S	4	S	3	S	>32	R
16CS0847-1	4	S	32	R	2	S	1,25	S	1,75	S	10	R	3	S	>32	R
16CS0847-2	2	S	16	R	1,5	S	1	S	1	S	6	R	3	S	>32	R
16CS0847-4	4	S	32	R	1,5	S	1	S	1	S	12	R	6	S	>32	R
16CS0847-5	1	S	0,12	S	0,38	S	0,5	S	1	S	6	R	3	S	>32	R
16CS0847-6	4	S	32	R	2	S	1,25	S	1,75	S	12	R	6	S	>32	R
16CS1043-1	3	S	0,06	S	2	S	1	S	1,5	S	8	R	2	S	>32	R
16CS1285-3	4	S	0,12	S	1	S	1	S	1	S	4	S	2	S	>32	R
16CS1285-4	2	S	32	R	2	S	2	S	1	S	12	R	4	S	>32	R
16CS1290-1	4	S	32	R	1,5	S	1,5	S	1,5	S	12	R	4	S	>32	R
16CS1292-3	4	S	32	R	1,5	S	16	R	1,5	S	12	R	6	S	>32	R
16CS1292-4	4	S	32	R	2	S	12	R	2	S	12	R	1,5	S	>32	R
17CS0830-1	4	S	0,12	S	1,5	S	1	S	1	S	4	S	3	S	>32	R
17CS0996-A	4	S	0,06	S	1	S	1	S	1	S	6	R	6	S	>32	R
17CS0996-B	2	S	0,06	S	1,5	S	1	S	1	S	6	R	4	S	>32	R
17CS1055-A	2	S	0,12	S	2	S	0,25	S	0,5	S	8	R	6	S	>32	R
17CS1055-B	1	S	8	R	1	S	1	S	1	S	12	R	2	S	>32	R
17CS1061	1	S	0,06	S	0,75	S	0,5	S	1	S	6	R	2	S	>32	R
17CS1201-1	2	S	0,12	S	2	S	1	S	1	S	6	R	1,5	S	8	R
17CS1201-2	0,5	S	0,06	S	0,75	S	0,5	S	2	S	6	R	3	S	>32	R

CIP – ciprofloxacin, DC – doxycycline, TC – tetracycline, GEN – gentamicin, STX – streptomycin, AMP – ampicillin, CTX – cefotaxime, S – susceptible, R – resistant.

while eight isolates also carried *bla*3 (RM4018p_14830). None of the strains carried the chloramphenicol acetyltransferase gene (*cat*3, RM4018p_07870). Isidro et al. (2020) detected a strong correlation between the presence of an OXA-15-like beta-lactamase gene (*bla*3) and ampicillin resistance. This beta-lactamase gene was detected in one strain (16CS0830-1). Contradictory, the strain was phenotypically susceptible to ampicillin *in vitro*. This may indicate that not only the presence of the beta-lactamase gene is important, but also its activity together with penicillin-binding proteins and outer-membrane permeability (Fanelli et al., 2019). The presence of the two beta-lactamase genes *bla*1 and *bla*2 in all 44 genomes might be the reason for the cefotaxime resistance. Resistance to ciprofloxacin in *Aliarcobacter* spp. is, as reported previously, caused by a point mutation in the quinolone resistance determining region (QRDR) at position 254 of the *gyr*A gene (Abdelbaqi et al., 2007). This mutation subsequently leads to an amino acid exchange from threonine to isoleucine (Thr-85-Ile). In this study, out of 16 resistant *A. cryaerophilus* strains, 15 exhibited this mutation (**Supplementary Table S5**). One strain, 16CS1285-4, was phenotypically resistant but did not carry this specific mutation or any other known mutation [e.g., Asp-89-Tyr (Ferreira et al., 2018)]. Interestingly, two susceptible strains had the reported mutation. These observations show that not in every case resistance to ciprofloxacin is due to a single mutation in the *gyr*A gene in *A. cryaerophilus*. Maybe a functional multidrug efflux pump e.g., *cme*ABC (RND) is also required as described before for *Campylobacter* (Shen et al., 2018). It is noteworthy, that the topoisomerase IV genes *par*C and *par*E which are also responsible for fluoroquinolone resistance were absent in the tested strains, suggesting that they are not involved in ciprofloxacin resistance in *A. cryaerophilus*.

Three of our four plasmid sequences carried at least one gene that is associated with antimicrobial resistance e.g., multidrug efflux systems *cme*ABC and *cme*DEF (RND) and a streptomycin-3-O-adenyltransferase (**Supplementary Table S6**), showing that *A. cryaerophilus* may be able to exchange antimicrobial resistance genes.

In silico Virulence Gene Profiling

As with antibiotic resistance genes, the database created by Isidro et al. (2020) was also used to identify potential virulence determinants in *A. cryaerophilus* genomes of cluster I. This database includes genes for the flagellum synthesis, chemotaxis system, and capsule as well as genes for adherence, invasion, iron uptake, type IV secretion system (T4SS), and an urease cluster.

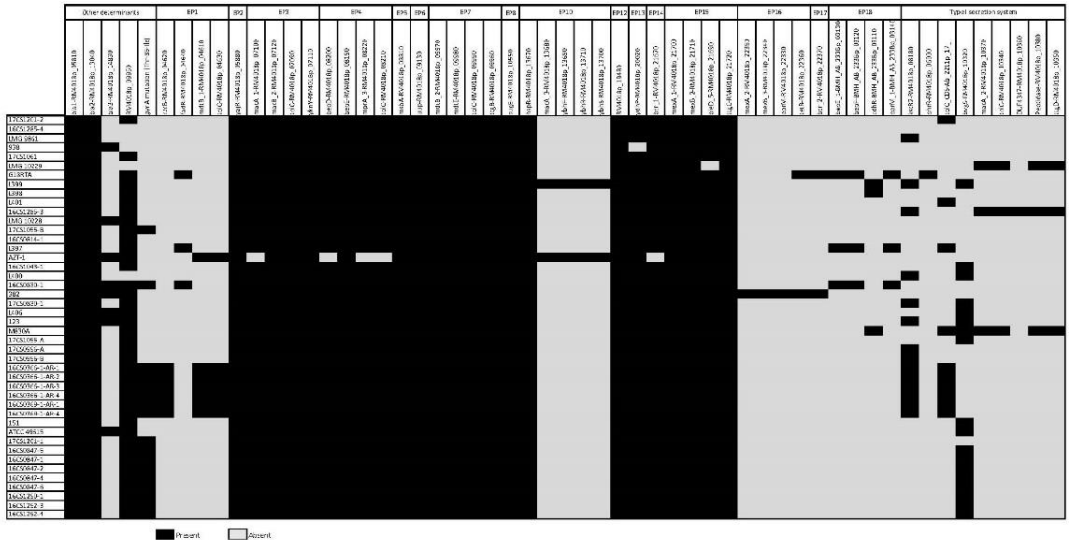

FIGURE 4 | Predicted antimicrobial resistance determinants in 44 *A. cryaerophilus* genomes of cluster I including 16 efflux pumps (EP) systems, a type I secretion system (T1SS), resistance genes and the *gyr*A mutation (Thr-85-Ile).

The survey showed that none of the 44 genomes carried the urease cluster (**Figure 5**). This finding was not surprising because *A. cryaerophilus* is reported to be non-ureolytic compared to *A. butzleri* (Miller et al., 2018).

Furthermore, only fragments of the T4SS were found, since 33 of 55 genes were detected in our strains. These 33 genes were either present in all isolates (e.g., PAS domain-containing protein, D4963p_10560) or just in one strain (e.g., hypothetical protein, D4963p_10940).

The potential capsule cluster was only found partially. Four out of 7 genes, namely *gmh*A2, *gmh*B, *hdd*A, and *hdd*C, were detected. While strain L400 (accession: GCF_001573005.1) carried all 4 genes, the *gmh*A2 gene was the only gene present in all tested genomes.

Aliarcobacter cryaerophilus is a motile bacterium with a polar flagellum like *A. butzleri*. It was therefore not surprising that flagellum genes were detectable. However, three genes, namely *fla*B (minor flagellin subunit), *flh*A (flagellar biosynthesis protein), and *hag* (another gene encoding flagellin), out of 34 flagellar genes could not be found in any of the 44 genomes. Of the remaining 31 genes, 22 were detected in all isolates, with strain AZT-1 having the fewest flagellum genes. Nevertheless, the missing flagellar genes could have an impact on the assembly or function of the flagellum.

We highlight the presence of the chemotaxis system genes (*che*A-*che*Y) in all tested *A. cryaerophilus* isolates apart from the strains AZT-1 and 938. While *che*Y2 was not found in strain 938, the genes *che*A and *che*V were not found in strain AZT-1. This result shows that *A. butzleri* is not the only *Aliarcobacter* spp. that carries a full chemotaxis system (Miller et al., 2007; Isidro et al., 2020). Interestingly, the chemotaxis-associated genes *doc*A and *lux*S were also present in all investigated isolates. Only strain AZT-1 had no *doc*A gene.

The following virulence determinants associated with cellular adhesion and invasion were present in all 44 genomes: *cad*F, *cj*1349 (both fibronectin-binding proteins), *tly*A (hemolysin), *iam*A (invasion-associated gene), *mvi*N (inner membrane protein for peptidoglycan biosynthesis). The genes *cia*B (host cell invasion) and *pld*A (outer membrane phospholipase A) were present in all strains except in LMG 10229^{T} and AZT-1, respectively. The gene *htr*A–a chaperon involved in adhesins folding (Isidro et al., 2020) – was present in 43 isolates, but not in strain 17CS1201-1. While *hec*A (filamentous hemagglutinin) was only present in G18RTA (accession: GCF_008086605.1), *hec*B (hemolysin activation protein) occurred in both, G18RTA and L401. Of the two genes, namely *cfr*B and *fur*, possibly involved in iron uptake, *fur* was detected in all strains but not in 17CS1201-1. The *cfr*B gene was found to be present in 24 isolates. The genes *irg*A and *iro*E which have been associated with the uropathogenicity of *E. coli* (Miller et al., 2007) were both detected in five genomes, with *irg*A being present in three additional strains.

Previous studies reported that the virulence genes *cia*B, *cad*F, *cj*1349, *pld*A, and *mvi*N are more frequently detected in *A. cryaerophilus* strains than the other virulence determinants e.g., *tly*A, *irg*A, iroE, *hec*A, and *hec*B (Douidah et al., 2012; Zacharow et al., 2015; Sekhar et al., 2017; Brückner et al., 2020). This is mostly in line with our data. Here, only strain G18RTA carried all 14 virulence genes associated with adherence, invasion, and iron uptake. This is consistent with previous reports, as it is very rare to find all virulence genes in every *A. cryaerophilus* isolate (Zacharow et al., 2015; Sekhar et al., 2017; Brückner et al.,

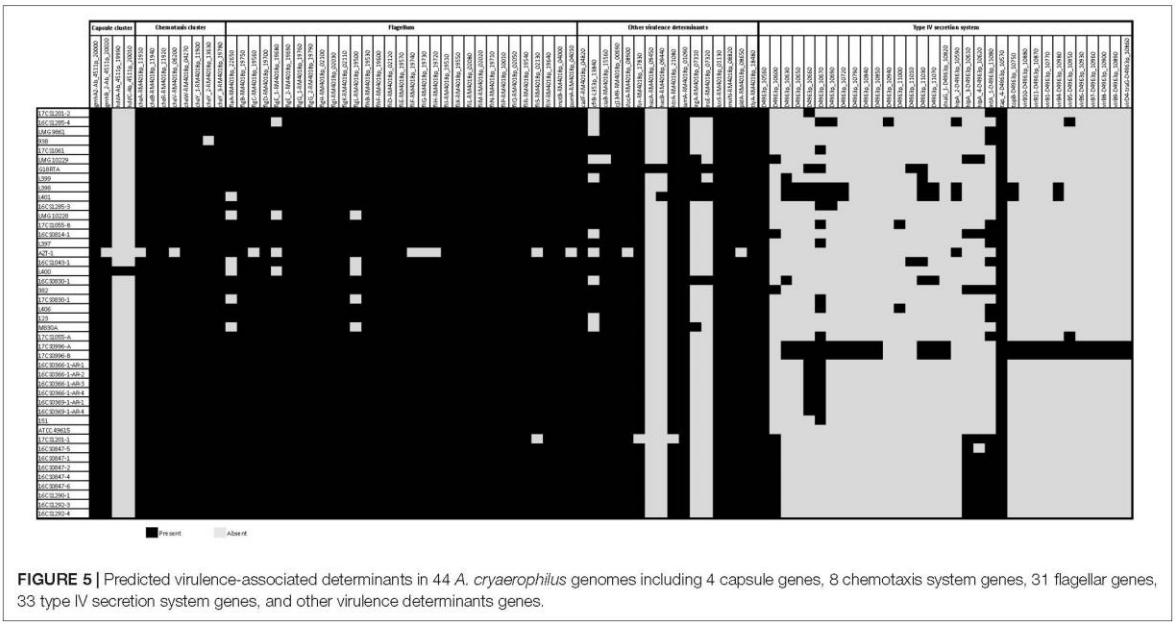

FIGURE 5 | Predicted virulence-associated determinants in 44 *A. cryaerophilus* genomes including 4 capsule genes, 8 chemotaxis system genes, 31 flagellar genes, 33 type IV secretion system genes, and other virulence determinants genes.

2020). In fact, previous studies reported that the phenotypic urease test in *A. cryaerophilus* was negative. Therefore it could be hypothesized that *A. cryaerophilus* is not able to metabolize urea and may not be able to survive in acidic surroundings, e.g., in the urinary tract (Pérez-Cataluña et al., 2018b, 2019).

Additionally, the findings of the virulence-associated genes – previously found on the virulence plasmid from an *A. cryaerophilus* strain (BankIt2207814 M830MA_plasmid MK715471) (On et al., 2019) – in all *A. cryaerophilus* sequences used in the present study are summarized in **Supplementary Table S7**. While one virulence-associated gene was found in 14 strains, five strains carried two genes. These findings indicate that *A. cryaerophilus* may also be able to exchange virulence factors.

Although *A. cryaerophilus* has a large repertoire of virulence genes, the investigated strains were isolated from healthy animals, probably indicating that a complex mechanism of virulence exists and that the sole presence of *A. cryaerophilus* in the gut may not be sufficient for pathogenicity.

CONCLUSION

To the best of our knowledge, this study presents the first report of whole-genome sequencing data of *A. cryaerophilus* from Germany. The genomic information on *A. cryaerophilus* is currently limited and as of to date no in-depth comparative genomic analysis has been conducted. In this study, a total of 27 *A. cryaerophilus* strains were isolated from seven poultry farms in Germany. These strains belong to the *A. cryaerophilus* cluster I following a recent taxonomic proposal. A high genetic diversity between *A. cryaerophilus* from Germany was observed. This is despite that the strains were restricted to a single federal state (Thuringia, Germany), a particular host (water poultry), and were collected over a short period of time (2 years). Additionally, the phylogenetic analysis of cluster I did not support distinct clustering based on the geographical or ecological niche of the strains.

The genomic features of cluster I *A. cryaerophilus* strains include: a chromosome densely packed with coding sequences (91.5–93.4% of the chromosome size); variable presence of mobile elements such as IS, GI and prophages. Furthermore, the alignment of the chromosomes from our four circularized genomes compared with the genome of strain ATCC 49615 revealed a considerable degree of synteny, however, inversion and shifts were observed.

The genetic prediction of virulence and antibiotic resistance showed that *A. cryaerophilus* has a large virulome and that the genetic antimicrobial resistance is only to a limited extent consistent with the phenotypic characterization. Therefore, antimicrobial susceptibility should continue to be tested phenotypically. Surprisingly, *A. cryaerophilus* appears to be more resistant to ciprofloxacin than *A. butzleri*. Although a partial correlation has been found between the presence of the *gyr*A mutation (Thr-85-Ile) and ciprofloxacin resistance, this does not apply to all resistant strains. It is noteworthy, that a functional multidrug efflux pump like *cme*ABC could also be a possible resistance mechanism against ciprofloxacin. The presence of two beta-lactamases (*bla*1, *bla*2) in all *A. cryaerophilus* genomes from cluster I may correlate with the resistance to cefotaxime. Additionally, to our knowledge, this is the first report of the detection of antimicrobial resistance determinants in *A. cryaerophilus* plasmids, which indicates the possibility of exchanging resistance genes between different strains.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are publicly available. This data can be found here: DDBJ/ENA/GenBank; BioProject: PRJNA632720. Publicly available datasets were analyzed in this study. This data can be found here: DDBJ/ENA/GenBank; BioProject: PRJNA66819, PRJNA302819, PRJNA307600, PRJNA294644, PRJNA294645, PRJNA294646, PRJNA308312, PRJNA294642, PRJNA369468, and PRJNA431460.

AUTHOR CONTRIBUTIONS

EM, HT, and MA-G designed the work. CA provided the fecal samples and metadata. EM, IH, and MA-G performed the analyses. EM and MA-G interpreted the data and wrote the manuscript. HH and HT supervised the analyses. All authors contributed to the revision of the manuscript, read, and approved the submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.01549/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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3.2 Stellungnahme zum Eigenanteil an den Arbeiten zur Publikation 2

"*Aliarcobacter butzleri* from Water Poultry: Insights into Antimicrobial Resistance, Virulence and Heavy Metal Resistance"

Müller E., Abdel-Glil M.Y., Hotzel H., Hänel I. und Tomaso H. (2020) *Aliarcobacter butzleri* from Water Poultry: Insights into Antimicrobial Resistance, Virulence and Heavy Metal Resistance. Genes. 11:1104

An der Konzeption des Themas waren mein Betreuer PD Dr. Herbert Tomaso und Dr. Mostafa Y. Abdel-Glil maßgeblich beteiligt.

Der Manuskriptentwurf (inklusive Literaturrecherche) wurde eigenständig verfasst.

Die im Material- und Methodenteil erwähnten Untersuchungen hinsichtlich der DNA-Extraktion sowie der bioinformatischen Analysen habe ich selbstständig durchgeführt. Bei der Antibiotika-Empfindlichkeitstestung wurde ich von Frau Sandra Thierbach unterstützt.

Die Auswertung und Interpretation der Daten im Ergebnisteil erfolgten ebenfalls selbstständig.

Bei Revision des Manuskripts erhielt ich Hilfe von den anderen Co-Autoren.

Die zur Publikation gehörenden Supplements befinden sich im Anhang.

3.2.1 Publikation 2

genes

MDPI

Article

Aliarcobacter butzleri from Water Poultry: Insights into Antimicrobial Resistance, Virulence and Heavy Metal Resistance

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Abstract: *Aliarcobacter butzleri* is the most prevalent *Aliarcobacter* species and has been isolated from a wide variety of sources. This species is an emerging foodborne and zoonotic pathogen because the bacteria can be transmitted by contaminated food or water and can cause acute enteritis in humans. Currently, there is no database to identify antimicrobial/heavy metal resistance and virulence-associated genes specific for *A. butzleri*. The aim of this study was to investigate the antimicrobial susceptibility and resistance profile of two *A. butzleri* isolates from Muscovy ducks (*Cairina moschata*) reared on a water poultry farm in Thuringia, Germany, and to create a database to fill this capability gap. The taxonomic classification revealed that the isolates belong to the *Aliarcobacter* gen. nov. as *A. butzleri* comb. nov. The antibiotic susceptibility was determined using the gradient strip method. While one of the isolates was resistant to five antibiotics, the other isolate was resistant to only two antibiotics. The presence of antimicrobial/heavy metal resistance genes and virulence determinants was determined using two custom-made databases. The custom-made databases identified a large repertoire of potential resistance and virulence-associated genes. This study provides the first resistance and virulence determinants database for *A. butzleri*.

Keywords: *Aliarcobacter*; emerging pathogen; antibiotic susceptibility; whole-genome sequencing; antimicrobial resistance; virulence; heavy metal

1. Introduction

The genus *Arcobacter* was included in the family *Campylobacteraceae* in 1991 [1]. The genus *Arcobacter* has recently been reorganized and is now separated into six different genera: *Arcobacter*, *Aliarcobacter*, *Halarcobacter*, *Malaciobacter*, *Poseidonibacter*, and *Pseudarcobacter* [2–5]. The twenty-nine validly published *Arcobacter* spp. are distributed over those genera, with *Arcobacter butzleri* (now *Aliarcobacter butzleri*) belonging to the genus *Aliarcobacter* (*A.*) [2–4,6,7].

A. butzleri usually causes self-limiting acute enteritis associated with watery diarrhea, nausea, abdominal pain and sometimes fever in humans [8,9]. Diarrhea occurs due to epithelial barrier dysfunction induced through changes in tight-junction proteins and the induction of epithelial apoptosis [10]. A long-term study showed that *A. butzleri* is the fourth most common *Campylobacter*-like organism isolated from human feces [11]. In rare cases, *A. butzleri* may cause bacteremia [8,12]. In animals, *A. butzleri* has been associated with enteritis and/or diarrhea in pigs, cattle, and horses, but has also been found in feces of healthy animals [9,13,14].

A. butzleri is the most prevalent *Aliarcobacter* species detected in food [15]. These bacteria have been isolated in products of animal origin such as poultry meat, pork and beef meat, but also in water,

milk, dairy products, shellfish, and vegetables [9,16–19]. The consumption of contaminated food or water is the most probable route of transmission to humans and animals [8,9,20–22]. Contact with companion animals is also a possible way of transmission to humans as *A. butzleri* has been found in the oral cavity of cats [9,23]. Therefore, *A. butzleri* is not only an emerging foodborne and zoonotic pathogen [8,9], it is also considered as a serious hazard to human health [24]. However, in animals, horizontal and vertical transmission have been described [19,25].

Previous studies mostly examined the antibiotic susceptibility of *A. butzleri* phenotypically, but in the last few years the underlying antimicrobial resistance (AMR) genes have also been studied more closely [18,26–30]. Recently, more virulence-associated genes have been identified in *A. butzleri* in addition to the previously known homologous genes found in *Campylobacter jejuni* (*pldA*, *mviN*, *irgA*, *iroE*, *ciaB*, *hecA*, *hecB*, *cj1345*, *cadF*, *tlyA*) [18,26,27,31]. The resistance of *A. butzleri* to heavy metals has hardly been investigated to date [18,27].

Herein, we describe the antibiotic susceptibility profiles and the genomic characterization of two *A. butzleri* strains isolated from Muscovy ducks (*Cairina moschata*) from a water poultry farm in Thuringia, Germany. In addition, we describe the presence of putative AMR and heavy metal resistance genes as well as potential virulence determinants. Furthermore, we provide a database containing all currently known resistance and virulence-associated genes, which is easily accessible for all researchers to enable faster resistance profiling of *A. butzleri*.

2. Materials and Methods

The strains 16CS0817-2 and 16CS0821-2 were isolated from two fecal samples obtained from a water poultry farm in Thuringia, Germany, in 2016. Each isolate originated from a single fecal sample of a Muscovy duck (*C. moschata*). The fecal samples were taken by a veterinarian with the permission of the animal owners.

For this study, no ethical review process was required, as there were no experiments with animals as defined by the German Animal Protection Law (Tierschutzgesetz) and the Animal Welfare Laboratory Animal Regulation (Tierschutz-Versuchstierordnung).

2.1. Culturing and Identification

The *Aliarcobacter* isolates from fecal samples were cultivated in *Arcobacter* broth (Oxoid GmbH, Wesel, Germany) which was supplemented with three different antibiotics (cefoperazone, amphotericin, and teicoplanin (CAT), Oxoid GmbH). The broth was then spread on plates (Mueller–Hinton agar/CAT/5% defibrinated bovine blood, Sifin GmbH, Berlin, Germany). The incubation criteria for each step were: 48–72 h, 30 °C and microaerophilic atmosphere (5% O_2, 10% CO_2, and 85% N_2). Suspicious colonies were further cultivated and then identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as described before [32,33]. IVD Bacterial Test Standard and Biotyper 3.1 software were used (Bruker Daltonik GmbH, Bremen, Germany). DNA was purified using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions, and the species identification was confirmed with a multiplex PCR assay [34].

2.2. Antimicrobial Susceptibility Testing

The antibiotic susceptibility was determined by using the gradient strip diffusion method (E-Test™, bioMérieux, Nürtingen, Germany) following the manufacturer's instructions. Briefly, the *Aliarcobacter* strains were transferred to Mueller–Hinton agar plates (Sifin GmbH) and incubated for 48–72 h at 30 °C under microaerophilic conditions. Then, the bacterial colonies were added to five milliliters of *Arcobacter* broth and incubated at 30 °C under microaerophilic conditions for a further 48–72 h. Subsequently, 750 µL were spread on Mueller–Hinton agar plates and one antibiotic gradient strip was placed on each plate. Each strain was tested against erythromycin (0.015–256 µg/mL, MA0108F, Oxoid GmbH), ciprofloxacin (0.002–32 µg/mL, MA0104F, Oxoid GmbH), streptomycin

(0.064–1.024 µg/mL, REF: 526800, bioMérieux), gentamicin (0.06–1.024 µg/mL, MA0117F, Oxoid GmbH), tetracycline (0.015–256 µg/mL, MA0105F, Oxoid GmbH), doxycycline (0.016–256 µg/mL, REF: 142328, bioMérieux), ampicillin (0.016–256 µg/mL, REF: 412253, bioMérieux) and cefotaxime (0.002–32 µg/mL, REF:412281, bioMérieux). The minimum inhibitory concentration (MIC) was determined after 48 h of incubation at 30 °C under microaerophilic conditions. The strain *A. butzleri* DSM 8739 was used as a control.

In this study, the cut-off values for *Campylobacter* spp. provided by EUCAST [35] were used for erythromycin, ciprofloxacin, doxycycline, and tetracycline, as no specific breakpoints are available for *Aliarcobacter* spp. For gentamicin, ampicillin, and cefotaxime, we used the 2019 EUCAST breakpoints for *Enterobacterales*. For streptomycin, the cut-off values for *Campylobacter* spp. provided by the European Food Safety Authority were used [36]. The bacterial strains were classified as sensitive or resistant.

2.3. DNA Extraction and Whole-Genome Sequencing

Colony material from one to two plates was needed to obtain sufficient bacterial mass for DNA preparation. Each plate was washed with 2 mL of phosphate-buffered saline (PBS) and the liquid was collected in a 2-mL tube. The tubes were centrifuged for 20 min at 5400 rpm and the supernatant was discarded. The remaining content was washed at least twice with PBS buffer until the supernatant was clear. The resulting pellet was used for DNA recovery using the High Pure PCR Template Preparation Kit. The concentration of the double-stranded DNA (dsDNA) was examined with a Qubit 3 Fluorometer using the Qubit™ dsDNA HS Assay Kit (both Invitrogen™, ThermoFischer Scientific, Berlin, Germany). The Nextera XT DNA Library Preparation Kit (Illumina, Inc., San Diego, CA, USA) was used according to the manufacturer's instructions to generate a sequencing library. Sequencing was done with an Illumina MiSeq instrument (Illumina, Inc., San Diego, CA, USA).

2.4. Bioinformatic Analyses

The raw data were assembled using SPAdes version 3.12.0 [37] after removing low-quality reads and sequencing primers with Trimmomatic (parameters are PE -phred33 LEADING:10 TRAILING:10 MINLEN:30 ILLUMINACLIP:adapters.fa:1:30:11) [38]. Only contigs larger than 500 base pairs (bp) and with a k-mer coverage > 5 were kept using an in-house script. The quality was then assessed with QUAST version 4.3 [39]. The Prokka annotation pipeline 1.14 was used in standard settings for annotation [40].

To confirm the species identity of the assembled data, taxonomic classification was performed using Kraken2 version 2.0.7 [41]. Furthermore, the average nucleotide identity (ANI) of the German strains was calculated in comparison to 11 *A. butzleri* genomes (including the *A. butzleri* reference genome RM4018), the reference genomes of *A. cryaerophilus* ATCC 43158^T and *A. trophiarum* LMG 25534^T, as well as to the out-group genomes *Campylobacter* (C.) *jejuni* subsp. *jejuni* NCTC 11168^T and *Helicobacter* (*H.*) *pylori* NCTC 11637^T, which were downloaded from the NCBI repository, using pyani version 0.2.9 [42]. In silico DNA–DNA hybridization (DDH) was done using the Genome-to-Genome Distance Calculator (GGDC) software [43]. In this study, the recommended formula 2 was used for analysis [43].

Multilocus sequence typing (MLST) based on the whole-genome sequences was done using the PubMLST database (pubmlst.org/arcobacter/) and the MLST tool version 2.15.2 with default settings [44].

In this study, public AMR databases were searched for known AMR genes and mutations, using ABRicate version 0.8.10, which uses the BLASTN algorithm. ABRicate includes, besides others, the databases ResFinder, CARD, ARG-ANNOT, and NCBI [45–48].

For the construction of the databases, previously described AMR, virulence-associated and heavy metal resistance genes were used [18,26,27]. Additionally, more resistance genes were added after screening the Prokka annotated assemblies of the genomes RM4018p, Ab_2211p, Ab_4511p,

BMH_AB_233Bp, BMH_AB_246Bp, D4963p and L353p provided by Isidro et al. [26]. The genes derived from previous studies and the genes identified in this study were blasted with BLASTX using the non-redundant protein sequences (nr) database. Afterwards, all genes were extracted from those genomes with Geneious Prime® 2019.2.3 [49], and were put together into two custom databases (ARCO_IBIZ_AMR; ARCO_IBIZ_VIRULENCE; both available at https://gitlab.com/FLI_Bioinfo_pub) within ABRicate. The here generated whole-genome sequences were screened within this database to identify the presence or absence of the genes. With a detection value of at least 50% coverage and 75% identity, a gene was considered to be present.

In addition, the *gyrA* gene, the 23S rRNA gene, the *rplV* gene and the *rplD* gene of the two strains were extracted using Geneious Prime® 2019.2.3 to identify any known mutations.

The original contributions presented in this study are publicly available. The data has been deposited at DDBJ/ENA/GenBank under the accession WCIX00000000 and WCIY00000000. The version described in this study is version WCIX01000000 and WCIY01000000 (Bioproject: PRJNA575341).

3. Results and Discussion

3.1. Identification and Antimicrobial Susceptibility Testing

The two strains were identified by MALDI-TOF MS and multiplex PCR as *A. butzleri*. The species identification using MALDI-TOF MS (scores > 2.3) was reliable as there were sufficient spectra available for *A. butzleri* in the database for comparison, and the multiplex PCR assay was able to identify the species *A. butzleri* with 100% reliability [34,50].

As shown in Table 1, the tested *A. butzleri* isolates were susceptible to ciprofloxacin, ampicillin and gentamicin.

Table 1. Antimicrobial resistance profiles of *A. butzleri* strains 16CS0817-2 and 16CS0821-2.

Strain	ERY	CIP	DC	TET	GM	SM	AMP	CTX
16CS0817-2	S (2 mg/L)	S (0.03 mg/L)	S (1.5 mg/L)	S (2 mg/L)	S (1.5 mg/L)	R (6 mg/L)	S (4 mg/L)	R (32 mg/L)
16CS0821-2	R (40 mg/L)	S (0.38 mg/L)	R (4 mg/L)	R (3 mg/L)	S (1.5 mg/L)	R (12 mg/L)	S (4 mg/L)	R (32 mg/L)

S = sensitive; R = resistant; minimal inhibitory concentration in brackets (ERY = erythromycin, CIP = ciprofloxacin, DC = doxycycline, TET = tetracycline, GM = gentamicin, SM = streptomycin, AMP = ampicillin, CTX = cefotaxime).

Most studies reported that *A. butzleri* is susceptible or shows low resistance rates to gentamicin, which is in line with our results [18,51–57]. Some studies reported that *A. butzleri* is susceptible to ciprofloxacin [18,31,52,58], whereas other studies reported resistance [8,51,54,55]. Contradictory to our results, previous studies described *A. butzleri* isolates as highly resistant to ampicillin [18,51–54,56,57]. Strain 16CS0821-2 showed resistance to erythromycin, doxycycline, tetracycline, cefotaxime and streptomycin, whereas strain 16CS0817-2 was only resistant to streptomycin and cefotaxime. Most studies have described resistance against erythromycin [18,31,51,54]. Only a few studies reported susceptibility of *A. butzleri* to erythromycin [52,57,58]. In this study, both phenotypes occurred. The same applies to doxycycline and tetracycline. Strain 16CS0817-2 was susceptible to both antibiotics, while strain 16CS0821-2 was resistant. Similar observations were made in previous studies, in which both phenotypes were described [18,52–54,56]. The strains tested here were both resistant to streptomycin. This is in line with the results of some studies, although *A. butzleri* was also found to be susceptible to streptomycin by others [18,51,53,56]. Resistance to cefotaxime has been described before [18,54,56]. The resistance against cefotaxime in *A. butzleri* is well known and is the reason why this antimicrobial agent is often used to supplement culture media to inhibit the growth of unwanted bacteria [56].

However, it is difficult to compare our results with those of previous studies, because of the small number of investigated strains in this study and the different methods used for antimicrobial susceptibility testing. Nevertheless, previous studies already noticed that there is still a lack of standardized methods, specific breakpoints and epidemiological cut-off values regarding antimicrobial resistance of *A. butzleri* isolates [8,21,59].

3.2. Genome Assembly

Whole-genome sequencing was done using the Illumina MiSeq instrument, generating 468,526–3,042,934 paired-end reads with a mean sequencing depth of 35–301 reads. The assemblies of the genomes consisted of 89 and 52 contigs with a GC content of 26.97% and 27.01%, respectively (Table 2). The total sequence length was 2,121,905 bp for 16CS0821-2 and 2,432,983 bp for 16CS0817-2.

Table 2. Summary of the assembly results of the *A. butzleri* strains 16CS0817-2 and 16CS0821-2 using SPAdes (v. 3.12.0), Quast (v. 4.3) and Prokka (v. 1.14).

Assembly	16CS0817-2	16CS0821-2
Total length	2,432,983	2,121,905
GC (%)	26.97	27.01
Nr. of contigs	89	52
Largest contig	159,485	177,585
N50	62,983	69,691
Predicted genes	2500	2159
CDS	2451	2110
rRNA	3 (5S,16S, 23S)	3 (5S, 16S, 23S)
tRNA	46	46
tmRNA	1	1

3.3. Taxonomic Classification of the Whole-Genome Sequence Data

The bioinformatic tool Kraken2 taxonomically classified the two isolates as *A. butzleri*. In order to confirm the taxonomic classification, ANI was calculated between each genome pair of the *A. butzleri* group (Table S1), based on the whole genome sequences. The genomes of *C. jejuni* subsp. *jejuni* NCTC 11168^T and *H. pylori* NCTC 11637^T were used as outgroups (Table S1). The analysis showed that the two German sequences of *A. butzleri* were very similar (>95%) to the other 11 *A. butzleri* genomes used in this study (Figure 1, Table S2).

In previous studies, it has been shown that if the targeted genomes belong to the same species, the ANI threshold is above 95% [60,61]. The 13 *A. butzleri* sequences had an average pairwise ANI value of 97.6% (range: 96.97 to 99.99%). 16CS0817-2 and 16CS0821-2 shared a mean nucleotide identity of 97.81%. The nearest relatives for 16CS0817-2 were the strains 7h1h and L352 (ANI of 98.28% and 98.25%, respectively), and for 16CS0821-2 the strains L354 and L352 (ANI of 97.96% and 97.89%, respectively). The German isolates had 97.40% and 97.53% nucleotide identity with the reference genome RM4018. The analysis also showed that all *A. butzleri* strains had less than 67% and 64% nucleotide identity with *C. jejuni* subsp. *jejuni* NCTC 11168^T and *H. pylori* NCTC 11637^T, respectively, and less than 78% nucleotide identity with the more closely related species *A. cryaerophilus* ATCC 43158^T and *A. trophiarum* LMG 25534^T (Table S2). Based on these results, we concluded that the German strains belong to the species *A. butzleri*. In addition, an in silico DDH analysis was performed, which showed DDH values of over 70% when comparing the two German strains and the other 10 *A. butzleri* strains with the reference genome RM4018 (Table 3).

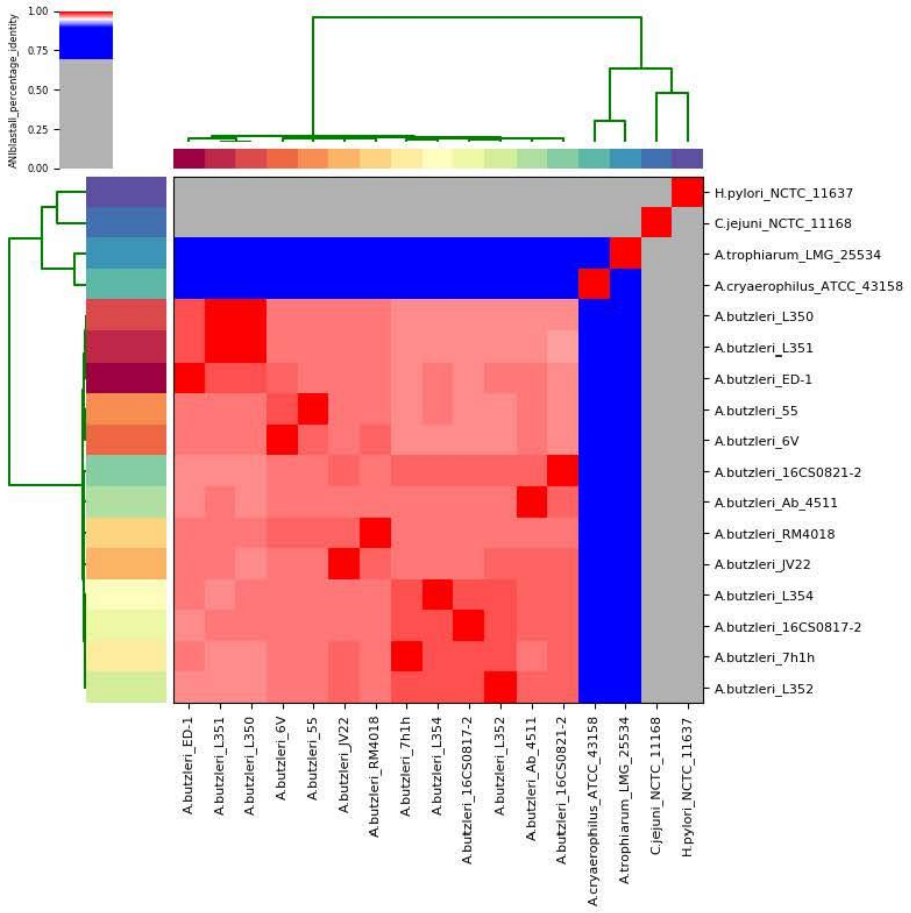

Figure 1. Results of the average nucleotide identity (ANI) analysis. The cells in the heatmap corresponding to an ANI value of 95% and higher are stained red. This indicates that the corresponding strains belong to the same species. Blue colored cells indicate that the corresponding strains do not belong to the same species. The dendrograms (in green; above and on the left side), which were constructed by the simple linkage of the ANIm percentage identities, correspond to the results of the clustering of the ANI values between the used strains [42].

A DDH value of 70% is the recommended standard for delineating distinct species [62,63]. The DDH values of the out-group genomes *C. jejuni* subsp. *jejuni* and *H. pylori* dropped to 21.80% and 25.60%, respectively, when compared to the *A. butzleri* reference genome RM4018, confirming that these strains do not belong to *A. butzleri* (Table 3). Interestingly, the DDH values of *A. cryaerophilus* ATCC 43158^{T} and *A. trophiarum* LMG 25534^{T} were even lower (21.70% and 20.90%, respectively). This result proves that these two strains also do not belong to the species *A. butzleri*. Because of the above mentioned ANI, *A. cryaerophilus* ATCC 43158^{T} and *A. trophiarum* LMG 25534^{T} are more closely related to *A. butzleri* than *C. jejuni* subsp. *jejuni* and *H. pylori*. Therefore, the DDH results supported the result of the ANI analysis, since the recommended DDH cut-off is known to correspond very well to an ANI value of 95% [60,61]. Based on these results, we concluded that our strains belong to the *Aliarcobacter* gen. nov. as *A. butzleri* comb. nov. [2–4].

Table 3. Results of the in silico DNA–DNA hybridization (DDH).

Strain	Species	*Aliarcobacter butzleri* RM4018		
		Identities/HSP Length		Difference in % G + C (≤1 Either Distinct or Same Species; >1 Distinct Species)
		Distance	DDH Estimate (GLM-Based)	
16CS0817-2	*A. butzleri*	0.0248	78.80% [75.8–81.4%]	0.07
16CS0821-2	*A. butzleri*	0.0238	79.70% [76.7–82.3%]	0.04
6V	*A. butzleri*	0.0222	81.00% [78–83.6%]	0.20
55	*A. butzleri*	0.0219	81.20% [78.3–83.8%]	0.26
7h1h	*A. butzleri*	0.0242	79.30% [76.3–81.9%]	0.01
Ab_4511	*A. butzleri*	0.0239	79.50% [76.6–82.2%]	0.09
ED-1	*A. butzleri*	0.0247	78.90% [75.9–81.6%]	0.16
JV22	*A. butzleri*	0.0218	81.40% [78.5–83.9%]	0.03
L350	*A. butzleri*	0.0254	78.30% [75.3–81%]	0.10
L351	*A. butzleri*	0.0253	78.40% [75.4–81.1%]	0.06
L352	*A. butzleri*	0.0237	79.70% [76.8–82.4%]	0.03
L354	*A. butzleri*	0.0252	78.50% [75.5–81.2%]	0.11
ATCC 43158	*A. cryaerophilus*	0.2025	21.70% [19.4–24.1%]	0.42
LMG 25534	*A. trophiarum*	0.2101	20.90% [18.7–23.3%]	1.17
NCTC 11168	*C. jejuni* subsp. *jejuni*	0.2010	21.80% [19.6–24.3%]	3.50
NCTC 11637	*H. pylori*	0.1699	25.60% [23.2–28.1%]	11.78

HSP = high-scoring segment pair; GLM = generalized linear model; ATCC = American Type Culture Collection; LMG = Bacterial Collection of the Laboratory of Microbiology of the University of Ghent; NCTC = National Collection of Type Cultures; A. = *Aliarcobacter*; C. = *Campylobacter*; H. = *Helicobacter*.

The MLST analysis revealed that the two sequenced strains belong to different sequence types (ST) (Table 4). In each strain, we detected a new allele for the *glyA* locus whose sequences were not identical. Currently, the PubMLST database (accessed on 27.05.2020) contains sequence data of 736 *A. butzleri* isolates from 18 countries with MLST data of only two isolates from Germany and 16 isolates of unknown origin. Of those 736 *A. butzleri* strains, 725 were typed into 680 STs, indicating a high genetic diversity among the *A. butzleri* species. Of note, since January 2019 the PubMLST database for *Aliarcobacter* is no longer curated. Therefore, it was not possible to upload our two sequences and assign them to new STs.

Table 4. Results of the multilocus sequence typing (MLST). Allelic profiles of the *A. butzleri* isolates 16CS0817-2 and 16CS0821-2.

Strain	*aspA*	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST
16CS0817-2	177	39	40	123	new allele	194	24	new
16CS0821-2	47	217	4	129	new allele	123	37	new

3.4. AMR and Heavy Metal Resistance Genes

Although the investigated *A. butzleri* strains showed phenotypic resistance to various antibiotics, no antimicrobial resistance genes were predicted using the public AMR databases. This is probably due to the lack of known antimicrobial resistance-associated genes in *Aliarcobacter* spp. Therefore, we have developed a database specifically for *A. butzleri*, which is based on the current knowledge aboutAMR genes of this species.

The AMR database created here (ARCO_IBIZ_AMR) contains both specific AMR genes and heavy metal resistance genes, as described before for *A. butzleri* [18,26,27]. In addition, genes were added which, due to their Prokka annotation, were presumed to belong to one of these groups (see Materials and Methods). The final database contains 92 putative AMR genes and 27 potential heavy metal genes.

Of these 119 genes, 77 and 80 genes were detected in 16CS0817-2 and 16CS0821-2, respectively (Table S3). In both tested genomes, 15 efflux pump (EP) systems belonging to different families were identified: a) EP2, EP9, EP12, EP13, EP14, and EP17 (all members of the major facilitator superfamily (MFS)); b) EP8 (belongs to the small multidrug resistance (SMR) family; c) EP3, EP5, EP6

and EP10 (all three belong to the ATP-binding cassette (ABC) family); and d) EP4, EP11, EP15 and EP16 (all members of the resistance modulation division (RND) family) [26]. It is worth mentioning that EP14 and EP17 each consist of only one gene, the *bcr* gene, which is involved in resistance to sulfonamides and bicyclomycin in *Escherichia* (*E.*) *coli* [64]. EP9 confers resistance to fosmidomycin because it contains the *fsr* gene [27]. Of the 19 EP systems described before by Isidro et al., only four (including the Type I secretion system) were not detected [26]. Nevertheless, these findings show that *A. butzleri* harbors all major families of efflux transporters with the exception of the multidrug and toxic efflux (MATE) family [65]. Furthermore, the transcriptional regulator *ohrR* (organic hydroperoxide resistance transcriptional regulator) of the MarR family, which is involved in the response to oxidative stress and the regulation of virulence in *E. coli* [66], and the transcriptional regulator *kstR2* of the TetR/AcrR family, which is associated with cholesterol degradation in *Mycobacterium tuberculosis*, were found but only in the genome of 16CS0821-2 [67]. The survey of the other antibiotic determinants revealed that our two genomes contain additional putative multidrug export ATP-binding/permease proteins (ABU_RS05540, *ybiT1*, *ylmA*, *macB1*) as well as parts of putative EP systems (*acrB*, *tolC*). Strain 16CS0821-2 also carried an outer membrane protein (*oprF3*) and another putative ATP-binding/permease protein (ABU_RS02345) for multidrug export.

Efflux pump mechanisms, ribosomal protection and the enzymatic inactivation of tetracyclines are considered to be the causes of tetracycline resistance [68]. Both the tetracycline efflux pump encoded by the *tetA* gene and the ribosomal protection proteins encoded by, for example, the *tet*(*O*) gene as already described for *Campylobacter* spp., were not present in either genome [69,70]. Therefore, the reason for the resistance of strain 16CS0821-2 to doxycycline and tetracycline must be different. It would be very interesting to investigate this issue in more detail in the future.

Furthermore, we identified five genes, namely *bla2* (putative metallo-hydrolase), *hcpC* (putative β-lactamase), *mrdA*, *pbpB*, and *pbpF* (all penicillin-binding proteins) in both German strains. These genes are suspected to be the reason for the phenotypic resistance to cefotaxime in both isolates. Resistance to β-lactams in Gram-negative bacteria has been described as a combination of the presence and activity of β-lactamase genes and penicillin-binding proteins together with reduced membrane permeability [71]. However, this would contradict the phenotypically determined sensitivity of both strains to ampicillin in this study. An explanation for this result could be the absence of the *bla3* gene, as its presence is associated with ampicillin resistance [26].

Resistance to ciprofloxacin in *Aliarcobacter* spp. is caused by a point mutation (C254T) in the quinolone resistance-determining region (QRDR) of the *gyrA* gene, resulting in an amino acid exchange from threonine to isoleucine (Thr-85-Ile) [29]. In this study, both strains carried neither this characteristic mutation nor any other known mutation (e.g., Asp-89-Tyr [72]). These findings correlate with the phenotype since both isolates were susceptible to ciprofloxacin. However, the *relE* gene, a toxic component of the type II toxin–antitoxin system, was present in both genomes. Overexpression of this gene is associated with the development of persistent resistance to ciprofloxacin and ampicillin [73,74]. Due to the phenotypic susceptibility of both strains to ciprofloxacin and ampicillin, it can be hypothesized that there was no overexpression of the *relE* gene.

Erythromycin resistance could be caused, as previously described for *Campylobacter* spp., by point mutations in the 23S rRNA gene (A2074G, A2074C, A2075G) and/or by amino acid changes in the *rplD* gene (Gly-57-Asp/Val, Gly-67-Val, Ala-71-Asp, Arg-72-Ile, Gly-74-Asp) or *rplV* gene (Gly-74-Ala, Gly-86-Glu, Ala-88-Glu, Ala-105-Met, Thr-109-Ala) [69,70,75,76]. Therefore, we screened our sequences for these modifications. None of the mutations/alterations mentioned above could be detected, not even in the erythromycin-resistant strain 16CS0821-2. The involvement of EP3 was discussed in a previous study because it contains two macrolide export proteins encoded by the *macA1* gene and *macB2* gene [18]. Although the presence of these export proteins could explain the phenotypic resistance of 16CS0821-2 to erythromycin, it contradicts the phenotype of 16CS0817-2. However, the susceptible phenotype of 16CS0817-2 could be explained by the fact that the genes were present,

but not expressed. Alternatively, these genes could have a single amino acid substitution that may render them non-functional.

It is noteworthy that the screening for further antibiotic determinants identified additional genes in both isolates that are potentially responsible for resistance to certain antibiotics: *arnB* and *eptA* (resistance to polymyxin), and *rlmN* (resistance to various classes of antibiotics) [18,27]. Two genes, namely *cat3* and *wbpD*, are responsible for resistance to chloramphenicol, but only the *wbpD* gene was detected in strain 16CS0821-2 [26,27]. While three *hipA* genes (*hipA2*, *hipA3* and *hipA4*) were present in strain 16CS0817-2, only the *hipA2* gene could be identified in 16CS0821-2. These *hipA* genes encode a serine/threonine-protein kinase, which is another toxic component of the type II toxin–antitoxin system and is involved in multidrug resistance [77].

The investigation for heavy metal resistance genes revealed a putative copper and arsenic cluster in both strains. This result is in line with previous studies [18,27]. The copper cluster consists of six genes, namely: *copA1* (copper-exporting P-type ATPase A), *copA2* (putative copper-importing P-type ATPase A), *copR* (transcription activator protein), *copZ* (copper chaperone), *csoR* (copper-sensing transcriptional repressor) and cusS (sensor kinase). The putative arsenic cluster comprises four genes coding for an arsenic pump membrane protein (*arsB*), an arsenate reductase (*arsC1*), a glutaredoxin arsenate reductase (*ars*C2) and an arsenic resistance protein (ABU_RS02800). Simple arsenic clusters or *ars* operons have been described in a variety of Gram-negative bacteria [78].

Furthermore, an almost complete ABC-type transport system for molybdate was detected, which has been described for *E. coli* and *Staphylococcus carnosus* [79,80]. This transport system usually contains three genes: *modA* (molybdate-binding periplasmic protein), *modB* (molybdenum transport system permease protein) and *modC* (cytoplasmic ATPase). Although no *modC* gene was identified in this study, we were able to detect the *mopA* gene (regulator of *modABC*), which was previously described in *Rhodobacter capsulatus* [81].

In addition, we detected a mechanism for the export of cadmium, zinc and cobalt in both genomes, which included: a cadmium, zinc and cobalt transporting ATPase (*cadA*) and a cadmium, cobalt and zinc/H^+-K^+ antiporter (*czcD*). These results are concordant with previous studies [18,27]. The cobalt–zinc–cadmium resistance proteins encoded by the *czcA* and *czcB* genes were not present in either genome. However, three genes (*czcR1*, *czcR2*, *czcR3*) for the transcription activator protein CzcR were identified. In both strains, the genes of four additional transporters were present: a mercuric transporter (*merT*), a zinc transporter (*zntB*, *ctpC*) and a magnesium and cobalt efflux protein (*corC*) [27].

3.5. Virulence-Associated Genes

In this study, a second database was compiled: ARCO_IBIZ_VIRULENCE. This database contains 148 potential virulence determinants including flagellar genes (n = 36), chemotaxis system genes (n = 8), urease cluster genes (n = 6), putative capsule cluster genes (n = 7), type IV secretion system (T4SS) genes (n = 55), lipid A cluster genes (n = 12) and other virulence genes relevant for adherence, invasion and iron absorption (n = 24). Most of those virulence genes have been described before for *A. butzleri* [18,26,27]. Of these 148 putative virulence genes, 85 and 78 were present in 16CS0817-2 and 16CS0821-2, respectively (Table S4).

The detection of flagellar genes was expected because *A. butzleri* is a motile bacterium due to the polar flagellum. While in strain 16CS0817-2, all 36 flagellar genes were present, strain 16CS0821-2 only carried 35 genes as the *flaA* gene was not detected. Since the *flaA* gene encodes the same component, the flagellin, as the *flaB* gene, it can be hypothesized that it is not essential for the function of the flagellum. These results are similar to those obtained in previous studies [18,26].

Both genomes presented a complete chemotaxis and urease cluster. The existence of both clusters is consistent with the results of previous studies [26,31]. Although the chemotaxis system was present, only one of the two chemotaxis-associated genes (*luxS*, *ccp* (formerly *docA*)) was detected in both strains—the *luxS* gene. The detection of the urease cluster supports the result of the phenotypic urease assay in a previous study [26]. Therefore, it could be hypothesized that *A. butzleri*, in contrast to

A. cryaerophilus, can metabolize urea and survive in an environment with a low pH level, similar to *H. pylori* [82].

We highlight the detection of a putative lipid A cluster in both strains. This cluster contains genes encoding the enzymes for the biosynthesis of lipid A as well as those for the regulatory proteins. Together with an oligosaccharide core and the O-antigen polysaccharide, lipid A forms the lipopolysaccharide (LPS), an endotoxin, in Gram-negative bacteria [83]. Lipid A is the only region of the LPS that is recognized by the innate immune system and is capable of triggering a strong immune response in humans and animals [84]. The putative lipid A cluster contains eight genes that are responsible for lipid A biosynthesis: *lpxA*, *lpxB*, *lpxC*, *lpxD*, *lpxH*, *lpxK*, *lpxP*, and *waaA*. *LpxP* is a paralogue of *lpxL* and encodes the palmitoleolytransferase, which is induced by low temperatures. In *A. butzleri*, the presence of *lpxP* could be responsible for the adaptation of growth to low temperatures, thus enabling survival outside a host, as described for *E. coli* [85]. In a previous study, it was reported that several Gram-negative bacteria can modify their lipid A structure. Modifications can lead to higher resistance to cationic antimicrobial peptides of the host and to lower receptor recognition. The regulation of such modifications is mediated by a two-component system called PhoP–PhoQ [83]. Only one of the *phoP* genes, *phoP3* (encoding the virulence transcriptional regulatory protein), was present in our strains. This finding, as well as the absence of the *phoQ* gene, suggests that our strains were not able to change their lipid A structure or use a hitherto unknown mechanism.

The two heptosyltransferases I and II encoded by *rfaC* (formerly *waaC*) and *rfaF* (formerly *waaF*) were detected in both investigated strains. These heptosyltransferases are involved in the biosynthesis of the LPS core, as described in previous publications [27,86]. Although heptosyltransferases are not essential, their absence leads to structural changes in the outer membrane (reduced protein content; increased sensitivity towards hydrophobic agents) and sometimes to a rough phenotype [87].

The putative capsule cluster, as described before, was not present in our genomes [26]. A complete T4SS was also not found in both strains as only a few genes were present: eight in 16CS0817-2 and three in 16CS0821-2.

The following virulence genes associated with cell adhesion and cell invasion were present in both genomes: *ciaB* (host cell invasion protein), *oprF2* (formerly *cadF*; fibronectin-binding protein), *cj1349* (fibronectin-binding protein), *degP* (formerly *htrA*; chaperone involved in adhesion folding), *tlyA* (hemolysin), *pldA* (outer membrane phospholipase A), *iamA* (invasion-associated gene) and *murJ* (formerly *mviN*; integral membrane protein of murein biosynthesis). The presence of virulence-associated genes was first described in connection with the presentation of the complete genome sequence of *A. butzleri* RM4018 [31]. In this study, homologs of the virulence genes *cadF*, *ciaB*, *cj1349*, *mviN*, *pldA*, and *tlyA* of *C. jejuni* were detected in *A. butzleri*. In addition, four further virulence-associated genes (*irgA*, *iroE*, *hecA*, *hecB*) were discovered [31]. In subsequent studies, the same six virulence-associated genes were repeatedly detected in almost all *A. butzleri* strains [30,56,88,89]. Only Fanelli et al. found *iroE* in two isolates together with *ciaB*, *oprF2*, *cj1349*, *murJ*, *pldA*, and *tlyA* [18]. Our results are therefore more or less in agreement with those of previous studies. According to already existing studies, the virulence determinants *irgA* (now *cirA1*) and *iroE* (now *besA*), which are associated with uropathogenicity in *E. coli*, are detected less frequently than the six virulence genes mentioned above [26,31]. This is consistent with our results since only the *besA* gene was present in both strains. Interestingly, another *cirA* gene, the *cirA2* gene, was detected in both strains. This gene had the same Prokka annotation as *cirA1*—colicin I receptor, indicating that these two genes may be involved in iron uptake [90]. Surprisingly, a third *cirA* gene, *cirA3* (formerly *cfrB*), was identified in 16CS0817-2. The former *cfrB* gene is also involved in iron absorption [26]. The ferric uptake is regulated by the *fur* gene, which was present in both genomes [26,90]. In this study, neither the *hecA* (now *cdiA*; filamentous hemagglutinin) nor the *hecB* (now *shlB*; hemolysin transporter) were detected. These genes are rare, although previous studies do not provide consistent data on the presence of *hecB* [18,30,56,88,89].

Finally, we highlight the detection of a) the conserved virulence factor B (*cvfB*), which is known to regulate the expression of virulence factors in *Staphylococcus aureus* [91], b) the virulence protein

(*voc*), and c) the virulence regulator transcription activator (*virF*), which has been associated with the regulation of plasmid-transmitted virulence genes in *Shigella flexneri* [92,93], in both analyzed genomes. In contrast, the virulence sensor protein encoded by the *bvgS* gene was not present [27].

4. Conclusions

In this study, two *A. butzleri* isolates from a water poultry farm in Germany were sequenced and the taxonomical analyses of their whole-genome sequences revealed that both strains belong to *Aliarcobacter* gen. nov. as *A. butzleri* comb. nov.

To the best of our knowledge, this study presents the first AMR and virulence database for *A. butzleri* only. These databases enable researchers to predict genetic virulence and antimicrobial resistance faster in the future. Nevertheless, the phenotypic determination of antimicrobial resistance should continue to be carried out, as the genotype only corresponds to the phenotype to a limited extent.

Additionally, this is, to the best of our knowledge, the first report about the identification of a lipid A cluster in *A. butzleri*.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4425/11/9/1104/s1, Table S1: Genomes used in this study, Table S2: Results of the ANI analysis, Table S3: Presence and absence of AMR genes in the *A. butzleri* strains 16CS0817-2 and 16CS0821-2, Table S4: Presence and absence of virulence genes in the *A. butzleri* strains 16CS0817-2 and 16CS0821-2.

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3.3 Stellungnahme zum Eigenanteil an den Arbeiten zur Publikation 3

"Antimicrobial Resistance and *in silico* Virulence Profiling of *Aliarcobacter butzleri* Strains from German Water Poultry"

Müller E., Hotzel H., Linde J., Hänel I. und Tomaso H. (2020) Antimicrobial Resistance and *in silico* Virulence Profiling of *Aliarcobacter butzleri* Strains from German Water Poultry. Front. Microbiol. 11:617685.

An der Konzeption des Themas war mein Betreuer PD Dr. Herbert Tomaso maßgeblich beteiligt.

Der Manuskriptentwurf (inklusive Literaturrecherche) wurde eigenständig verfasst.

Die im Material- und Methodenteil erwähnten Untersuchungen hinsichtlich Urease-Test und DNA-Extraktion sowie die bioinformatischen Analysen habe ich selbstständig durchgeführt. Bei der Antibiotika-Empfindlichkeitstestung wurde ich von Frau Sandra Thierbach unterstützt.

Die Auswertung und Interpretation der Daten im Ergebnisteil erfolgten ebenfalls selbstständig.

Bei Revision des Manuskripts erhielt ich Hilfe von den anderen Co-Autoren.

Die zur Publikation gehörenden Supplements befinden sich im Anhang.

3.3.1 Publikation 3

frontiers in Microbiology

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Antimicrobial Resistance and *in silico* Virulence Profiling of *Aliarcobacter butzleri* Strains From German Water Poultry

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Aliarcobacter butzleri is an emerging foodborne and zoonotic pathogen that is usually transmitted via contaminated food or water. *A. butzleri* is not only the most prevalent *Aliarcobacter* species, it is also closely related to thermophilic *Campylobacter*, which have shown increasing resistance in recent years. Therefore, it is important to assess its resistance and virulence profiles. In this study, 45 *Aliarcobacter butzleri* strains from water poultry farms in Thuringia, Germany, were subjected to an antimicrobial susceptibility test using the gradient strip diffusion method and whole-genome sequencing. In the phylogenetic analysis, the genomes of the German strains showed high genetic diversity. Thirty-three isolates formed 11 subgroups containing two to six strains. The antimicrobial susceptibility testing showed that 32 strains were resistant to erythromycin, 26 to doxycycline, and 20 to tetracycline, respectively. Only two strains were resistant to ciprofloxacin, while 39 strains were resistant to streptomycin. The *in silico* prediction of the antimicrobial resistance profiles identified a large repertoire of potential resistance mechanisms. A strong correlation between a *gyr*A point mutation (Thr-85-Ile) and ciprofloxacin resistance was found in 11 strains. A partial correlation was observed between the presence of the *bla*3 gene and ampicillin resistance. *In silico* virulence profiling revealed a broad spectrum of putative virulence factors, including a complete lipid A cluster in all studied genomes.

Keywords: *Aliarcobacter*, emerging pathogen, heavy metal resistance, virulence, antimicrobial resistance, whole-genome sequencing, antibiotic susceptibility

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INTRODUCTION

The species *Aliarcobacter* (*A.*) *butzleri* (former *Arcobacter butzleri*) belongs to the genus *Aliarcobacter*, a member of the *Campylobacteraceae* family, together with *A. cryaerophilus*, *A. skirrowii*, *A. thereius*, *A. cibarius*, *A. lanthieri*, *A. faecis*, and *A. trophiarum* (Pérez-Cataluña et al., 2018a,b, 2019).

A. butzleri is an emerging foodborne and zoonotic pathogen that has been considered as a serious hazard to human health (ICMSF, 2002; Collado and Figueras, 2011; Ramees et al., 2017). In humans, *A. butzleri* infections are associated with self-limiting enteritis, diarrhea and rarely bacteremia; in animals, it has been associated with abortions, mastitis, and gastrointestinal diseases (Logan et al., 1982; Vandamme et al., 1992; Woo et al., 2001; Lau et al., 2002; Vandenberg et al., 2004; Collado and Figueras, 2011; Chieffi et al., 2020).

A. butzleri is the most prevalent foodborne *Aliarcobacter* species, followed in frequency by *A. cryaerophilus* and *A. skirrowii* (Lehner et al., 2005; Collado and Figueras, 2011; Ramees et al., 2017; Caruso et al., 2020). The bacteria have been found in water, vegetables, meat (especially poultry), milk, dairy products, and shellfish (Ho et al., 2006; Hausdorf et al., 2013; Yesilmen et al., 2014; Ramees et al., 2017; Fanelli et al., 2019; Caruso et al., 2020; Chieffi et al., 2020). Since *A. butzleri* is frequently isolated from food-producing animals, these animals should be considered as an important reservoir (Rahimi, 2014; Giacometti et al., 2015; Rathlavath et al., 2017; Sekhar et al., 2017; Caruso et al., 2018; Chieffi et al., 2020). However, this would require successful colonization of the intestines and contamination during the slaughter process (Chieffi et al., 2020).

The consumption of contaminated food/feed or water is the main transmission route for humans and animals. The contamination of food products is probably a consequence of unhygienic procedures (Chieffi et al., 2020). Contact from person-to-person and companion animals-to-person are also possible ways of transmission to humans (Fera et al., 2009; Collado and Figueras, 2011; Shah et al., 2012a; Giacometti et al., 2015; Ferreira et al., 2016; Ramees et al., 2017; Chieffi et al., 2020).

The antimicrobial susceptibility of *A. butzleri* has been frequently investigated phenotypically (Harrass et al., 1998; Fera et al., 2003; Houf et al., 2004; Ho et al., 2006; Vandenberg et al., 2006; Abay et al., 2012; Ferreira et al., 2013, 2017; Shah et al., 2013; Rahimi, 2014; Shirzad Aski et al., 2016; Van den Abeele et al., 2016; Pérez-Cataluña et al., 2017; Vicente-Martins et al., 2018; Fanelli et al., 2019, 2020; Parisi et al., 2019). The underlying genetic resistance mechanisms have been studied in more detail in recent years but data is still limited (Abdelbaqi et al., 2007; Merga et al., 2013a; Fanelli et al., 2019, 2020; Parisi et al., 2019; Isidro et al., 2020). In addition to virulence-associated genes that are homologs of genes previously found in *Campylobacter* (*C.*) *jejuni* (*pld*A, *mvi*N, *irg*A, *iro*E, *cia*B, *hec*A, *hec*B, *cj*1345, *cad*F, *tly*A), further potential virulence-associated genes have been discovered (Miller et al., 2007; Fanelli et al., 2019, 2020; Isidro et al., 2020). Only little is known about the resistance of *A. butzleri* to heavy metals (Fanelli et al., 2019, 2020).

Here, we report antibiotic susceptibility profiles of 45 *A. butzleri* strains isolated from water poultry in Thuringia, Germany, and present insights into their phylogeny. Furthermore, we have complemented these data with 30 *A. butzleri* genomes from public databases, and described the presence of putative antimicrobial resistance genes, potential heavy metal resistance genes and virulence-associated genes in all genomes.

MATERIALS AND METHODS

Bacterial Strains, Culturing, and Identification

In 2016 and 2017, 188 fecal samples were collected from clinically healthy animals from seven water poultry farms in Thuringia, Germany. In detail, 88 fecal samples were collected in 2016 from five water poultry farms from 60 geese (*Anser anser*), 13 Muscovy ducks (*Cairina moschata*), and 15 mulard ducks (*Cairina moschata* × *Anas platyrhynchos domesticus*). In 2017, 100 fecal samples were gathered from 50 geese, 25 Muscovy ducks, 15 mulard ducks, and 10 Pekin ducks (*Anas platyrhynchos domesticus*) from seven water poultry farms. A veterinarian collected the fecal samples with the permission of the animal owners.

For this study, no ethical review process was required as there were no experiments with animals as defined by the German Animal Protection Law (Tierschutzgesetz) and the Animal Welfare Laboratory Animal Regulation (Tierschutz-Versuchstierverordnung).

Culturing and identification was performed as described previously (Müller et al., 2020a). Briefly, the *Aliarcobacter* isolates were cultivated in *Arcobacter* broth (Oxoid GmbH, Wesel, Germany), and then spread on plates (Mueller-Hinton-agar/5% defibrinated bovine blood; Sifin GmbH, Berlin, Germany). Nutrient broth and agar plates were supplemented with antibiotics (cefoperazone, amphotericin, and teicoplanin (CAT), SR0174, Oxoid GmbH). The incubation criteria were: 48–72 h at 30°C in microaerophilic atmosphere (5% O_2, 10% CO_2, and 85% N_2). Suspicious colonies were further cultivated and subsequently identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) as described before (El-Ashker et al., 2015; Hänel et al., 2018). DNA was purified using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions and confirmation of the species identification was done with a multiplex PCR assay (Houf et al., 2000).

Antibiotic Susceptibility Testing

Antibiotic susceptibility was determined using the gradient strip diffusion method (E-Test™, bioMérieux, Nürtingen, Germany) as previously described (Müller et al., 2020a). Each strain was tested two times against erythromycin, ciprofloxacin, streptomycin, gentamicin, tetracycline, doxycycline, ampicillin, and cefotaxime. The minimum inhibitory concentration (MIC) was determined after 48 h of incubation at 30°C under microaerophilic conditions. The *A. butzleri* type strain DSM 8739 was used as a control. In this study, the cut-off values for *Campylobacter* spp. provided by EUCAST (2019) were used for erythromycin, ciprofloxacin, doxycycline, and tetracycline because currently no specific breakpoints are available for *Aliarcobacter* spp. For gentamicin, ampicillin, and cefotaxime, we used the breakpoints for *Enterobacterales* provided by EUCAST (2019). For streptomycin, the cut-off values for *Campylobacter* spp. provided by the EFSA Journal 2019 were used [European Food Safety Authority (EFSA) et al., 2019]. The bacterial strains were classified as sensitive, intermediate, or resistant.

Urease Test

For the urease test, the following ingredients of a urea broth (Merck KgaA, Darmstadt, Germany) were added to one liter of *Arcobacter* broth: potassium hydrogen phosphate (7.63 g/L), disodium hydrogen phosphate (9.59 g/L), urea (20.0 g/L), and phenol red (0.012 g/L). The final broth was inoculated with

colony material from fresh *A. butzleri* cultures and incubated at 30°C for 48 h under microaerophilic conditions to increase bacterial growth. Then, the broth was further incubated at 37°C for at least 72 h. A color change from orange to pink was considered as a positive result. The urease assay was done for the 45 *A. butzleri* strains investigated in this study as well as for two strains (16CS0817-2 and 16CS0821-2) for which culture material was available as they were previously examined by our institute (Müller et al., 2020a). Each strain was subjected to the urease assay twice.

DNA Extraction and Whole-Genome Sequencing

DNA extraction was performed for 45 *A. butzleri* isolates as described previously (Müller et al., 2020a). Briefly, the DNA was extracted from fresh *A. butzleri* cultures using the Qiagen Genomic-tip 20/G (Qiagen GmbH, Hilden, Germany). The concentration of the double-stranded DNA (dsDNA) was examined with a Qubit 3 Fluorometer using the Qubit™ dsDNA HS Assay Kit (both Invitrogen™, ThermoFischer Scientific, Berlin, Germany). The Nextera XT DNA Library Preparation Kit (Illumina, Inc., San Diego, CA, United States) was used according to the manufacturer's instructions to generate a paired-end sequencing library. Whole-genome sequencing was done with an Illumina MiSeq instrument (Illumina, Inc.) generating reads of 300 base pairs (bp) in length.

Bioinformatic Analyses

The 45 genomes sequenced in this study were analyzed together with 29 European *A. butzleri* genomes (France = 1, UK = 1, Italy = 2, Germany = 4, and Portugal = 21) (Fanelli et al., 2019; Isidro et al., 2020; Müller et al., 2020a) and the *A. butzleri* reference genome (NCTC 12481^T, accession: GCF_900187115.1) (**Table 1**, **Supplementary Table 1**). Those additional *A. butzleri* genomes were downloaded from the NCBI GenBank database (last accessed on 08.07.2020) (Benson et al., 2013). For 24 publicly available *A. butzleri* genomes raw sequencing reads were available and downloaded from the Sequence Read Archive (SRA, last accessed on 08.07.2020) (Leinonen et al., 2011). The raw reads together with the raw data of the 45 *A. butzleri* isolates sequenced herein were *de novo* assembled. For the analyses, we used the WGSBAC pipeline (https://gitlab.com/FLI_Bioinfo/WGSBAC) as described previously (Garcia-Soto et al., 2020; Wareth et al., 2020). WGSBAC assembled the raw data using shovill version 1.0.4 (https://github.com/tseemann/shovill) after adapter trimming with Trimmomatic (Bolger et al., 2014). The quality of all assemblies was then assessed with QUAST version 5.0.2 (Gurevich et al., 2013). The software Prokka version 1.14.5 (Seemann, 2014) in default settings was used to annotate the assembled genomes.

To confirm the species identity, taxonomic classification was performed using Kraken2 version 2.0.7_beta with the database Kraken2DB (Wood et al., 2019). The average nucleotide identity (ANI) was calculated using pyani version 0.2.9 (module ANIblastall) (Pritchard et al., 2016) for all *A. butzleri* strains (including the reference genome NCTC 12481^T) in comparison to the reference genomes of *A. cryaerophilus* ATCC 43158^T (accession: GCF_003660105.1) and *A. trophiarum* LMG 25534^T (accession: GCF_003355515.1) as well as to the out-group genome *C. jejuni* subsp. *jejuni* NCTC 11168 (accession: GCF_000009085.1), which were downloaded from the NCBI repository. *In silico* DNA-DNA hybridization (DDH) was done using the Genome-to-Genome Distance Calculator (GGDC) software (available at https://ggdc.dsmz.de). In this study, the recommended formula 2 was used for analysis (Meier-Kolthoff et al., 2013). Multilocus sequence typing (MLST) based on the whole-genome sequences was performed using the mlst software version 2.19.0 (https://github.com/tseemann/mlst) and the PubMLST database (pubmlst.org/arcobacter; last accessed on 27.05.2020) (Jolley and Maiden, 2010). Phylogenetic analyses were performed using Roary version 3.13.0 (Page et al., 2015) as previously applied for *A. butzleri* (Pérez-Cataluña et al., 2018b) and Snippy version 4.3.6 (https://github.com/tseemann/snippy). The phylogenetic tree, based on the Roary output, was built using FastTree version 2.1.9 (Price et al., 2009, 2010) and visualized with iTOL version 5.6.3 (Letunic and Bork, 2007).

For further investigations, two custom-made databases specific for *A. butzleri* (available at https://gitlab.com/FLI_Bioinfo_pub, Müller et al., 2020a) were used to determine the presence of potential antimicrobial and heavy metal resistance genes as well as the presence of virulence-associated genes within ABRicate version 0.8.10 (https://github.com/tseemann/abricate). A gene was considered to be present with a detection value of at least 50% coverage and 75% identity. In addition, the *gyr*A gene, the 23S rRNA gene, the *rplV* gene and the *rplD* gene of all *A. butzleri* genomes were extracted and analyzed using Geneious Prime® 2019.2.3 (Kearse et al., 2012) to identify any known point mutations or amino acid changes that are known to exist in *Campylobacter* spp. (Perez-Boto et al., 2010; Bolinger and Kathariou, 2017; Shen et al., 2018; Elhadidy et al., 2020).

Plasmid prediction was performed for all *A. butzleri* strains using BLASTn (Altschul et al., 1990) and the NCBI RefSeq plasmid database (https://ftp.ncbi.nlm.nih.gov/refseq/release/plasmid/). With a detection value of at least 55% coverage and 85% identity, a plasmid was considered to be present.

RESULTS AND DISCUSSION

Bacterial Strains and Whole-Genome Sequencing

Out of 188 fecal samples, 29 were positive for *A. butzleri*, 10 in 2016 and 19 in 2017. These were obtained from 15 geese, eight Muscovy ducks, five Pekin ducks, and one mulard duck (**Table 1**). Because of the different morphology of the suspected *A. butzleri* colonies on the culture plates, one to three single colonies were picked and processed separately. In total, 45 *A. butzleri* strains were identified by MALDI-TOF MS and multiplex PCR assay. Since sufficient spectra for *A. butzleri* were available in the database, species identification by MALDI-TOF MS (scores > 2.3) was reliable (Hänel et al., 2018). The multiplex PCR assay

TABLE 1 | The metadata of 75 *A. butzleri* strains used in this study.

WGS	Strain	BioProject	Isolation source	Year of isolation	Geographic location	Farm	Sample No.	References
SRR12709624	16CS0847-3	PRJNA665330	Fecal sample (goose)	2016	Germany	A	1	This study
SRR12709623	17CS0824	PRJNA665330	Fecal sample (goose)	2017	Germany	A	2	This study
SRR12709612	17CS1193-1	PRJNA665330	Fecal sample (mulard duck)	2017	Germany	A	3	This study
SRR12709601	17CS1193-2	PRJNA665330	Fecal sample (mulard duck)	2017	Germany	A	3	This study
SRR12709635	17CS1193-3	PRJNA665330	Fecal sample (mulard duck)	2017	Germany	A	3	This study
SRR12709629	17CS1197	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	A	4	This study
SRR12709628	17CS1197-2	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	A	4	This study
SRR12709627	17CS1197-3	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	A	4	This study
SRR12709626	17CS1198-1	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	A	5	This study
SRR12709625	17CS1198-2	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	A	5	This study
SRR12709622	17CS1198-3	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	A	5	This study
SRR12709621	16CS0367-AR	PRJNA665330	Fecal sample (goose)	2016	Germany	B	6	This study
SRR12709620	16CS0367-1-AR	PRJNA665330	Fecal sample (goose)	2016	Germany	B	6	This study
SRR12709619	16CS0370-1	PRJNA665330	Fecal sample (goose)	2016	Germany	B	7	This study
SRR12709618	16CS0375-1	PRJNA665330	Fecal sample (goose)	2016	Germany	B	8	This study
SRR12709617	16CS0375-2	PRJNA665330	Fecal sample (goose)	2016	Germany	B	8	This study
SRR12709616	16CS0831-1-1	PRJNA665330	Fecal sample (goose)	2016	Germany	B	9	This study
SRR12709615	16CS0831-4-1	PRJNA665330	Fecal sample (goose)	2016	Germany	B	9	This study
SRR12709614	17CS1200-1	PRJNA665330	Fecal sample (Pekin duck)	2017	Germany	B	10	This study
SRR12709613	17CS1201	PRJNA665330	Fecal sample (Pekin duck)	2017	Germany	B	11	This study
SRR12709611	17CS1205-1	PRJNA665330	Fecal sample (Pekin duck)	2017	Germany	B	12	This study
SRR12709610	17CS1205-2	PRJNA665330	Fecal sample (Pekin duck)	2017	Germany	B	12	This study
SRR12709609	17CS1206-1	PRJNA665330	Fecal sample (Pekin duck)	2017	Germany	B	13	This study
SRR12709608	17CS1208-3	PRJNA665330	Fecal sample (Pekin duck)	2017	Germany	B	14	This study
SRR12709607	17CS1167	PRJNA665330	Fecal sample (goose)	2017	Germany	C	15	This study
SRR12709606	17CS1168	PRJNA665330	Fecal sample (goose)	2017	Germany	C	16	This study
SRR12709605	17CS1056	PRJNA665330	Fecal sample (goose)	2017	Germany	D	17	This study
SRR12709604	17CS1066	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	D	18	This study
SRR12709603	17CS1067	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	D	19	This study
SRR12709602	17CS1068-1	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	D	20	This study
SRR12709600	17CS1068-2	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	D	20	This study
SRR12709599	17CS0916-1	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	E	21	This study
SRR12709598	17CS0916-2	PRJNA665330	Fecal sample (Muscovy duck)	2017	Germany	E	21	This study
SRR12709597	16CS0857-1	PRJNA665330	Fecal sample (goose)	2016	Germany	F	22	This study
SRR12709596	16CS0861-1	PRJNA665330	Fecal sample (goose)	2016	Germany	F	23	This study
SRR12709595	17CS1092-1	PRJNA665330	Fecal sample (goose)	2017	Germany	F	24	This study
SRR12709594	17CS1092-2	PRJNA665330	Fecal sample (goose)	2017	Germany	F	24	This study
SRR12709593	17CS1095	PRJNA665330	Fecal sample (goose)	2017	Germany	F	25	This study
SRR12709637	16CS0815-2	PRJNA665330	Fecal sample (goose)	2016	Germany	G	26	This study
SRR12709636	16CS0820-1	PRJNA665330	Fecal sample (Muscovy duck)	2016	Germany	G	27	[1], this study
SRR12709634	16CS0820-2	PRJNA665330	Fecal sample (Muscovy duck)	2016	Germany	G	27	This study
SRR12709633	16CS0823-1	PRJNA665330	Fecal sample (Muscovy duck)	2016	Germany	G	28	This study
SRR12709632	16CS0823-2	PRJNA665330	Fecal sample (Muscovy duck)	2016	Germany	G	28	[1], this study
SRR12709631	17CS0965-1	PRJNA665330	Fecal sample (goose)	2017	Germany	G	29	This study
SRR12709630	17CS0965-B	PRJNA665330	Fecal sample (goose)	2017	Germany	G	29	This study
SRR10215626	16CS0817-2	PRJNA575341	Fecal sample (Muscovy duck)	2016	Germany	G	–	This study
SRR10215625	16CS0821-2	PRJNA575341	Fecal sample (Muscovy duck)	2016	Germany	G	–	This study
GCF_900187115.1	NCTC 12481	PRJEB6403	Diarrheic stool (human)	1992	USA	–	–	NA, [9]$^{\#}$, [10]§
GCF_004283125.1	55	PRJNA489574	Mussel	2014	Italy	–	–	[2]

(Continued)

TABLE 1 | Continued

WGS	Strain	BioProject	Isolation source	Year of isolation	Geographic location	Farm	Sample No.	References
GCF_004283115.1	6V	PRJNA489609	Clams	2014	Italy	–	–	[2]
GCF_013363865.1	RMI	PRJNA637480	Raw milk	2018	Germany	–	–	NA
GCF_013363855.1	RMIII	PRJNA637480	Raw milk	2018	Germany	–	–	NA
GCF_000215345.2	7h1h	PRJNA67167	Fecal sample (beef)	2007	UK	–	–	[3, 6]
ERR3523212	Ab_1426_2003	PRJEB34441	Diarrheic stool (human)	2003	France	–	–	[4, 6]
ERR3523199	Ab_1711	PRJEB34441	Poultry slaughterhouse equipment surface	2011	Portugal	–	–	[5, 6]
ERR3523202	Ab_2211	PRJEB34441	Slaughterhouse surface	2011	Portugal	–	–	[5, 6]
ERR3523203	Ab_2811	PRJEB34441	Carcass neck skin (poultry)	2011	Portugal	–	–	[5, 6]
ERR3523197	Ab_3711	PRJEB34441	Caecum (poultry)	2011	Portugal	–	–	[5, 6]
ERR3523210	Ab_4211	PRJEB34441	Carcass drippings (poultry)	2011	Portugal	–	–	[5, 6]
ERR3523201	Ab_4511	PRJEB34441	Carcass drippings (flock)	2011	Portugal	–	–	[5, 6]
ERR3523204	Ab_A103	PRJEB34441	River water	2016	Portugal	–	–	[6]
ERR3523214	Ab_A111	PRJEB34441	River water	2016	Portugal	–	–	[6]
ERR3523198	Ab_CR1132	PRJEB34441	Ready-to-eat vegetables	2016	Portugal	–	–	[6, 7]
ERR3523196	Ab_CR1143	PRJEB34441	Meat (poultry)	2016	Portugal	–	–	[6, 7]
ERR3523205	Ab_CR424	PRJEB34441	Meat (poultry)	2015	Portugal	–	–	[6, 7]
ERR3523200	Ab_CR461	PRJEB34441	Meat (fish)	2015	Portugal	–	–	[6, 7]
ERR3523213	Ab_CR502	PRJEB34441	Meat (poultry)	2015	Portugal	–	–	[6, 7]
ERR3523209	Ab_CR604	PRJEB34441	Meat (beef)	2015	Portugal	–	–	[6, 7]
ERR3523206	Ab_CR641	PRJEB34441	Meat (poultry)	2015	Portugal	–	–	[6, 7]
ERR3523216	Ab_CR891	PRJEB34441	Meat (poultry)	2016	Portugal	–	–	[6, 7]
ERR3523215	Ab_CR892	PRJEB34441	Meat (poultry)	2016	Portugal	–	–	[6, 7]
ERR3523207	Ab_DQ20dA1	PRJEB34441	Milk (goat)	2015	Portugal	–	–	[6, 8]
ERR3523217	Ab_DQ31A1	PRJEB34441	Milk (sheep)	2015	Portugal	–	–	[6, 8]
ERR3523211	Ab_DQ40A1	PRJEB34441	Dairy plant equipment surface	2015	Portugal	–	–	[6, 8]
ERR3523208	Ab_DQ64A1	PRJEB34441	Dairy plant equipment surface	2015	Portugal	–	–	[6, 8]

[1] - Müller et al. (2020a); [2] - Fanelli et al. (2019); [3] - Merga et al. (2013b); [4] - Ferreira et al. (2014); [5] - Ferreira et al. (2013); [6] - Isidro et al. (2020); [7] - Vicente-Martins et al. (2018); [8] - Ferreira et al. (2017); [9] - Miller et al. (2007); [10] - Miller et al. (2009); NA, not available; # antimicrobial susceptibility values and result of the urease assay were taken from reference [9]; § results of the MLST analysis were taken from reference [10].

identified the species *A. butzleri* with 100% reliability (Houf et al., 2000; Levican and Figueras, 2013).

In the present study, whole-genome sequencing of 45 *A. butzleri* strains was performed. The Illumina sequencing yielded an average number of 0.86 million reads per strain (range: 219,906–3,042,034 reads) and an average depth of coverage of 73.9-fold (range: 23 to 176-fold). For the 45 genomes, an average N50 value of 137.0 kbp (range: 22.9–255.9 kbp) and an average of 49 contigs per strain (range: 17–191 contigs) was calculated (**Table 2**).

Taxonomic Classification of *A. butzleri* From Germany

The German *A. butzleri* strains sequenced herein were taxonomically classified at species level, based on their reads, with an average of 89.9% (range: 72.2–94.7%) as *A. butzleri* using the bioinformatic tool Kraken2. To confirm this result, ANI was calculated between each genome pair based on the whole-genome sequences. The analysis showed that the German *A. butzleri* strains were highly similar (>95%) to the other European *A. butzleri* strains used in this study, confirming that those genomes belong to the same species (Goris et al., 2007; Richter and Rossello-Mora, 2009) (**Figure 1**, **Supplementary Table 2**). All *A. butzleri* strains ($n = 74$) had an average pairwise ANI value of 97.9% (range: 96.75 to 100.0%) and shared a mean nucleotide identity of 97.5% (range: 97.15 to 97.82%) with the *A. butzleri* reference genome NCTC 12481^T. The closest relative for all 74 *A. butzleri* genomes was *A. cryaerophilus* ATCC 43158^T with a mean ANI value of 78.09%. Interestingly, all *A. butzleri* strains were also closely related to *A. trophiarum* LMG 25534^T (ANI of 77.22%). The analysis also showed that *A. butzleri* genomes had less than 67% nucleotide identity with *C. jejuni* subsp. *jejuni* NCTC 11168. Additionally, an *in silico* DDH analysis was performed, revealing DDH values above 76% when comparing all 74 *A. butzleri* strains with the reference genome NCTC 12481^T (**Supplementary Table 3**). The recommended standard for delineating distinct species is a DDH threshold of 70% (Wayne et al., 1987; On et al., 2017). *C. jejuni* subsp. *jejuni* NCTC 11168, *A. cryaerophilus* ATCC 43158^T, and *A. trophiarum* LMG 25534^T do not belong to the species *A. butzleri* due to their low DDH values (21.80, 21.70, and 20.90%, respectively). The DDH results supported

TABLE 2 | Sequencing statistics, assembly statistics, and annotation of 45 German *A. butzleri* strains.

ID	Sequencing statistics				Assembly statistics				Annotation		
	Sequencing platform	Total number of reads (X1000)	Average read length (bp)	Coverage depth	Genome size	No. contigs	N50	GC%	Total CDS	No. rRNA	No. tRNA
16CS0847-3	Illumina MiSeq	539.2	230	52	2.12 Mbp	30	114,025	26.96	2,101	3	46
17CS0824	Illumina MiSeq	917.0	208	80	2.19 Mbp	35	135,672	26.92	2,155	3	47
17CS1193-1	Illumina MiSeq	368.9	222	34	2.23 Mbp	20	201616	26.93	2,223	3	46
17CS1193-2	Illumina MiSeq	554.0	233	54	2.23 Mbp	17	237,465	26.93	2,223	3	46
17CS1193-3	Illumina MiSeq	473.0	233	46	2.19 Mbp	22	209,048	26.89	2,181	3	45
17CS1197	Illumina MiSeq	337.4	219	31	2.21 Mbp	73	63,178	27.00	2,216	3	46
17CS1197-2	Illumina MiSeq	533.7	225	50	2.21 Mbp	47	117,432	26.99	2,224	3	46
17CS1197-3	Illumina MiSeq	823.1	218	75	2.21 Mbp	47	105,309	26.99	2,225	3	46
17CS1198-1	Illumina MiSeq	885.7	245	91	2.16 Mbp	35	181,112	26.90	2,164	3	45
17CS1198-2	Illumina MiSeq	910.2	217	83	2.16 Mbp	35	181,142	26.90	2,166	3	45
17CS1198-3	Illumina MiSeq	789.5	209	69	2.16 Mbp	36	165,442	26.90	2,166	3	46
16CS0367-AR	Illumina MiSeq	1,001.3	237	99	2.19 Mbp	79	54,432	26.96	2,204	3	46
16CS0367-1-AR	Illumina MiSeq	219.9	257	23	2.20 Mbp	25	251,305	26.94	2,214	3	45
16CS0370-1	Illumina MiSeq	1,038.6	224	97	2.20 Mbp	25	251,305	26.94	2,213	3	45
16CS0375-1	Illumina MiSeq	630.7	259	68	2.20Mbp	26	251,306	26.94	2,213	3	46
16CS0375-2	Illumina MiSeq	1,009.2	215	91	2.20 Mbp	36	137,063	26.94	2,214	3	46
16CS0831-1-1	Illumina MiSeq	1,566.2	173	113	2.20 Mbp	34	157,075	26.94	2,211	3	46
16CS0831-4-1	Illumina MiSeq	759.8	231	73	2.26 Mbp	24	170,136	26.91	2,255	3	47
17CS1200-1	Illumina MiSeq	792.7	235	78	2.12 Mbp	31	186,556	26.95	2,122	3	46
17CS1201	Illumina MiSeq	1,015.7	210	89	2.12 Mbp	34	157,092	26.95	2,122	3	46
17CS1205-1	Illumina MiSeq	315.2	202	26	2.24 Mbp	61	81,235	27.06	2,311	3	47
17CS1205-2	Illumina MiSeq	823.7	222	76	2.24 Mbp	42	130,656	26.88	2,316	3	47
17CS1206-1	Illumina MiSeq	903.2	214	81	2.24 Mbp	53	103,432	26.88	2,315	3	47
17CS1208-3	Illumina MiSeq	333.4	209	29	2.24 Mbp	64	117,355	26.89	2,311	3	47
17CS1167	Illumina MiSeq	451.7	232	44	2.21 Mbp	31	117,108	26.91	2,170	3	45
17CS1168	Illumina MiSeq	1,422.1	246	147	2.17 Mbp	40	103,522	26.92	2,143	3	46
17CS1056	Illumina MiSeq	279.2	208	24	2.19 Mbp	191	22,951	26.99	2,167	3	45
17CS1066	Illumina MiSeq	459.5	265	51	2.21 Mbp	71	66,964	26.93	2,158	3	46
17CS1067	Illumina MiSeq	410.9	241	41	2.21 Mbp	102	38,333	26.93	2,160	3	46
17CS1068-1	Illumina MiSeq	1,215.5	217	110	2.21 Mbp	29	160,528	26.91	2,170	3	46
17CS1068-2	Illumina MiSeq	368.6	201	31	2.21 Mbp	105	32,597	26.94	2,148	3	45
17CS0916-1	Illumina MiSeq	1,019.0	221	94	2.03 Mbp	24	204,435	27.07	2,066	3	46
17CS0916-2	Illumina MiSeq	2,003.3	210	176	2.03 Mbp	25	204,435	27.07	2,066	3	46
16CS0857-1	Illumina MiSeq	812.9	234	80	2.06 Mbp	37	168,312	26.99	2,082	3	46
16CS0861-1	Illumina MiSeq	2,967.4	124	154	2.18 Mbp	64	69,382	26.92	2,149	3	53
17CS1092-1	Illumina MiSeq	860.1	211	76	2.15 Mbp	44	111,096	27.07	2,177	3	45
17CS1092-2	Illumina MiSeq	1,008.8	204	86	2.15 Mbp	46	102,709	27.07	2,174	3	46
17CS1095	Illumina MiSeq	818.2	213	73	2.11 Mbp	48	117,56	27.10	2,127	3	46
16CS0815-2	Illumina MiSeq	841.5	228	80	2.19 Mbp	27	154,166	26.86	2,191	3	46
16CS0820-1	Illumina MiSeq	1,353.3	179	101	2.12 Mbp	21	18,643	26.98	2,117	3	45
16CS0820-2	Illumina MiSeq	1,178.6	200	99	2.12 Mbp	22	199,264	27.05	2,124	3	46
16CS0823-1	Illumina MiSeq	572.6	226	54	2.12 Mbp	23	255,949	27.05	2,122	3	46
16CS0823-2	Illumina MiSeq	563.8	229	54	2.13 Mbp	35	135,934	27.04	2,142	3	46
17CS0965-1	Illumina MiSeq	849.5	218	77	2.43 Mbp	60	95,695	26.84	2,493	3	46
17CS0965-B	Illumina MiSeq	327.8	227	31	2.18 Mbp	147	26,561	26.99	2,166	3	46

the results of the ANI analysis because the recommended DDH cut-off value corresponds very well to an ANI value of 95% (Goris et al., 2007; Richter and Rossello-Mora, 2009). Therefore, we concluded that the species of the newly sequenced German strains had been correctly identified as *A. butzleri*.

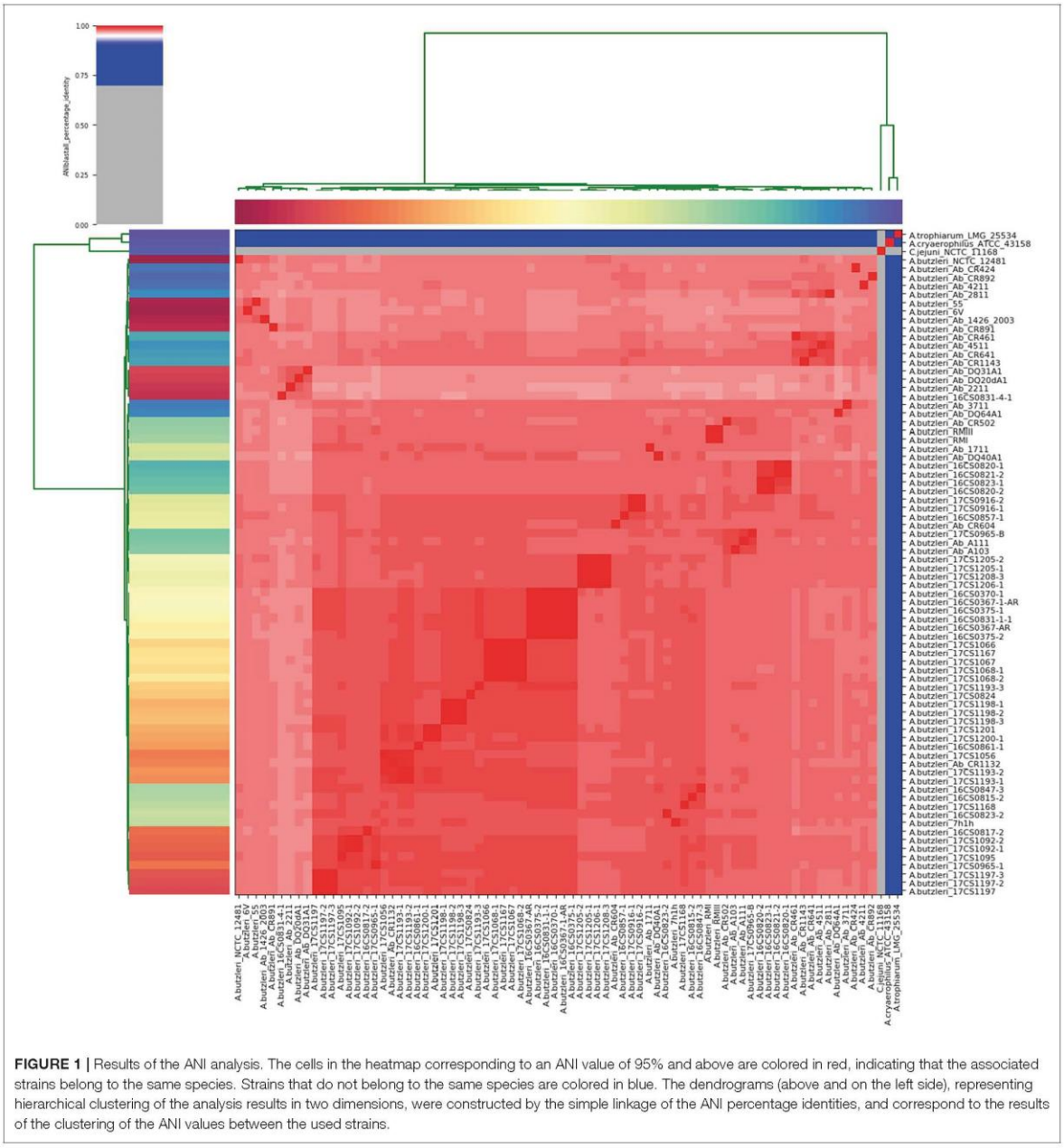

FIGURE 1 | Results of the ANI analysis. The cells in the heatmap corresponding to an ANI value of 95% and above are colored in red, indicating that the associated strains belong to the same species. Strains that do not belong to the same species are colored in blue. The dendrograms (above and on the left side), representing hierarchical clustering of the analysis results in two dimensions, were constructed by the simple linkage of the ANI percentage identities, and correspond to the results of the clustering of the ANI values between the used strains.

Phylogenetic Relatedness of the German *A. butzleri* Strains

Figure 2 visualizes the phylogenetic tree based on the core genome alignment (including all *A. butzleri* genomes) done by Roary, which has been used before for this species (Pérez-Cataluña et al., 2018b). Most of the tree branches present high bootstrap values (average: 0.97; range: 0.89–1.00). The identified core genome consists of 1,295 genes corresponding to a size of ∼1.18 Mbp, which represents half of the average *A. butzleri* genome. We observed that after adding about 50 genomes, the size of the core genome did not decrease any further (data not shown). The *A. butzleri* accessory genome comprised 10,435 genes. These findings lead to the conclusion that *A. butzleri* harbors an open pan-genome with a total of

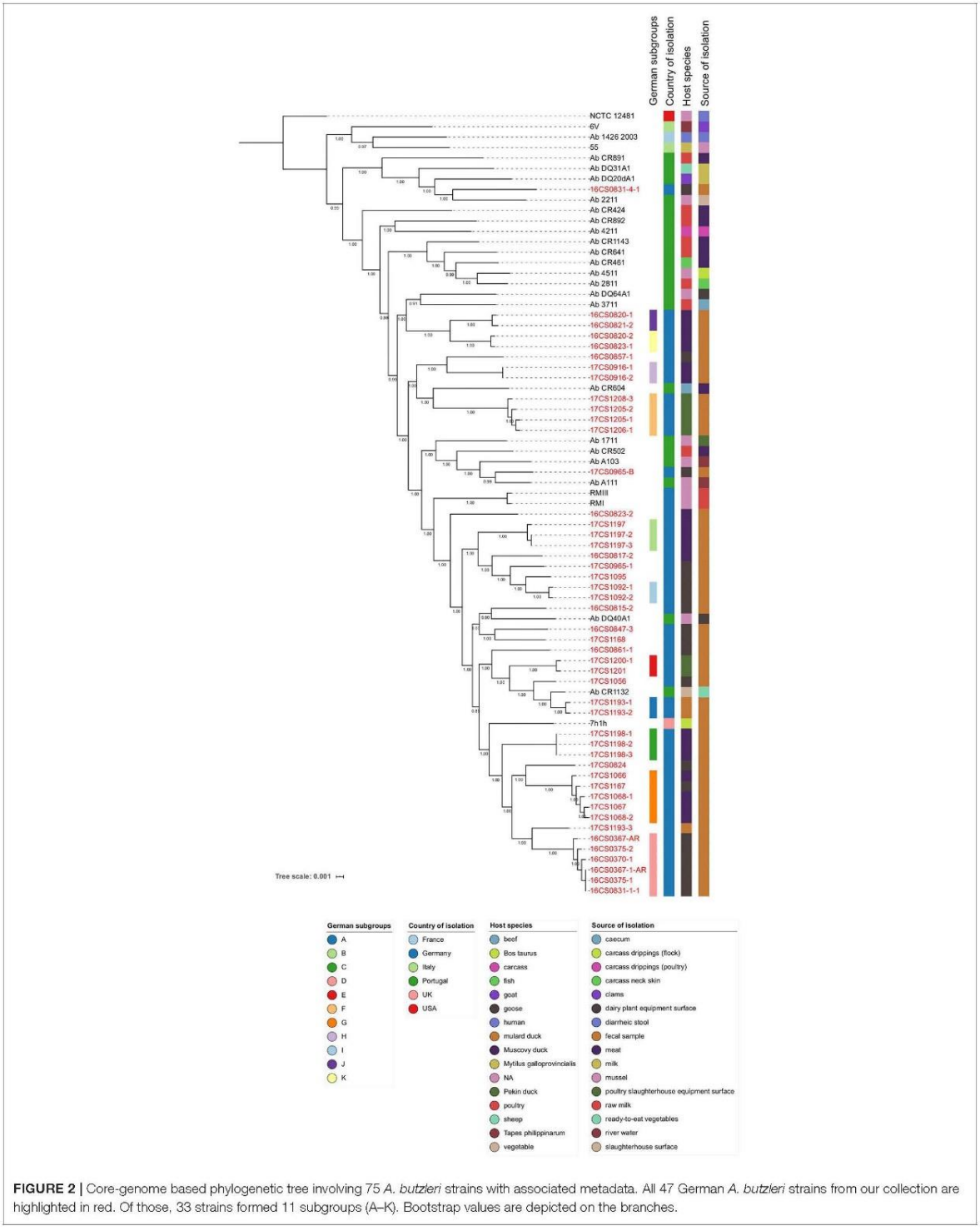

FIGURE 2 | Core-genome based phylogenetic tree involving 75 *A. butzleri* strains with associated metadata. All 47 German *A. butzleri* strains from our collection are highlighted in red. Of those, 33 strains formed 11 subgroups (A–K). Bootstrap values are depicted on the branches.

11,730 genes. This result is comparable to a previous study (Isidro et al., 2020).

The core genome SNP analysis revealed that the mean pairwise genetic distance between our German *A. butzleri* isolates ($n = 47$) was 10,923 SNPs (range: 0–19,930 SNPs) (**Supplementary Table 4**). While the majority of our whole-genome sequences has sufficient coverage (>30-fold), four strains (16CS0367-1-AR, 17CS1056, 17CS1205-1, 17CS1208-3) have low coverage (23 to 29-fold). This might be an explanation for the differences between the observed SNP-inferred phylogeny and the epidemiology. Recombination might lead to false-positive SNPs but according to Isidro et al. (2020), they do not affect the phylogeny of *A.* butzleri. However, the distances between the genomes are reduced by 30% (Isidro et al., 2020). Thirty-three German strains could be grouped into 11 subgroups (A to K) (**Figure 2**). In those groups, the individual strains, which had been isolated from a single sample or multiple samples collected in the same water poultry farm, were less than 50 SNPs apart. The remaining German strains ($n = 14$) could not be assigned to any subgroup. Subgroup G included isolates ($n = 5$) that were recovered from fecal samples collected from two different farms in 2017, four from farm D (four samples from ducks), and one from farm C (one sample from a goose). Those isolates were distant to each other by less than 12 SNPs (**Supplementary Table 4**). The finding of highly similar strains in two different farms may indicate a possible epidemiological relatedness between them. We speculate that this could be due to a common source of animals or personnel. A fifth sample from farm D was collected from a goose in 2017. But the isolate 17CS1056 retrieved from this sample did not group with the other isolates from farm D, which could be due to the low coverage of the whole-genome sequence of this isolate or due to genetic diversity. In five subgroups (A, B, C, H, and I) the isolates were retrieved from the same sample and were less than or equal to 2 SNPs distant from each other, indicating a clonal-relatedness. Although the remaining 5 subgroups (D, E, F, J, and K) included isolates of two or more samples from the same farm, they were distant to each other by less than 35 SNPs. A possible explanation for this result could be that there is a clone circulating in the water poultry farm's herd. Subgroup A contains two isolates (17CS1193-1 and 17CS1193-2) retrieved from a fecal sample of a duck that was collected from farm A in 2017. The third isolate of this sample, 17CS1193-3, is distant by more than 8,500 SNPs to the two strains of subgroup A. This finding might be due to the presence of two *A. butzleri* strains which are not clonally related. High divergence was also observed in two isolates (16CS0831-1-1, 16CS0831-4-1) from farm B, isolated in 2016 from a goose; a distance of 19,445 SNPs was detected. The same applied for four isolates retrieved out of 2 different samples from ducks in 2016 from farm G. While the isolates 16CS0820-1 and 16CS0820-2 were distant by 6,973 SNPs, the other two isolates (16CS0823-1, 16CS0823-2) were distant by 13,490 SNPs. The same farm had been sampled once more in 2017, and from this additional sample (from a goose), two isolates were recovered. These were ~12,580 SNPs distant from each other. This phenomenon has also been described before for *A. cryaerophilus* isolates from German water poultry (Müller et al., 2020b).

Furthermore, the MLST analysis showed that out of 45 German *A. butzleri* strains sequenced herein, only four were assigned to a known sequence type (ST) (**Table 3**). The remaining 43 strains most likely belong to new STs. Nevertheless, most of the German *A. butzleri* strains assigned to a subgroup have the same MLST profile. Therefore, we hypothesize that strains with the same allelic profile also belong to the same ST. Since January 2019, the PubMLST database for *Aliarcobacter* is no longer curated, therefore, it was not possible to upload the sequences and assign them to new STs. The PubMLST database contains MLST data of 736 *A. butzleri* isolates from 18 countries. Of these, 725 *A. butzleri* strains were assigned to 680 different STs, showing a high genetic diversity of this species. This high genetic diversity of *A. butzleri* may be the reason why we could not assign 43 of the German genomes to a specific ST. In this study, the MLST results of some European strains varied from the MLST results of a previous study which is most likely due to different assembly approaches (**Table 3**). In fact, when the available assembly data of the European strains were used for the MLST analysis, the same alleles were identified as in the study conducted by Isidro et al. (2020). However, in some genomes ($n = 10$) the accurate assignment was not possible due to the presence of paralogs for the *gly*A and *glt*A loci (i.e., multiple copies in the same genome). Similar observations were made for strains of the species *A. butzleri* and *A. cryaerophilus* (Isidro et al., 2020; Müller et al., 2020b), although the paralogs found here were not identical except for the *glt*A locus. While developing the first MLST scheme for *Aliarcobacter*, Miller et al. (2009) reported the presence of two *gly*A loci, *gly*A1 and *gly*A2. At the end, the *gly*A1 locus was integrated into the scheme due to its discriminatory power. Consequently, the here identified paralogs of the *gly*A locus could represent the alleles of both *gly*A loci. This is supported by the fact that the detected alleles (1; 142) for the *gly*A locus of the *A. butzleri* reference strain NCTC 12481^T are identical with the alleles identified by Miller et al. (2009). In that study, allele 1 was assigned to the *gly*A1 locus and allele 142 to the *gly*A2 locus (Miller et al., 2009). We conclude that the *gly*A locus is either not suitable for MLST analysis since it can lead to incorrect allele calling, or new bioinformatic approaches need to be developed to tackle this problem (Isidro et al., 2020; Müller et al., 2020b).

In summary, our results indicate a high genetic diversity among *A. butzleri* strains, although the sample pool of German strains was limited to one federal state (Thuringia, Germany), water poultry as host, and a short study period (2 years). Since similar isolates were identified in geese and ducks (e.g., as in subgroup G), strain diversity within *A. butzleri* seems to be independent of the host species. This was also supported by the overall phylogeny, as it was not possible to identify major clusters related to the host species. The phylogenetic analysis did also not support clustering based on either the isolation source or geographical location of the genomes. Similar observations have been reported before for *A. cryaerophilus* (Müller et al., 2020b).

TABLE 3 | Results of the MLST analysis of 75 *A. butzleri* strains.

Strain	Subgroup	*aspA*	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST
16CS0367-1-AR	D	4	4	40	19	471, 534	194	158	New
16CS0367-AR	D	4	4	40	19	471, 534	194	158	New
16CS0370-1	D	4	4	40	19	471, 534	194	158	New
16CS0375-1	D	4	4	40	19	471, 534	194	158	New
16CS0375-2	D	4	4	40	19	471, 534	194	158	New
16CS0815-2	–	182	4	40	136	413?	211	87	New
16CS0817-2	–	177	39	40	123	~511	194	24	New
16CS0820-1	J	47	217	4	129	~694	123	37	New
16CS0820-2	K	47	217	9	129	~615	123	37	New
16CS0821-2	J	47	217	4	129	694?	123	37	New
16CS0823-1	K	47	217	9	129	~632	123	37	New
16CS0823-2	–	~229	4	34	4	488	102	63	New
16CS0831-1-1	D	4	4	40	19	471, 534	194	158	New
16CS0831-4-1	–	81	69	26	66	182	124	86	205
16CS0847-3	–	4	12	1	19	346	~103	24	New
16CS0857-1	–	20	7	20	7	186	8	7	New
16CS0861-1	–	4	12	1	19	~418	213	160	New
17CS0824	–	4	58	44	19	150	4	89	New
17CS0916-1	H	20	7	20	23	186?	45	37	New
17CS0916-2	H	20	7	20	23	186?	45	37	New
17CS0965-1	–	153	39	1	19	184	11	~9	New
17CS0965-B	–	20	39	11	11	513	26	55	New
17CS1056	–	37	133	44	12	~379	278	160	New
17CS1066	G	4	4	4	4	~139	4	89	New
17CS1067	G	4	4	4	4	3	4	89	17
17CS1068-1	G	4	4	4	4	139	4	89	18
17CS1068-2	G	4	4	4	4	~139	4	89	New
17CS1092-1	I	153	4	40	19	497?	98	158	New
17CS1092-2	I	153	4	40	19	489?	98	158	New
17CS1095	–	153	4	40	19	528?	98	158	New
17CS1167	G	4	4	4	4	139	4	89	18
17CS1168	–	~69	12	9	19	410	290	165	New
17CS1193-1	A	4	4	44	12	376	4	160	New
17CS1193-2	A	4	4	44	12	376	4	160	New
17CS1193-3	–	4	4	51	126	471	20	89	New
17CS1197	B	~15	4	40	19	~677	63	9	New
17CS1197-2	B	~15	4	40	19	~677	63	9	New
17CS1197-3	B	~15	4	40	19	~677	63	9	New
17CS1198-1	C	4	4	4	19	351	219	158	New
17CS1198-2	C	4	4	4	19	351	219	158	New
17CS1198-3	C	4	4	4	19	351	219	158	New
17CS1200-1	E	41	133	51	19	510	4	160	New
17CS1201	E	41	133	51	19	510	4	160	New
17CS1205-1	F	37	23	7	~165	659	26	New	New
17CS1205-2	F	37	23	7	~165	659	26	New	New
17CS1206-1	F	37	23	7	~165	659	26	New	New
17CS1208-3	F	37	23	7	~165	659	26	New	New
Ab_CR1143	–	23	5	24	44	112	35	55	New
Ab_3711	–	20	2	1	~15	~449	71	165	New
Ab_CR1132	–	240	133	40	12	541	278	160	519

(Continued)

TABLE 3 | Continued

Strain	Subgroup	*aspA*	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST
Ab_1711	–	13	4	40	15,15	**~536** (545)	102	158	New
Ab_CR461	–	5	5	24	27	80	62	20	New
Ab_4511	–	23	44	24	44	112	35	20	510
Ab_2211	–	234	15	26	164	375	260	176	460
Ab_2811	–	23	44	24	2	**769?** (137)	62	20	**New** (107)
Ab_A103	–	20	~39	11	11	98	87	178	New
Ab_CR424	–	23	7	33	38	**156** (~745)	~120	174	New
Ab_CR641	–	23	44	24	15	124	55	20	108
Ab_DQ20dA1	–	59	47	26	53	~205	88	60	New
Ab_DQ64A1	–	10	20	11	19	192	**123** (134)	11	New
Ab_CR604	–	26	22	4	44	~24	55	165	New
Ab_4211	–	20	25	7	2	**~595** (~465)	32	2	New
Ab_DQ40A1	–	4	164	40	19	**418** (528)	102	4	New
Ab_1426_2003	–	8	8	8	8	**191** (9)	9	8	**New** (47)
Ab_CR502	–	20	39	11	15	**~103** (~666)	~26	2	New
Ab_A111	–	20	12	11	19	**112?** (~692)	127	88	New
Ab_CR892	–	10	14	41	30	**747?** (~602)	~263	~31	New
Ab_CR891	–	21	22	21	24	48	27	25	94
Ab_DQ31A1	–	55	45	26	48	113	85	59	172
7h1h	–	150	4	1	122	~370	194	52	New
6V	–	236	161	1	183	521	296	207	537
55	–	332	8	1	166	685	367	292	675
RMIII	–	5	5	5	15	66,176	11	10	New
RMI	–	5	5	5	15	66,176	11	10	New
NCTC 12481	–	1	1	1	1	1,142	1	1	New

The allele loci that do not agree with previous results are written in bold. The alleles identified by Isidro et al. (2020) are shown in brackets. (~ = novel full length allele similar to a known allele; ? = allele partial matches to a known allele).

Antibiotic Susceptibility Testing

The 45 German *A. butzleri* strains were resistant to cefotaxime (**Supplementary Table 1**). Resistance to cefotaxime in *A. butzleri* is well-known, and therefore, cefotaxime is often added to selective media to inhibit the growth of accompanying bacteria (Shah et al., 2013; Rathlavath et al., 2017; Fanelli et al., 2019; Müller et al., 2020a). None of the strains was resistant to gentamicin but 26 strains showed intermediate resistance. These findings are in line with earlier studies (Abay et al., 2012; Kayman et al., 2012; Shah et al., 2012b; Ferreira et al., 2013, 2019; Van den Abeele et al., 2016; Rathlavath et al., 2017; Fanelli et al., 2019; Isidro et al., 2020). Our data shows that gentamicin can still be used for the treatment of *Aliarcobacter* infections like it has been suggested before (Ramees et al., 2017; Rathlavath et al., 2017), but the increasing resistance has to be kept in mind. In our study, only two isolates (17CS0916-1, 17CS0916-2) were resistant to ciprofloxacin. This result is consistent with previous studies (Miller et al., 2007; Kayman et al., 2012; Ferreira et al., 2017; Fanelli et al., 2019, 2020), although there are studies which have reported higher resistance rates (Collado and Figueras, 2011; Shah et al., 2012b; Ferreira et al., 2013, 2019; Isidro et al., 2020). From a total of 45 *A. butzleri* isolates tested in this study, 32 were resistant to erythromycin, 26 to doxycycline and 20 to tetracycline. These results are in line with previous studies, but resistance to erythromycin has been described controversially (Miller et al., 2007; Abay et al., 2012; Kayman et al., 2012; Shah et al., 2012b; Van den Abeele et al., 2016; Ferreira et al., 2017; Rathlavath et al., 2017; Fanelli et al., 2019, 2020; Isidro et al., 2020). Similar resistance rates for ciprofloxacin, tetracyclines, and erythromycin have been observed for *Campylobacter* spp. in poultry (Nobile et al., 2013; Marotta et al., 2019). Rising resistance in *Campylobacter* spp. has been connected with the inappropriate use or overuse of antibiotics in animal husbandry (Endtz et al., 1991; Marotta et al., 2019). We assume, that this might also be true for tetracyclines and erythromycin and *Aliarcobacter* spp. as they are admitted for treatment of bacterial infections in poultry in Germany. According to existing studies *A. butzleri* isolates are highly resistant to ampicillin (Abay et al., 2012; Kayman et al., 2012; Shah et al., 2012b; Van den Abeele et al., 2016; Rathlavath et al., 2017; Fanelli et al., 2019, 2020). However, in this study more than half of the *A. butzleri* strains ($n = 28$) were susceptible to ampicillin *in vitro*. Nearly all studied strains were resistant to streptomycin ($n = 39$), although in previous studies *A. butzleri* strains were usually sensitive to streptomycin (Abay et al., 2012; Rathlavath et al., 2017; Fanelli et al., 2019, 2020). This indicates an increase in resistance. Therefore, we do not recommend the use of streptomycin for the treatment of *A. butzleri* infections in both veterinary and human medicine.

In previous studies, the antimicrobial susceptibility of *A. butzleri* has been determined with different methods e.g., disk diffusion, broth microdilution, or agar diffusion. However, according to a study conducted by Van den Abeele et al. (2016), the gradient strip method should be preferred over the disk diffusion method. Since both methods' agreement stands at only 60%, our results can be compared with those of earlier studies to a limited extent. This shows the need for a standardized method for antimicrobial susceptibility testing of *Aliarcobacter* spp., and for the interpretation of the results as already noticed by other authors (Ferreira et al., 2016; Ramees et al., 2017; Riesenberg et al., 2017).

In silico Prediction of Antimicrobial Resistance Genes

To determine the underlying genotypic antimicrobial resistance (AMR) profile, we screened all 75 *A. butzleri* genomes with a custom-made database (ARCO_IBIZ_AMR) as described previously (Müller et al., 2020a). This database contains 92 putative AMR genes and 27 putative heavy metal resistance genes.

The survey revealed that all tested genomes contained an average of 80 potential antimicrobial and heavy metal resistance genes. Strain NCTC 12481^T harbored most of the genes ($n = 104$) and strain Ab_3711 the fewest ($n = 66$) (**Supplementary Table 5**).

Out of 19 efflux pumps (EP) that have been described before for *A. butzleri* (Isidro et al., 2020), 17 EP systems were found in the *A. butzleri* genomes tested in this study. The two missing efflux pump systems are EP18 and EP19 [both belong to the resistance-nodulation-division (RND) family]. Eight EPs were present in all genomes: (a) EP2, EP12, EP13, and EP14 [all members of the major facilitator superfamily (MFS)]; (b) EP5, EP6, and EP10 [belonging to the ATP-binding cassette (ABC) family]; and (c) EP8 [belongs to the small multidrug resistance (SMR) family]. This result is concordant with those of previous studies (Isidro et al., 2020; Müller et al., 2020a). The remaining nine EPs belong to the ABC (EP3), RND (EP4, EP7, EP11, EP15, EP16), and MFS families (EP9, EP17) and were present in 16 *A. butzleri* genomes. These findings showed that *A. butzleri* harbors all major families of efflux transporters which are present in prokaryotes except for the multidrug and toxic efflux (MATE) family (Webber and Piddock, 2003). Although they are classified into different families, EPs can confer multidrug resistance as they can export a variety of different substrates (Van Bambeke et al., 2000; Sun et al., 2014; Alcalde-Rico et al., 2016). It is worth mentioning that EP5, EP6, EP8, EP9, EP12, EP13, EP14, and EP17 each consist of only one gene. Hereby, EP9, EP14, and EP17 are of particular interest. Both, EP14 and EP17, contain the same gene, the *bcr* gene which is associated with resistance to sulfonamides and bicyclomycin in *Escherichia coli* (*E. coli*) (Nichols and Guay, 1989). EP9 contains the *fsr* gene that confers resistance to fosmidomycin (Fanelli et al., 2020). The presence of an EP does not necessarily mean that resistance to a particular antibiotic is also present; the resistance rather depends on the expression level of the EP (Alcalde-Rico et al., 2016).

Type 1 secretion systems (T1SS), which are responsible for the transport of unfolded substrates across the inner and outer membrane, are usually found in Gram-negative bacteria (Green and Mecsas, 2016). The putative T1SS described for *A. butzleri* (Isidro et al., 2020), was only present in six strains.

The analysis of potential antibiotic determinants revealed that all tested genomes carried additional putative multidrug export ATP-binding/permease proteins (RM4018p_11130, *ybi*T1, *ylm*A, *mac*B1) and parts of putative EP systems (*acr*B, *tol*C). The outer membrane protein gene, *opr*F3, and the putative multidrug export ATP-binding/permease protein, RM4018p_04700, were also present in all *A. butzleri* genomes except strain 16CS0817-2.

Resistance to tetracyclines is probably due to efflux pump mechanisms, ribosomal protection and enzymatic inactivation (Grossman, 2016). Although the *tet*A gene—encoding the tetracycline EP as described for *Campylobacter* spp. (Shen et al., 2018)—was present in 37 genomes, the phenotype did not correspond to the genotype as both, sensible and resistant isolates carried this gene (**Table 4**). However, as mentioned above, the phenotype may depend on the expression level of the EP. Ribosomal protection proteins encoded by, for example the *tet*(O) gene, were not present, which is not surprising as they are usually located on plasmids (Elhadidy et al., 2020). Although the plasmid prediction in this study revealed the possible presence of a known *A. butzleri* plasmid in seven strains (**Supplementary Table 6**), none of the suggested plasmid carried a potential resistance or virulence-associated gene. Therefore, *A. butzleri* strains either carry a hitherto unknown plasmid or harbor an unknown resistance mechanism against doxycycline and tetracycline. Further studies on this topic are needed in the near future.

In this study, five genes which might cause phenotypic resistance to cefotaxime were identified in all genomes: *bla*2 (putative metallo-hydrolase), *hcp*C (putative beta-lactamase), *mrd*A, *pbp*B, and *pbp*F (all penicillin-binding proteins). Penicillin-binding proteins are the target of beta-lactam antibiotics and are therefore not a resistance mechanism as such, unless they have a low affinity for beta-lactams (Macheboeuf et al., 2006; Zapun et al., 2008). As already described by Georgopapadakou (1993), resistance to beta-lactams in Gram-negative bacteria is the result of a combination of the presence and activity of beta-lactamase genes and penicillin-binding proteins as well as reduced membrane permeability. However, this would contradict the phenotype determined against ampicillin (**Table 4**). Isidro et al. (2020) noticed a strong correlation between the ampicillin resistance and the presence of the *bla*3 gene (OXA-15 beta-lactamase). This hypothesis is partly supported by the results of this study. Here, only two strains (16CS0831-1-1, 16CS0820-1) that did not carry the *bla*3 gene were resistant to ampicillin, and of 33 genomes carrying the *bla*3 gene, only four isolates (17CS1167, 16CS0820-2, Ab_CR1132, Ab_DQ64A1) were susceptible to ampicillin.

As reported previously, resistance to ciprofloxacin in *Aliarcobacter* spp. is due to a point mutation (C254T) in the quinolone resistance-determining region (QRDR) at position 254 of the *gyr*A gene (Abdelbaqi et al., 2007). This transition leads to the substitution of threonine to isoleucine (Thr-85-Ile). In this

TABLE 4 | Genotype-phenotype correlations regarding antibiotic resistance detected in this study.

Strain	DC	*tetA_ gene*	TET	CIP	*gyrA_ gene**	AMP	*bla2_ gene*	*bla3_ gene*	*hcpC_ gene*	*mrdA_ gene*	*pbpB_ gene*	*pbpF_ gene*	CTX	Urease assay	Urease cluster
16CS0847-3	S		S	S		S							R	+	
17CS0824	R		R	S		S							R	+	
17CS1193-1	S		S	S		R							R	+	
17CS1193-2	S		S	S		R							R	+	
17CS1193-3	S		S	S		S							R	+	
17CS1197	R		R	S		S							R	+	
17CS1197-2	R		R	S		S							R	+	
17CS1197-3	R		R	S		S							R	+	
17CS1198-1	S		S	S		S							R	+	
17CS1198-2	S		S	S		S							R	+	
17CS1198-3	S		S	S		S							R	Ø	
16CS0367-AR	R		R	S		S							R	+	
16CS0367-1-AR	R		R	S		S							R	+	
16CS0370-1	R		R	S		S							R	+	
16CS0375-1	R		R	S		S							R	+	
16CS0375-2	R		R	S		S							R	+	
16CS0831-1-1	R		R	S		R							R	+	
16CS0831-4-1	R		R	S		R							R	Ø	
17CS1200-1	S		S	S		S							R	+	
17CS1201	S		S	S		S							R	+	
17CS1205-1	S		S	S		R							R	+	
17CS1205-2	R		S	S		R							R	+	
17CS1206-1	R		S	S		R							R	+	
17CS1206-3	R		S	S		R							R	+	
17CS1167	R		S	S		S							R	+	
17CS1168	S		S	S		S							R	+	
17CS1056	S		S	S		S							R	+	
17CS1066	S		S	S		R							R	+	
17CS1067	S		S	S		R							R	+	
17CS1068-1	R		R	S		R							R	+	
17CS1068-2	R		R	S		R							R	+	
17CS0916-1	S		R	R		R							R	+	
17CS0916-2	R		R	R		R							R	+	
16CS0857-1	R		R	S		R							R	+	
16CS0861-1	R		S	S		S							R	+	
17CS1092-1	R		R	S		S							R	+	
17CS1092-2	S		S	S		S							R	+	
17CS1095	S		S	S		S							R	+	
16CS0815-2	S		S	S		S							R	+	
16CS0817-2	S		S	S		S							R	+	
16CS0820-1	R		R	S		R							R	+	
16CS0820-2	R		R	S		S							R	+	
16CS0821-2	R		R	S		S							R	+	
16CS0823-1	R		R	S		R							R	+	
16CS0823-2	R		S	S		S							R	+	
17CS0965-1	S		S	S		S							R	+	
17CS0965-B	R		S	S		S							R	+	

*The determined phenotype of the 47 German strains is presented next to the associated genotype, their presence/absence is marked by colored/ white boxes. *Presence of the mutation Thr-85-Ile. R, resistant phenotype; S, susceptible phenotype; +, positive urease assay; Ø, negative urease assay; DC, doxycycline; TET, tetracycline; CIP, ciprofloxacin; AMP, ampicillin; CTX, cefotaxime.*

study, 11 *A. butzleri* genomes showed this mutation, two German strains (17CS0916-1 and 17CS0916-2) and nine Portuguese strains (Ab_1711, Ab_2811, Ab_3711, Ab_4211, Ab_A103, Ab_CR1143, Ab_CR502, Ab_CR892, and Ab_DQ20dA1). Those *A. butzleri* strains were also phenotypically resistant to ciprofloxacin (**Table 4**, **Supplementary Table 1**). This result is concordant with earlier studies, although higher resistance rates have been reported (Fera et al., 2003; Ferreira et al., 2013, 2017; Van den Abeele et al., 2016; Rathlavath et al., 2017; Vicente-Martins et al., 2018).

Point mutations in the 23S rRNA gene and amino acid changes in the *rplD* and/or *rplV* gene are considered to be the cause of resistance to erythromycin, as described previously for *Campylobacter* spp. (Alfredson and Korolik, 2007; Perez-Boto et al., 2010; Bolinger and Kathariou, 2017; Shen et al., 2018; Elhadidy et al., 2020). All used genomes were screened for modifications, but none was detected in any strain. Therefore, the resistance mechanism in *A. butzleri* needs to be different. In a previous study, it has been hypothesized that the protein size of the regulator TetR (RM4018p_22360), part of EP16, might correlate with the resistance to erythromycin. Truncating mutations in the *tet*R gene could lead to overexpression of EP16 and thus to increased excretion of erythromycin (Isidro et al., 2020). In another study, the involvement of EP3 has been discussed because it contains two macrolide export proteins (encoded by *mac*A1 and *mac*B2 gene) (Fanelli et al., 2019). Although the presence of those export proteins could explain the phenotype of the resistant isolates, it contradicts the phenotype of the susceptible isolates. The fact that these genes were present but not expressed, might explain the susceptible phenotype.

Furthermore, the screening for other antibiotic determinants identified additional genes in all isolates that are potentially responsible for resistance to certain antibiotics: *arn*B and *ept*A (conferring resistance to polymyxin), and *rlm*N (resistance to various classes of antibiotics) (Fanelli et al., 2019, 2020). The two genes, *cat*3 and *wbp*D, which confer resistance to chloramphenicol were detected in 11 and 27 genomes, respectively. While nine isolates carried three *hip*A genes (*hip*A2, *hip*A3, and *hip*A4), was the *hip*A1 gene only present in two and the *hip*A2 gene in three genomes. The *hip*A genes encode a serine/threonine-protein kinase—a toxic component of the type II toxin-antitoxin system—and are suspected to be involved in multidrug resistance (Schumacher et al., 2009).

In silico Prediction of Heavy Metal Resistance

The survey for heavy metal resistance genes revealed the presence of a putative cluster of genes coding for arsenic resistance in all isolates (**Supplementary Table 5**). This arsenic cluster consists of four genes: *ars*B (arsenic pump membrane protein), *ars*C1 (arsenate reductase), *ars*C2 (glutaredoxin arsenate reductase), and an arsenic resistance protein (ABU_RS02800). Simple arsenic clusters or *ars* operons have been reported for various Gram-negative bacteria (Ben Fekih et al., 2018). Also a putative copper cluster was found in 59 genomes, consisting of six genes: *cop*A1 (copper-exporting P-type ATPase A), *cop*A2 (putative copper-importing P-type ATPase A), *cop*R (transcription activator protein), *cop*Z (copper chaperone), *cso*R (copper-sensing transcriptional repressor), and *cus*S (sensor kinase). Furthermore, a molybdate cluster (ABC-type transport system) that has been described for *E. coli* and *Staphylococcus carnosus*, was present in all strains (Rech et al., 1995; Neubauer et al., 1999). However, the cluster was incomplete because the *mod*C gene (encoding the cytoplasmic ATPase) was not present. Instead of the *mod*C gene, we identified the *mop*A gene (regulator of *mod*ABC) which has been described in *Rhodobacter capsulatus* (Wiethaus et al., 2006). These findings are mostly in line with the results of previous studies (Fanelli et al., 2019, 2020).

The export mechanism for cadmium, zinc, and cobalt represented by *cad*A (a cadmium, zinc and cobalt transporting ATPase) and *czc*D (a cadmium, cobalt and zinc/H^+-K^+ antiporter) was present in all genomes except for Ab_3711. This is concordant with previous studies (Fanelli et al., 2019, 2020). The genes *czc*A and *czc*B, encoding cobalt-zinc-cadmium resistance proteins, were found in eight and 10 strains, respectively. Interestingly, three genes (*czc*R1, *czc*R2 and *czc*R3) responsible for the transcription activator protein CzcR were found in all strains, while the *czc*R4 gene was only present in 22 genomes.

Furthermore, all *A. butzleri* genomes carried four additional transporters: a mercuric transporter (*mer*T), a zinc transporter (*znt*B), a putative manganese/zinc-exporting P-type ATPase (*ctp*C) and a magnesium and cobalt efflux protein (*cor*C) (Fanelli et al., 2020).

The detection of heavy metal resistance clusters/transporters was expected, as the co-resistance and co-selection of AMR and heavy metal resistance, as well as the risk of simultaneous horizontal transmission between bacteria, is driven by their genetic linkage (Bengtsson-Palme and Larsson, 2015; Fanelli et al., 2019, 2020; Zhao et al., 2019). However, this also means that in an environment polluted with heavy metals, this mechanism will lead to the promotion of antibiotic resistance even without the presence of antimicrobials (Fanelli et al., 2020).

In silico Virulence Profiling

The virulence-associated genes of all 75 *A. butzleri* genomes were determined using the database ARCO_IBIZ_VIRULENCE (Müller et al., 2020a). This database can identify 148 virulence-associated genes, including flagellar genes ($n = 36$), chemotaxis system genes ($n = 8$), urease cluster genes ($n = 6$), putative capsule cluster genes ($n = 7$), type IV secretion system (T4SS) genes ($n = 55$), lipid A cluster genes ($n = 12$), and other virulence determinants ($n = 24$) described in earlier studies (Fanelli et al., 2019, 2020; Isidro et al., 2020; Müller et al., 2020a). The analysis showed that all investigated genomes carried an average of 78 putative virulence-associated genes (**Supplementary Table 7**). While strain 17CS0965-1 had most of the genes ($n = 87$), strains Ab_2811 and Ab_A103 had the fewest ($n = 71$).

The detection of virulence-associated genes was first reported in the context of the description of the complete genome sequence of *A. butzleri* RM4018 (Miller et al., 2007). In that study, Miller and his colleagues discovered homologs of the virulence genes *cad*F, *cia*B, *cj*1349, *mvi*N, *pld*A, and *tly*A of *C. jejuni* as well as four other virulence determinants (*irg*A, *iro*E, *hec*A, *hec*B) in *A. butzleri* RM4018 (Miller et al., 2007). Of these virulence-associated genes, the following genes associated with cell adhesion and invasion were present in all 75

genomes: *cia*B (host cell invasion protein), *opr*F2 (fibronectin-binding protein), *cj*1349 (fibronectin-binding protein), *mur*J (integral membrane protein of murein biosynthesis), *pld*A (outer membrane phospholipase A), *tly*A (hemolysin), and additionally *deg*P (chaperone involved in adhesion folding; Isidro et al., 2020). These results are largely consistent with those of previous studies (Douidah et al., 2012; Karadas et al., 2013; Rathlavath et al., 2017; Fanelli et al., 2019, 2020; Parisi et al., 2019; Isidro et al., 2020). The virulence determinants *cir*A1 and *bes*A, which have been associated with the uropathogenicity in *E. coli*, were detected in 11 and 57 genomes, respectively. This is also consistent with earlier studies, as both genes are found less frequently in *A. butzleri* strains (Miller et al., 2007; Fanelli et al., 2019, 2020; Isidro et al., 2020). Three additional *cir*A genes (*cir*A2, *cir*A3, *cir*A4) were identified in some genomes. While *cir*A2 and *cir*A4 had the same annotation made by Prokka as *cir*A1—colicin I receptor—suggesting that both may be involved in iron uptake (Griggs et al., 1987), the *cir*A3 gene represents the former *cfr*B gene, which is involved in iron absorption (Isidro et al., 2020). The *fur* gene, which regulates the ferric uptake, was found in all tested genomes (Griggs et al., 1987; Isidro et al., 2020). According to existing studies, the genes *cdi*A (filamentous hemagglutinin) and *shl*B (hemolysin transporter protein) are rarely found. However, there are no consistent data on the presence of *shl*B (Douidah et al., 2012; Karadas et al., 2013; Rathlavath et al., 2017; Fanelli et al., 2019, 2020; Isidro et al., 2020). This is supported by the results of our study since *cdi*A was found in only two genomes (NCTC 12481^T, Ab_DQ20dA1) and *shl*B in 11 genomes, respectively.

A. butzleri is a motile bacterium due to the polar flagellum. Therefore, the presence of flagellar genes was expected. All 36 flagellar genes were present in 62 genomes. Among the remaining 13 genomes, the *fla*A and *fla*B genes (both coding for flagellin) were absent in seven genomes, while in six genomes, only the *fla*A gene was missing. Similar results have been reported in previous studies (Fanelli et al., 2019; Isidro et al., 2020). The absence of both *fla* genes could have consequences for the assembly and/or function of the flagellum (Isidro et al., 2020).

Each of the 75 tested genomes contained a complete chemotaxis system. This is consistent with the results of earlier studies (Miller et al., 2007; Isidro et al., 2020). Of the two chemotaxis-associated genes, the *lux*S gene was present in all genomes, while the *ccp* gene was detected in only four genomes (Isidro et al., 2020).

The complete urease cluster was detected in 59 *A. butzleri* genomes. Forty-seven German *A. butzleri* isolates (45 strains from this study and two strains from a previous study of the authors; Müller et al., 2020a) underwent a functional urease test. The assay revealed that 45 strains were urease-positive. The urease-negative phenotype of strain 16CS0831-4-1 was consistent with the genotype (**Table 4**) because of the absence of the urease cluster in this strain. While the urease cluster was present in strain 17CS1198-3, the urease assay of this strain was negative. A detailed examination of the urease cluster of this isolate showed several changes in the amino acid sequence of the *ure*E gene (encoding the urease accessory protein) which could justify the observed discrepancy. Although 17CS0965-B did not carry the urease cluster, this strain was phenotypically positive. So far, we do not have a sufficient explanation for this finding. Overall, these results are concordant with previous results (Isidro et al., 2020). Consequently, we hypothesize that there is a correlation between the occurrence of the urease cluster and the urease-positive phenotype. While the ability of *A. butzleri* to metabolize urea is well-known (Miller et al., 2007), the presence of the urease enzyme may also be a threat to a potential host. Due to the degradation of urea to ammonium and carbon dioxide, *A. butzleri* might be able to survive in an acidic environment as observed for *H. pylori* (Gupta et al., 2019).

As part of lipopolysaccharide (LPS), an endotoxin in Gram-negative bacteria, lipid A is recognized by the innate immune system and can trigger a strong immune response in humans and animals (Emiola et al., 2014). Therefore, it should be considered as an important virulence factor. We highlight the detection of a complete putative lipid A cluster in all 75 *A. butzleri* genomes. The putative lipid A cluster contains eight genes that are necessary for the biosynthesis of lipid A: *lpx*A, *lpx*B, *lpx*C, *lpx*D, *lpx*H, *lpx*K, *lpx*P, and *waa*A (Opiyo et al., 2010; Emiola et al., 2014; Steimle et al., 2016). Although a ninth gene, the *lpx*M gene (encoding myristoyltransferase), is described, its presence is usually not essential (Raetz et al., 2009; Emiola et al., 2014; Steimle et al., 2016). The presence of the *lpx*P gene (a paralogue of *lpx*L) in all genomes could explain the adaptation of growth to low temperatures and the survival of *A. butzleri* outside a host because the palmitoleolytransferase encoded by the *lpx*P gene is induced by low temperatures (Carty et al., 1999; Opiyo et al., 2010). Previous studies reported that some Gram-negative bacteria have the ability to change their lipid A structure (Ernst et al., 2001; Ramachandran, 2014; Steimle et al., 2016). These changes are regulated by a two-component system called PhoP-PhoQ and can lead, for example, to increased resistance to cationic antimicrobial peptides and reduced receptor recognition (Ramachandran, 2014). Two genomes, NCTC 12481^T and 6V, carried the two-component system consisting of three *pho*P genes (*pho*P1, *pho*P2, and *pho*P3) and the *pho*Q gene. While the PhoP-PhoQ system was detected in four strains (16CS0831-4-1, Ab_2211, Ab_DQ20dA1, and Ab_DQ31A1) without the *pho*P2 gene, *pho*P3, and *pho*Q were the only genes found in 17CS0965-1. Since no data were available in the current literature on how many *pho*P genes are necessary for the functionality of the PhoP-PhoQ system, it remains unclear if these seven strains might be able to modify their lipid A structure. All the other genomes had at least one *pho*P gene (*pho*P3) but no *pho*Q gene, suggesting that the two-component system was not functional. These *A. butzleri* strains were therefore either unable to modify their lipid A structure or used a hitherto unknown mechanism.

In this study, the genes coding for heptosyltransferases I and II, namely *rfa*C and *rfa*F, were identified in all 75 *A. butzleri* genomes. These heptosyltransferases are possibly responsible for the assembly and the phosphorylation of the inner-core region of the LPS (Rovetto et al., 2017; Fanelli et al., 2020). Although heptosyltransferases are not essential, their absence may affect the structure of the outer membrane of Gram-negative bacteria, and thus lead to a rough phenotype (Gronow et al., 2000).

Furthermore, the following genes were detected in all analyzed genomes: (a) *cvf* B (conserved virulence factor B), which is known to regulate the expression of virulence factors in *Staphylococcus aureus* (Matsumoto et al., 2007); (b) *voc* (virulence protein);

and (c) *vir*F (virulence regulator transcription activator), which has been associated with the regulation of plasmid-transmitted virulence genes in *Shigella flexneri* (Adler et al., 1989; Tobe et al., 1993). In contrast, the *bvg*S gene (virulence sensor protein) was only present in six *A. butzleri* strains.

CONCLUSION

In this study, 45 *A. butzleri* strains isolated from seven water poultry farms in Thuringia, Germany, were sequenced and correctly identified as *Aliarcobacter butzleri*.

We observed a high genetic diversity between the German *A. butzleri* strains, even though the isolates were limited to one federal state (Thuringia), a certain host (water poultry), and a short study period (2 years). However, the phylogenetic analysis did not support clustering based on the isolation source or geographical location of the strains.

Here, we were able to identify a comprehensive profile of potential resistance and virulence-associated genes in all investigated *A. butzleri* genomes. The antimicrobial genotype, however, only corresponds partially to the determined phenotype. Therefore, phenotypic antimicrobial susceptibility testing remains necessary and important. A strong correlation was found between the presence of the *gyr*A mutation (Thr-85-Ile) and the phenotypic ciprofloxacin resistance, as well as between the presence of the urease cluster and urease-positive phenotype. Only a partial correlation was observed between the presence of the *bla*3 gene and ampicillin resistance. In addition, we would like to highlight the identification of a lipid A cluster in all studied genomes, which may allow some strains a certain amount of flexibility to the host's immune response due to the ability to modify their lipid A structure.

DATA AVAILABILITY STATEMENT

The original contributions generated for this study are publicly available. This data can be found here: DDBJ/ENA/GenBank; BioProject: PRJNA665330, and PRJNA575341. Publicly available datasets were analyzed in this study. This data can be found here: DDBJ/ENA/GenBank; BioProject: PRJEB6403, PRJNA489574, PRJNA489609, PRJNA637480, PRJNA67167, and PRJEB34441.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because there were no experiments with animals as defined by the German Animal Protection Law (Tierschutzgesetz) and the Animal Welfare Laboratory Animal Regulation (Tierschutz-Versuchstierverordnung). Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

EM and HT conceived the work. IH provided the strains and metadata. EM and IH performed antimicrobial susceptibility testing. EM performed the urease test and bioinformatic analyses and wrote the manuscript. HH, JL, and HT supervised the data interpretation. All authors contributed to the revision of the manuscript, read and approved the submitted manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.617685/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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4 Diskussion

Im Rahmen dieser Studie wurden in den Jahren 2016 und 2017 insgesamt 250 Kotproben von 135 Gänsen (*Anser anser*), 45 Flugenten (*Cairina moschata*), 40 Mularden (*Cairina moschata × Anas platyrhynchos domesticus*) und 30 Pekingenten (*Anas platyrhynchos domesticus*) aus 10 verschiedenen Wassergeflügel-Betrieben in Thüringen, Deutschland, gesammelt und auf *Aliarcobacter* spp. untersucht. Aus 55 positiven Proben, konnten 27 *A. cryaerophilus*- und 47 *A. butzleri*-Stämme isoliert werden. Nach der Isolierung erfolgte die Bestimmung der phänotypsichen antimikrobiellen Empfindlichkeit gegenüber acht verschiedener Antibiotika mithilfe des Epsilometertests (E-Test). Durch die anschließende Gesamtgenom-Sequenzierung wurden potentielle antimikrobielle Resistenz- und Virulenzgene identifiziert. Des Weiteren wurde die phylogenetische Verwandtschaft der Isolate untersucht.

Die Untersuchungen in Bezug auf die phylogenetische Verwandtschaft der isolierten *Aliarcobacter*-Stämme ergaben, dass sowohl die untersuchten *A. cryaerophilus*-Genome als auch die *A. butzleri*-Genome eine durchschnittliche Nukleotid-Identität (Average Nucleotide Identity, ANI) von über 96% aufwiesen, sofern sie mit anderen Genomen bzw. dem Referenzgenom der jeweiligen Spezies verglichen wurden. Laut früheren Studien gehören die untersuchten Genome zur gleichen Spezies, wenn der ANI-Wert über dem Schwellenwert von 95% liegt (GORIS et al. 2007, RICHTER et al. 2009). Eine parallel durchgeführte *in silico* DNA-DNA-Hybridisierung (DDH) bestätigte die ANI-Ergebnisse. Dabei wurde festgestellt, dass der DHH-Wert der untersuchten *A. cryaerophilus*- und *A. butzleri*-Genome im Vergleich mit den jeweiligen Referenzgenomen deutlich über 70% lag. Ein DDH-Wert von 70% ist der empfohlene Standard für die Abgrenzung verschiedener Spezies (WAYNE et al. 1987, ON et al. 2017). Da der empfohlene DDH-Schwellenwert einem ANI-Wert von 95% entspricht (GORIS et al. 2007, RICHTER et al. 2009), unterstützen die DDH-Ergebnisse das Ergebnis der ANI-Analyse. Wurden die untersuchten Genome jedoch mit dem Referenzgenom einer anderen *Aliarcobacter*-Spezies oder einer noch weiter entfernten Spezies wie z. B. *Campylobacter jejuni* subsp. *jejuni* verglichen, so sanken die DDH-Werte ebenso wie die ANI-Werte auf unter 30% bzw. 70% ab. Anhand dieser Ergebnisse lässt sich schlussfolgern, dass die als *A. cryaerophilus* identifizierten Stämme der aktuell gültigen Taxonomie folgend zum Cluster I der Spezies *A. cryaerophilus* und somit zum Genomovar *A. cryaerophilus gv. pseudocryaerophilus* gehören und, dass die als *A. butzleri* identifizierten Isolate tatsächlich der Spezies *A. butzleri* angehören.

Ferner deuten die Untersuchungen zur phylogenetischen Verwandtschaft auf ein hohes Maß an genetischer Diversität unter den einzelnen *A. cryaerophilus*- und *A. butzleri*-Stämmen hin, obwohl die hier untersuchten Proben auf ein Bundesland (Thüringen), einen bestimmten Wirtstyp (Wassergeflügel) und eine kurze Studienzeit (2 Jahre) begrenzt waren. Die genetische Distanz der *A. cryaerophilus*- und *A. butzleri*-Stämme lag durchschnittlich bei 10.773 bzw. 10.923 Einzelnukleotid-Polymorphismen (SNP) und ist damit niedriger als die von ISIDRO et al. (2020) festgestellte Distanz von 24.600 SNPs für *A. butzleri*. Laut ISIDRO et al. (2020)

ist eine Gruppierung eng verwandter *A. butzleri*-Isolate in Übereinstimmung mit der geographischen Lage oder dem Ursprung der Isolate nicht möglich. Dies konnte durch die vorliegende Studie sowohl für *A. cryaerophilus* als auch für *A. butzleri* bestätigt werden. Dennoch wurde festgestellt, dass eine Vielzahl der untersuchten *Aliarcobacter*-Isolate Untergruppen bildeten. So formten 17 *A. cryaerophilus*-Isolate 3 Untergruppen und 33 *A. butzleri*-Isolate 11 Untergruppen. Allen 14 Untergruppen war folgendes gemein: Die Isolate einer Untergruppe stammten vorwiegend aus einer einzelnen Kotprobe. Daher ist anzunehmen, dass zwischen diesen Isolaten eine klonale Verwandtschaft besteht. Eine klonale Verwandtschaft besteht ebenfalls zwischen Isolaten, die aus unterschiedlichen Kotproben eines Betriebes stammen. Dies lässt den Schluss zu, dass mehrere Tiere einer Herde möglicherweise mit demselben bakteriellen Klon infiziert sind.

Sehr überraschend war die Tatsache, dass bei beiden *Aliarcobacter*-Spezies einzelne Isolate aus derselben Kotprobe sehr weit (über 8.500 SNPs) voneinander entfernt waren. Ferner gab es bei beiden *Aliarcobacter*-Spezies je eine Untergruppe in der Isolate von Kotproben aus verschiedenen Betrieben enthalten waren. Da die Distanz zwischen diesen Isolaten relativ gering war (kleiner als 12 SNPs), besteht die Möglichkeit einer epidemiologischen Verbindung zwischen den einzelnen Betrieben. Ursache hierfür könnte die gleiche Herkunft der Tiere, der Gerätschaften und/oder des Personals sein (SHANGE et al. 2019). Die Ergebnisse der vorliegenden Studie legen außerdem nahe, dass die genetische Diversität der *A. cryaerophilus* und *A. butzleri* unabhängig von der Wirtstierart ist, da ähnliche Stämme sowohl aus Enten- als auch Gänse-Kotproben isoliert werden konnten.

Angesichts der engen Verwandtschaft der *Aliarcobacter* spp. zu den thermophilen *Campylobacter* spp. ist die Bestimmung der antimikrobiellen Empfindlichkeit von großer Relevanz. Laut verschiedener Studien steht die Zunahme der Antibiotikaresistenz von *Campylobacter* spp. mit einem übermäßigen und/oder unangemessenen Gebrauch von Antibiotika in der Tierhaltung, insbesondere in der Geflügelhaltung, in Verbindung (ENDTZ et al. 1991, NOBILE et al. 2013, MAROTTA et al. 2019). Es ist daher davon auszugehen, dass in Zukunft die antimikrobielle Resistenz bei *Aliarcobacter* spp. ebenfalls zunehmen wird. Obwohl die antimikrobielle Empfindlichkeit bislang vorrangig für *A. butzleri* ermittelt wurde und für *A. cryaerophilus* nur wenige Vergleichswerte zur Verfügung stehen, fällt eine Gemeinsamkeit ins Auge: Die Cefotaxim-Resistenz (COLLADO et al. 2011, FERREIRA et al. 2019). Auch in der hier vorliegenden Studie waren alle getesteten *Aliarcobacter*-Isolate resistent gegenüber Cefotaxim. Es handelt dabei sich um eine bekannte Resistenz (SHAH et al. 2012, FERREIRA et al. 2017, RATHLAVATH et al. 2017, FANELLI et al. 2019, FANELLI et al. 2020, ISIDRO et al. 2020), die oft genutzt wird, um bei der Anzucht von *Aliarcobacter* spp. die eventuell vorhandene Begleitflora im Wachstum zu hemmen (COLLADO et al. 2011). Weiterhin waren mehr als drei Viertel der untersuchten *Aliarcobacter*-Isolate resistent gegenüber Streptomycin. Das ist insofern überraschend, da in der Literatur bisher nur über zwei Streptomycin-resistente *A. butzleri*-Stämme berichtet wurde (ABAY et al.

2012). Die in dieser Studie beobachtete hohe Streptomycin-Resistenzrate ist möglicherweise ein erstes Beispiel für die Zunahme der Antibiotikaresistenz unter den *Aliarcobacter* spp.

Während die 27 *A. cryaerophilus*-Stämme ein homogenes Resistenzprofil zeigten - alle Isolate waren sensibel gegenüber Erythromycin, Gentamicin und Ampicillin -, wiesen die 47 *A. butzleri*-Stämme ein überaus heterogenes Resistenzprofil auf. Die untersuchten *A. butzleri*-Isolate hatten eine deutlich höhere Resistenzrate gegenüber Doxycyclin und Tetracyclin als die *A. cryaerophilus*-Isolate. Wohingegen die *A. cryaerophilus*-Isolate vermehrt gegen Ciprofloxacin resistent waren. Daher stehen die Ergebnisse dieser Studie im Einklang mit denen vorheriger Untersuchungen (MILLER et al. 2007, ABAY et al. 2012, KAYMAN et al. 2012, SHAH et al. 2012, FERREIRA et al. 2013, SHAH et al. 2013, ÜNVER et al. 2013, VAN DEN ABEELE et al. 2016, FERREIRA et al. 2017, PÉREZ-CATALUÑA et al. 2017, RATHLAVATH et al. 2017, RIESENBERG et al. 2017, FANELLI et al. 2019, FERREIRA et al. 2019, FANELLI et al. 2020, ISIDRO et al. 2020). Erwähnenswert ist jedoch, dass in früheren Studien andere Methoden, wie z. B. der Plättchentest, der Agar-Diffusionstest oder der Mikrodilutionstest, zur Bestimmung der antimikrobiellen Empfindlichkeit angewandt wurden. Deshalb sind die Ergebnisse der vorliegenden Studie nur bedingt mit denen früherer Studien vergleichbar. Die in einer Vergleichsstudie gewonnene Erkenntnis, dass der E-Test einem Plättchentest vorzuziehen ist (VAN DEN ABEELE et al. 2016), hebt die Unerlässlichkeit einer standardisierten Methode zur Bestimmung der antimikrobiellen Empfindlichkeit von *Aliarcobacter* spp. und die Festlegung geeigneter Grenzwerte hervor. Diese Notwendigkeit wurde bereits von anderen Autoren aufgezeigt und eingefordert (FERREIRA et al. 2016, RAMEES et al. 2017, RIESENBERG et al. 2017).

Ein weiteres Ziel dieser Arbeit war die Identifizierung von potentiellen antimikrobiellen Resistenzgenen und Virulenzgenen in beiden *Aliarcobacter*-Spezies. Dies gelang durch den Nachweis von Genen, die in früheren Studien beschrieben wurden, sowie durch die Erstellung einer Resistenz- und Virulenzdatenbank. Für die Identifizierung potentieller Resistenz- und Virulenzgene in den *A. cryaerophilus*-Genomen wurden Gene verwendet, die zuvor von ISIDRO et al. (2020) für *A. butzleri* beschrieben worden waren. Im Verlauf der vorliegenden Studie wurden weitere Gene, die in anderen Studien beschrieben worden waren (FANELLI et al. 2019, FANELLI et al. 2020) bzw. die aufgrund ihrer Annotation mutmaßlich dazu gehören, in die Untersuchung eingebunden. Am Ende entstand eine Resistenz- und Virulenzdatenbank, die für *A. butzleri* spezifisch ist. Diese Datenbank ist der Öffentlichkeit zugänglich (https://gitlab.com/FLI_Bioinfo_pub/publicationdata_analysis-of-arcobacter-butzleri) und ermöglicht dadurch interessierten Wissenschaftlern eine unkomplizierte Bestimmung des genotypischen Resistenz- und Virulenzprofiles von *A. butzleri*-Isolaten.

Die Untersuchung bezüglich des Vorhandenseins antimikrobieller Resistenzgene ergab, dass sowohl *A. cryaerophilus* als auch *A. butzleri* mehrere Gene für verschiedene Effluxpumpen (EP) tragen. Effluxpumpen

stellen Transportproteine dar, die eine Vielzahl verschiedener Substrate (inklusive Antibiotika) aus der Bakterienzelle ausschleusen können (WEBBER et al. 2003). Ferner ist bekannt, dass Effluxpumpen die Ursache der intrinsischen Antibiotikaresistenz gramnegativer Bakterien sind (LI et al. 1994, WEBBER et al. 2003). Von den 19 Effluxpumpen, die zuvor für *A. butzleri* beschrieben worden waren (ISIDRO et al. 2020), konnten 16 in *A. cryaerophilus* identifiziert werden. Davon waren sechs Effluxpumpen (EP2, EP5, EP6, EP7, EP8, EP12) in jedem Genom vorhanden. Die übrigen zehn Effluxpumpen (EP1, EP3, EP4, EP10, EP13, EP14, EP15, EP16, EP17, EP18) waren in mindestens einem *A. cryaerophilus*-Genom präsent. Die untersuchten *A. butzleri*-Genome wiesen 17 der bekannten 19 Effluxpumpen auf. In jedem *A. butzleri*-Genom waren acht Effluxpumpen (EP2, EP5, EP6, EP8, EP10, EP12, EP13, EP14) zu finden. Die restlichen neun Effluxpumpen (EP1, EP3, EP4, EP7, EP9, EP11, EP15, EP16, EP17) waren in mindestens neun *A. butzleri*-Genomen vorhanden. Somit kommen fünf Effluxpumpen aus drei verschiedenen Familien in beiden *Aliarcobacter*-Spezies vor: EP2 und EP12 (MFS – major facilitator superfamily), EP5 und EP6 (ABC – ATP-binding cassette) und EP8 (SMR – small multidrug resistance). Des Weiteren besaßen alle untersuchten *Aliarcobacter*-Isolate, bis auf je einen *A. cryaerophilus*- und einen *A. butzleri*-Stamm, die EP15, welche zur RND (resistance-nodulation-division) Familie gehört. Das zeigt, dass sowohl *A. cryaerophilus* als auch *A. butzleri* nahezu alle wichtigen Familien der Effluxpumpen, die in Prokaryoten vorkommen, in sich tragen (WEBBER et al. 2003). Laut ALCALDE-RICO et al. (2016) bedeutet die Anwesenheit solcher Effluxpumpen aber nicht, dass gleichzeitig eine Resistenz gegen ein spezifisches Antibiotikum vorliegt. Die antimikrobielle Resistenz ist vielmehr von der Expressionshöhe der Effluxpumpen abhängig (ALCALDE-RICO et al. 2016).

Die von ISIDRO et al. (2020) aufgestellte Hypothese, dass die Anwesenheit des *bla*3-Genes die Resistenz gegenüber Ampicillin bedingt, trifft aufgrund der vorliegenden Resultate tatsächlich nur auf *A. butzleri* zu. Denn während dieses Gen bis auf wenige Ausnahmen in allen phänotypisch resistenten *A. butzleri*-Stämmen nachgewiesen werden konnte, wurde es in nur einem *A. cryaerophilus*-Stamm identifiziert, der zudem noch empfindlich gegenüber Ampicillin war. Es besteht jedoch die Möglichkeit, dass in gramnegativen Bakterien die Resistenz gegen β-Laktam-Antibiotika (z. B. Ampicillin und Cefotaxim) durch eine Kombination aus dem Vorhandensein und der Aktivität von β-Laktamase-Genen und Penicillin-bindenden Proteinen sowie einer verringerten Membranpermeabilität entsteht (GEORGOPAPADAKOU 1993). Dieser Umstand könnte die Anwesenheit des *bla*3-Genes in den Ampicillin-empfindlichen *A. butzleri*-Isolaten erklären.

Die Ciprofloxacin-Resistenz in *Aliarcobacter* spp. wird durch eine Punktmutation in der QRDR an Position 254 des *gyr*A-Genes verursacht (ABDELBAQI et al. 2007). Diese Mutation geht mit einem Aminosäureaustausch von Threonin zu Isoleucin (Thr-85-Ile) einher und führt anschließend zu einer Resistenz gegenüber Ciprofloxacin. Während bei *A. butzleri* ein eindeutiger Zusammenhang zwischen dem Auftreten der Mutation und der Resistenz besteht, so existiert dieser Zusammenhang bei *A. cryaerophilus* nur teilweise. Denn es traten sowohl resistente *A. cryaerophilus*-Stämme auf, die die genannte Mutation nicht aufwiesen, als auch sensible *A. cryaerophilus*-Stämme, welche die Mutation trugen. Daher ist für die Ausbildung einer

Ciprofloxacin-Resistenz in *A. cryaerophilus* höchstwahrscheinlich das Vorhandensein einer Effluxpumpe z.B. *cme*ABC wie bei *Campylobacter* spp. notwendig (SHEN et al. 2018).

Wie zuvor für *Campylobacter* spp. beschrieben, kann die Erythromycin-Resistenz durch Punktmutationen im 23S rRNA-Gen oder durch Veränderungen der Aminosäuresequenz des *rpl*D- und/oder *rpl*V-Genes verursacht werden (ALFREDSON et al. 2007, PEREZ-BOTO et al. 2010, BOLINGER et al. 2017, SHEN et al. 2018, ELHADIDY et al. 2020). Jedoch konnte keine der in den vorgenannten Studien beschriebenen Modifikationen in einem *A. butzleri*-Genom nachgewiesen werden, sodass mit hoher Wahrscheinlichkeit ein anderer Resistenzmechanismus ursächlich ist. ISIDRO et al. (2020) vermutet, dass die Proteingröße des Regulators TetR (ein Teil der EP16) mit dem Auftreten einer Erythromycin-Resistenz in Verbindung steht. Denn Mutationen im Regulator TetR, die ein frühzeitiges Stoppcodon zur Folge haben, führen zu einer Überexpression der EP16 und somit zu einer gesteigerten Exkretion von Erythromycin. Eine andere Studie hält die Beteiligung der EP3 für wahrscheinlich, da diese Effluxpumpe zwei Makrolid-Exportproteine enthält (FANELLI et al. 2019). Obwohl das Vorhandensein dieser Exportproteine den Phänotyp der resistenten *A. cryaerophilus*- bzw. *A. butzleri*-Isolate erklären könnte, widerspricht es dem Phänotyp der empfindlichen Isolate. Dieser Sachverhalt kann möglicherweise dadurch erklärt werden, dass die Gene zwar vorhanden, aber nicht exprimiert waren.

Effluxpumpen, ribosomaler Schutz und die enzymatische Inaktivierung von Tetracyclinen gelten als die Ursachen der Tetracyclin-Resistenz (GROSSMAN 2016). Die Tetracyclin-Effluxpumpe, die, wie zuvor bei *Campylobacter* spp. beschrieben (SHEN et al. 2018), durch das *tet*A-Gen kodiert wird, war in circa der Hälfte aller *A. butzleri*-Isolate zu finden. Dennoch entsprach der ermittelte Phänotyp selten dem zugrunde liegenden Genotyp, da sowohl empfindliche als auch resistente Isolate dieses Gen trugen. Wie eingangs beschrieben, kann der Phänotyp jedoch von der Expressionshöhe der Effluxpumpe abhängen (ALCALDE-RICO et al. 2016). Ribosomale Schutzproteine, die z. B. durch das *tet*(O)-Gen kodiert werden, waren weder in den *A. cryaerophilus*- noch in *A. butzleri*-Genomen vorhanden. Diese ribosomalen Schutzproteine sind meist auf Plasmiden lokalisiert (MARASINI et al. 2018, ELHADIDY et al. 2020). In der vorliegenden Studie konnten zwar in einigen *A. cryaerophilus*- und *A. butzleri*-Isolaten Plasmide nachgewiesen werden, aber in keinem war ein Gen für ein ribosomales Schutzprotein vorhanden. Daher ist davon auszugehen, dass die resistenten Stämme beider Spezies entweder einen noch unbekannten Resistenzmechanismus gegen Tetracycline beherbergen oder ein noch unbekanntes Plasmid tragen.

HARRASS et al. (1998) gelang es 1998 erstmalig innerhalb des Genus bei *A. butzleri* 21 Plasmiden mit einer Größe von bis zu fünf Kilobasenpaaren nachzuweisen. In den darauffolgenden Jahren gelang es sowohl DOUIDAH et al. (2014) als auch ON et al. (2019) Plasmide in *A. cryaerophilus*-Stämmen zu identifizieren. In der hier vorliegenden Studie, konnte in vier *A. cryaerophilus*-Genomen ebenfalls je ein Plasmid nachgewiesen werden. Davon beherbergten drei Plasmide mindestens ein potentielles antimikrobielles Resistenzgen. Ein

Beweis dafür, dass *A. cryaerophilus* möglicherweise in der Lage ist antimikrobielle Resistenzgene innerhalb und außerhalb der eigenen Spezies auszutauschen. Weiterhin wurde im Rahmen dieser Arbeit mithilfe einer bioinformatischen Methode in fünf deutschen *A. butzleri*-Genomen das Vorhandensein von je einem bekannten *A. butzleri*-Plasmid detektiert. Da diese bekannten *A. butzleri*-Plasmide, die 2014 von DOUIDAH et al. (2014) beschrieben worden waren, keine potentiellen Resistenz- oder Virulenzgene trugen, hatten sie mit hoher Wahrscheinlichkeit keinen Einfluss auf die vorliegende Resistenz und Virulenz dieser *A. butzleri*-Isolate.

Mit der Veröffentlichung der ersten vollständigen Genomsequenz von *A. butzleri* wurde erstmals über das Vorhandensein potentieller Virulenzgene berichtet (MILLER et al. 2007). Miller und seine Kollegen entdeckten Homologe der Virulenzgene *cad*F, *cia*B, *cj*1349, *mvi*N, *pld*A und *tly*A von *Campylobacter jejuni* sowie vier weitere Virulenzdeterminanten (*irg*A, *iro*E, *hec*A, *hec*B) im *A. butzleri*-Stamm RM4018 (MILLER et al. 2007). In der hier vorliegenden Studie konnten sowohl in *A. butzleri* als auch in *A. cryaerophilus* folgende potentielle Virulenzgene identifiziert werden: *opr*F2 (früher *cad*F), *cj*1349, *iam*A und *mur*J (früher *mvi*N). Darüber hinaus waren weitere mit der Zelladhäsion und -invasion assoziierte Virulenzgene in allen untersuchten *A. butzleri*-Genomen vorhanden: *cia*B, *deg*P (früher *htr*A), *tly*A und *pld*A. Diese Ergebnisse stimmen weitgehend mit denen früherer Studien überein (Douidah et al., 2012; Karadas et al., 2013; Rathlavath et al., 2017; Fanelli et al., 2019; Parisi et al., 2019; Fanelli et al., 2020; Isidro et al., 2020). Laut vorheriger Studien sind die potentiellen Virulenzgene *cir*A1 (früher *irg*A) und *bes*A (früher *iro*E), die mit der Uropathogenität von *E. coli* assoziiert werden, sowie *cdi*A (früher *hec*A) und *shl*B (früher *hec*B) seltener in *Aliarcobacter* spp. vorhanden als die oben genannten Virulenzgene (MILLER et al. 2007, DOUIDAH et al. 2012, KARADAS et al. 2013, ZACHAROW et al. 2015, RATHLAVATH et al. 2017, SEKHAR et al. 2017, FANELLI et al. 2019, BRÜCKNER et al. 2020, FANELLI et al. 2020, ISIDRO et al. 2020). Diese Beobachtungen werden durch die hier vorliegenden Ergebnisse unterstützt, da in beiden *Aliarcobacter*-Spezies die zuvor genannten Gene nur vereinzelt detektiert wurden. Im Gegensatz dazu wurden das *cir*A3-Gen (früher *cfr*B), welches an der Eisenaufnahme beteiligt ist (ISIDRO et al. 2020), sowie das *fur*-Gen, welches die Eisenaufnahme reguliert (GRIGGS et al. 1987, ISIDRO et al. 2020), in den untersuchten *A. butzleri*- und *A. cryaerophilus*-Stämmen häufiger identifiziert.

Sowohl *A. butzleri* als auch *A. cryaerophilus* besitzen ein polares Flagellum, welches für die Beweglichkeit des Bakteriums verantwortlich ist. Die für die Biosynthese des Flagellums verantwortlichen Gene konnten in beiden Spezies identifiziert werden. Jedoch wurden einzelne Gene nicht nachgewiesen. So fehlte in allen *A. cryaerophilus*-Genomen das *fla*B-Gen (kodiert die kleine Flagellin-Untereinheit), das *flh*A-Gen (kodiert das Flagellum-Biosyntheseprotein) und das *hag*-Gen (kodiert ebenfalls das Flagellin). Während in sieben *A. butzleri*-Genomen sowohl das *fla*A-Gen (kodiert die große Flagellin-Untereinheit) als auch das *fla*B-Gen nicht

vorhanden waren, konnte in sechs weiteren *A. butzleri*-Genomen nur das *fla*A-Gen nicht nachgewiesen werden. Über ähnliche Ergebnisse hatten FANELLI et al. (2019) und ISIDRO et al. (2020) in ihren Studien berichtet. Die Abwesenheit dieser Gene hat möglicherweise Auswirkungen auf die Biosynthese und/oder die Funktionalität der Geißel (ISIDRO et al. 2020).

In allen *A. butzleri*- und *A. cryaerophilus*-Genomen konnte ein komplettes Chemotaxis-System, bestehend aus acht Genen (*che*A, *che*B, *che*R, *che*W, *che*V, *che*Y1, *che*Y2, *che*Y3), identifiziert werden, welches 2007 erstmalig in *A. butzleri* entdeckt wurde (MILLER et al. 2007). Dieses Ergebnis beweist zum einen, dass *A. butzleri* nicht die einzige *Aliarcobacter*-Spezies ist (MILLER et al. 2007, ISIDRO et al. 2020), die ein Chemotaxis-System besitzt. Zum anderen zeigt es, dass *Aliarcobacter* spp. in der Lage sind mit ihrer Umwelt zu interagieren sowie sich gegebenenfalls widrigen Umweltbedingungen anzupassen und folglich zu überleben (MILLER et al. 2007).

Während *A. butzleri* die Fähigkeit besitzt, Harnstoff zu verstoffwechseln, gilt *A. cryaerophilus* als nicht-ureolytisch, da dieser Spezies das Enzym Urease fehlt (MILLER et al. 2007, MILLER et al. 2018). In der vorliegenden Studie wurde in keinem der untersuchten *A. cryaerophilus*-Genome das Urease-Cluster entdeckt, welches zuvor für *A. butzleri* beschrieben wurde (ISIDRO et al. 2020). Dieses Ergebnis untermauert somit die Beobachtung einer früheren Studie (MILLER et al. 2018). Im Gegensatz dazu besteht bei *A. butzleri* ein Zusammenhang zwischen dem Vorhandensein des Urease-Clusters und dem positiven Urease-Test. Sofern das Urease-Cluster nicht durch eine Mutation beeinträchtigt war, waren alle *A. butzleri*-Stämme, welche das Cluster trugen, im Urease-Test positiv. Dies bestätigt das Ergebnis der von ISIDRO et al. (2020) durchgeführten Studie. Das Vorhandensein des Urease-Enzyms in einigen *A. butzleri*-Isolaten stellt gleichzeitig aber auch ein Risiko für den potenziellen Wirt dar. Denn aufgrund des Abbaus von Harnstoff zu Ammonium und Kohlenstoffdioxid wären diese *A. butzleri*-Isolate in der Lage, im sauren Milieu zu überleben und im Wirt zu persistieren wie es bereits bei *Helicobacter pylori* beobachtet wurde (GUPTA et al. 2019).

Lipopolysaccharide, kurz LPS, stellen sowohl einen Bestandteil der äußeren Zellmembran als auch das Endotoxin gramnegativer Bakterien dar. Das LPS, wichtigster Virulenzfaktor gramnegativer Bakterien, besteht aus einem Oligosaccharidkern, einem O-Antigen-Polysaccharid und dem Lipid A (RAMACHANDRAN 2014). Da das Lipid A die einzige Region des LPS ist, die vom angeborenen Immunsystem erkannt wird und - selbst bei alleinigem Vorkommen - eine starke Immunantwort bei Mensch und Tier auslöst (EMIOLA et al. 2014), sollte es als eigenständiger Virulenzfaktor betrachtet werden. Insbesondere da in allen untersuchten *A. butzleri*-Genomen ein vollständiges Lipid-A-Cluster entdeckt wurde. Das Lipid-A-Cluster enthält acht Gene, die für die Lipid-A-Biosynthese verantwortlich sind: *lpx*A, *lpx*B, *lpx*C, *lpx*D, *lpx*H, *lpx*K, *lpx*P und *waa*A. *lpx*P ist ein Paralogon von *lpx*L und kodiert die Palmitoleoyltransferase, die durch niedrige Temperaturen induziert wird (CARTY et al. 1999). Die Anwesenheit von *lpx*P in allen *A. butzleri*-Genomen ist möglicherweise für die Anpassung des Wachstums an niedrige Temperaturen verantwortlich und ermöglicht so das Überleben

außerhalb eines Wirtes, wie es bereits für *E. coli* beschrieben wurde (OPIYO et al. 2010). In früheren Studien wurde darüber berichtet, dass einige gramnegative Bakterien die Fähigkeit besitzen, ihre Lipid-A-Struktur zu verändern (RAMACHANDRAN 2014, ERNST et al. 2001, STEIMLE et al. 2016). Die daraus resultierenden Modifikationen können z. B. eine höhere Resistenz gegen kationische antimikrobielle Peptide des Wirtes oder eine geringere Rezeptorerkennung bewirken. Die Regulation dieser Veränderungen obliegt einem Zweikomponentensystem namens PhoP-PhoQ (RAMACHANDRAN 2014). In der vorhandenen Literatur waren keine Daten darüber verfügbar, wie viele *pho*P-Gene für die Funktionalität des PhoP-PhoQ-Systems notwendig sind. Insofern bleibt unklar, ob die *A. butzleri*-Genome, welche mindestens ein *pho*P-Gen und das *pho*Q-Gen besitzen, in der Lage sind, ihre Lipid-A-Struktur zu modifizieren.

5 Zusammenfassung

Eva Müller

Untersuchungen zur Antibiotikaresistenz und Virulenz von *Aliarcobacter cryaerophilus*- und *Aliarcobacter butzleri*-Isolaten von Wassergeflügel aus Thüringen

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Schlüsselwörter: *Aliarcobacter*, antimikrobielle Empfindlichkeit, Resistenz, Virulenz, Gesamtgenom-Sequenzierung, Zoonose

Einleitung: *Aliarcobacter* (*A.*) *cryaerophilus* und *Aliarcobacter butzleri* gelten weltweit als lebensmittelbedingte und zoonotische Krankheitserreger, die vorrangig über kontaminierte Lebensmittel oder Trinkwasser übertragen werden. Beim Menschen stellt sich die Infektion meist als selbstlimitierende Enteritis dar, wohingegen sie bei Tieren neben gastrointestinalen Erkrankungen mit Mastitiden, Aborten und Reproduktionsstörungen assoziiert wird. Aufgrund der engen Verwandtschaft mit den thermophilen *Campylobacter* spp., deren antimikrobielle Resistenz in den letzten Jahren einen starken Anstieg verzeichnet, ist es wichtig, das Resistenz- und Virulenzprofil dieser beiden Spezies zu untersuchen.

Ziel der Untersuchungen: Ziel dieser Arbeit war die Bestimmung der phänotypischen und genotypischen Antibiotikaresistenz sowie die Ermittlung der Virulenzprofile von *Aliarcobacter cryaerophilus*- und *Aliarcobacter butzleri*-Stämmen, die aus Kotproben von Wassergeflügel aus Thüringen isoliert worden waren.

Tiere, Material und Methoden: In die Untersuchung gingen insgesamt 250 Kotproben von 135 Gänsen, 45 Flugenten, 40 Mularden und 30 Pekingenten ein, die in den Jahren 2016 und 2017 von zehn verschiedenen Wassergeflügel-Betrieben in Thüringen, Deutschland, gesammelt und auf *Aliarcobacter* spp. untersucht wurden. Die mit MALDI-TOF MS und multiplex-PCR identifizierten *Aliarcobacter*-Isolate wurden anschließend mithilfe des Epsilometertest (E-Test) auf ihre antimikrobielle Empfindlichkeit gegenüber acht verschiedenen Antibiotika getestet. Nach der DNA-Isolierung und Gesamtgenom-Sequenzierung wurde die phylogenetische Verwandtschaft sowie die Anwesenheit von potentiellen Resistenz- und Virulenzgenen mit Hilfe bioinformatischer Programme untersucht.

Ergebnisse: Aus 55 *Aliarcobacter*-positiven Kotproben wurden mittels MALDI-TOF MS und multiplex-PCR 27 *A. cryaerophilus*- und 47 *A. butzleri*-Isolate identifiziert. Die hier untersuchten *A. cryaerophilus*-Stämme gehören dem Cluster I und somit dem Genomovar *A. cryaerophilus gv. pseudocryaerophilus* an. Zusätzlich wurde durch die phylogenetischen Untersuchungen deutlich, dass beide *Aliarcobacter*-Spezies eine hohe

genetische Diversität aufweisen. Gleichzeitig bildeten einige *A. cryaerophilus*- und *A. butzleri*-Isolate Untergruppen. In diesen waren die einzelnen Bakterienstämme, welche zum Teil aus unterschiedlichen Betrieben stammten, nur wenige Einzelnukleotid-Polymorphismen (SNPs) voneinander entfernt. Die Bestimmung der phänotypischen antimikrobiellen Empfindlichkeit offenbarte, dass allen 74 *Aliarcobacter*-Isolaten die Cefotaxim-Resistenz gemein war. Ferner waren mehr als drei Viertel der *Aliarcobacter*-Stämme resistent gegen Streptomycin. Die 27 *A. cryaerophilus*-Stämme zeichneten sich durch eine Empfindlichkeit gegenüber Erythromycin, Gentamicin sowie Ampicillin und somit durch ein homogenes Resistenzprofil aus. Im Gegensatz dazu zeigten die 47 *A. butzleri*-Stämme ein heterogenes Resistenzprofil. Während die *A. butzleri*-Stämme eine deutlich höhere Resistenzrate gegenüber den Tetracyclinen aufwiesen, waren die *A. cryaerophilus*-Stämme vermehrt gegen Ciprofloxacin resistent.

In beiden *Aliarcobacter*-Spezies wurde ein großes Arsenal an potentiellen Resistenzgenen identifiziert. Neben mehreren Effluxpumpen wurden verschiedene β-Laktamase-Gene sowie die bekannte Ciprofloxacin-Resistenz vermittelnde Punktmutation an Position 254 in der Chinolon-Resistenz-bestimmenden Region im *gyr*A-Gen detektiert.

Des Weiteren wurde eine Vielzahl an potentiellen Virulenzgenen in beiden *Aliarcobacter*-Spezies ermittelt. Zum Repertoire gehören unter anderem ein komplettes Chemotaxis-System, ein Urease-Cluster, mehrere Flagellum-Gene sowie ein Lipid-A-Cluster.

Schlussfolgerungen: Obwohl die untersuchten Isolate auf eine Region (Thüringen), einen bestimmten Wirtstyp (Wassergeflügel) und eine kurze Studienzeit (2 Jahre) begrenzt waren, deuten die Ergebnisse der vorliegenden Arbeit auf ein hohes Maß an genetischer Diversität unter den *A. cryaerophilus*- und *A. butzleri*-Stämmen hin. Dabei ist die Stammdiversität innerhalb beider Spezies unabhängig von der geographischen Lage, dem Ursprung der Isolate und der Wirtsart. *Aliarcobacter*-Isolate, die aus dem selben Betrieb stammen, wurden aufgrund ihrer klonalen Verwandtschaft der gleichen Untergruppe zugeordnet. Die Existenz phylogenetisch ähnlicher Stämme aus mehreren Betrieben, weist auf eine mögliche epidemiologische Verbindung zwischen den einzelnen Betrieben hin. Durch die Untersuchungen wurde deutlich, dass auf die Bestimmung der phänotypischen antimikrobiellen Empfindlichkeit zugunsten der genotypischen Resistenzbestimmung nicht verzichtet werden kann, da der Genotyp nur in einem beschränken Umfang mit der phänotypischen Charakterisierung übereinstimmt. Ein direkter Zusammenhang zwischen der Anwesenheit einer bekannten Mutation im *gyr*A-Gen und der phänotypischen Ciprofloxacin-Resistenz konnte nur bei den *A. butzleri*-Stämmen festgestellt werden. Die im Rahmen dieser Studie erstellte Resistenz- und Virulenzdatenbank, ermöglicht eine unkomplizierte Bestimmung des genotypischen Resistenz- und Virulenzprofiles von *A. butzleri*-Stämmen.

6 Summary

Eva Müller

Analyses of antibiotic resistance and virulence of *Aliarcobacter cryaerophilus* and *Aliarcobacter butzleri* isolates of water poultry from Thuringia

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Keywords: *Aliarcobacter*, antimicrobial susceptibility, resistance, virulence, whole-genome sequencing, zoonosis

Introduction: *Aliarcobacter* (*A.*) *cryaerophilus* and *Aliarcobacter butzleri* are considered worldwide as foodborne and zoonotic pathogens that are predominantly transmitted via contaminated food or water. In humans, the infection usually appears as self-limiting enteritis, whereas in animals it is associated with mastitis, abortion, and reproductive disorders in addition to gastrointestinal diseases. Due to the close relatedness to thermophilic *Campylobacter* spp. whose antimicrobial resistance has shown a strong increase in recent years, it is important to investigate the resistance and virulence profiles of these two species.

Objectives: This thesis aimed to analyze the phenotypic and genotypic antibiotic resistance and the virulence profile of *Aliarcobacter cryaerophilus* and *Aliarcobacter butzleri* isolates from fecal samples of water poultry from Thuringia.

Animals, materials and methods: A total of 250 fecal samples from 135 geese, 45 Muscovy ducks, 40 mulards, and 30 Peking ducks were collected in 2016 and 2017 from ten different water poultry farms in Thuringia, Germany, and examined for *Aliarcobacter* spp. The *Aliarcobacter* isolates identified by MALDI-TOF MS and multiplex-PCR were subsequently tested for their antimicrobial susceptibility to eight different antibiotics using the epsilometer test (E-Test). After DNA extraction and whole-genome sequencing, the phylogenetic relatedness and the presence of potential resistance and virulence genes were investigated by using bioinformatic tools.

Results: Out of 55 positive fecal samples, 27 *A. cryaerophilus* and 47 *A. butzleri* isolates were identified using MALDI-TOF MS and multiplex-PCR. The studied *A. cryaerophilus* strains belong to cluster I and thus to the genomovar *A. cryaerophilus gv. pseudocryaerophilus*. In addition, the phylogenetic analyses revealed that both *Aliarcobacter* species display a high genetic diversity. However, some of the *A. cryaerophilus* and *A. butzleri* isolates formed subgroups. In these subgroups, the individual bacterial strains, which partly originated from different farms, were only a few Single Nucleotide Polymorphisms (SNPs) apart.

The analysis of the phenotypic antimicrobial susceptibility revealed that cefotaxime resistance was common to all 74 *Aliarcobacter* isolates. Additionally, more than three quarters of the *Aliarcobacter* strains were resistant to streptomycin. The 27 *A. cryaerophilus* strains were susceptible to erythromycin, gentamicin and ampicillin, and thus had a homogeneous resistance profile. In contrast, the 47 *A. butzleri* isolates showed a heterogeneous resistance profile. While the *A. butzleri* strains presented a higher resistance rate to tetracyclines, the *A. cryaerophilus* strains were more resistant to ciprofloxacin.

A large arsenal of potential resistance genes was identified in both *Aliarcobacter* species. In addition to multiple efflux pumps, several β-lactamase genes and a known ciprofloxacin resistance-determining point mutation at position 254 in the quinolone resistance determining region in the *gyr*A gene were detected. Furthermore, a large number of potential virulence genes in both *Aliarcobacter* species were identified. The repertoire includes a complete chemotaxis system, a urease cluster, several flagellum genes and a lipid A cluster.

Conclusion: Although the studied isolates were limited to one federal state (Thuringia), a specific host type (water poultry) and a short study period (2 years), the results of the present work indicate a high degree of genetic diversity among the *A. cryaerophilus* and *A. butzleri* strains. The strain diversity within both species is independent of the geographical location, the source of isolation and the host species. *Aliarcobacter* isolates originating from the same farm were assigned to the same subgroup based on their clonal relationship. The existence of phylogenetically similar strains from different farms indicates a possible epidemiological link between those farms. It became clear from the investigations that the determination of phenotypic antimicrobial susceptibility cannot be waived in favour of genotypic resistance determination, since the genotype only corresponded to a limited extent with the phenotypic characterization. A direct correlation between the presence of a known mutation in the *gyr*A gene and the phenotypic ciprofloxacin resistance could only be found in the *A. butzleri* strains. The resistance and virulence database established in this study allows an easier determination of the genotypic resistance and virulence profiles of *A. butzleri* strains.

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8 Anhang

8.1 Supplemente zur Publikation 1

Table S1 - Assembly data of the 17 *A. cryaerophilus* genomes belonging to Cluster I available at the NCBI GenBank database

WGS	Strain	Size (bp)	Contigs	Minimum (bp)	average (bp)	Maximum (bp)	N50	CDS	rRNA	tRNA	tmRNA
NZ_CP032825.1	ATCC 49615	2055914	1	2055914	2055914	2055914	2055914	2019	15	51	1
GCF_002080085.1	AZT-1	1850477	456	501	4058	58740	10361	2072	4	35	1
GCF_001572865.1	L397	2312134	93	514	24861	117496	56051	2322	3	28	1
GCF_001572855.1	L398	2034191	65	524	31295	210632	64420	2073	4	26	1
GCF_001573015.1	L399	2100483	88	524	23869	203577	54372	2156	5	31	-
GCF_001573005.1	L400	2207754	83	628	26599	202092	54663	2201	6	30	1
GCF_001572845.1	L401	2173179	77	584	28223	187324	58920	2172	3	26	1
GCF_001572875.1	L406	2019452	63	543	32054	186396	64258	2051	3	30	1
GCF_006503595.1	123	1987555	8	2332	248444	867616	656377	1987	4	40	1
GCF_006503545.1	151	2043164	12	1061	170263	815663	435621	2059	5	40	1
GCF_006503605.1	382	2256512	58	564	38905	204567	95369	2252	3	38	1
GCF_006503615.1	938	2146078	69	568	31102	148137	75027	2223	3	41	1
GCF_006508135.1	LMG 10228	2165012	127	801	17047	138385	38970	2157	3	38	1
GCF_002993045.1	LMG 10229	2066440	27	518	76534	271901	199142	2102	5	37	1
GCF_002993065.1	LMG 9861	2023111	32	750	63222	492973	178435	2037	7	41	-
GCF_008086605.1	G18RTA	2222529	47	548	47287	266087	137498	2240	3	37	1
GCF_008086685.1	M830A	2281561	55	518	41482	395448	128419	2245	3	40	1

Table S2 - Results of the *in silico* DNA-DNA hybridisation (DDH)

Strain	LMG 10229^T (Cluster I)			LMG 9065^T (Cluster II)			LMG 24291^T (Cluster III)			LMG 10210 (Cluster IV)		
	identities / HSP length		Difference in % G+C *	identities / HSP length		Difference in % G+C *	identities / HSP length		Difference in % G+C *	identities / HSP length		Difference in % G+C *
	Distance	DDH estimate		Distance	DDH estimate		Distance	DDH estimate		Distance	DDH estimate	
16CS0366-1-AR-1	0.0291	75.30% [72.3 - 78.1%]	0.18	0.0721	49.80% [47.2 - 52.4%]	0.19	0.0721	49.80% [47.2 - 52.4%]	0.07	0.0494	61.30% [58.4 - 64.1%]	0.42
16CS0366-1-AR-2	0.0291	75.30% [72.3 - 78.1%]	0.17	0.0720	49.80% [47.2 - 52.5%]	0.18	0.0721	49.80% [47.2 - 52.4%]	0.07	0.0494	61.30% [58.4 - 64.1%]	0.41
16CS0366-1-AR-3	0.0291	75.30% [72.3 - 78.1%]	0.17	0.0721	49.80% [47.2 - 52.4%]	0.19	0.0719	49.90% [47.2 - 52.5%]	0.07	0.0494	61.30% [58.4 - 64.1%]	0.41
16CS0366-1-AR-4	0.0291	75.30% [72.3 - 78%]	0.17	0.0721	49.80% [47.2 - 52.4%]	0.18	0.0721	49.80% [47.2 - 52.4%]	0.07	0.0493	61.30% [58.5 - 64.1%]	0.41
16CS0369-1-AR-1	0.0291	75.30% [72.3 - 78%]	0.17	0.0720	49.80% [47.2 - 52.5%]	0.19	0.0721	49.80% [47.2 - 52.4%]	0.07	0.0494	61.30% [58.4 - 64.1%]	0.41
16CS0369-1-AR-4	0.0290	75.30% [72.3 - 78.1%]	0.01	0.0719	49.90% [47.3 - 52.5%]	0.02	0.0718	49.90% [47.3 - 52.5%]	0.09	0.0494	61.30% [58.5 - 64.1%]	0.25
16CS0814-1	0.0315	73.40% [70.4 - 76.2%]	0.04	0.0734	49.20% [46.6 - 51.9%]	0.03	0.0740	49.00% [46.4 - 51.6%]	0.14	0.0503	60.80% [57.9 - 63.5%]	0.20
16CS0830-1	0.0299	74.70% [71.7 - 77.5%]	0.05	0.0721	49.80% [47.2 - 52.4%]	0.03	0.0727	49.60% [46.9 - 52.2%]	0.15	0.0497	61.10% [58.3 - 63.9%]	0.19
16CS0847-1	0.0296	74.90% [71.9 - 77.7%]	0.04	0.0721	49.80% [47.2 - 52.4%]	0.02	0.0727	49.60% [46.9 - 52.2%]	0.14	0.0493	61.40% [58.5 - 64.2%]	0.20
16CS0847-2	0.0297	74.80% [71.8 - 77.6%]	0.03	0.0724	49.70% [47 - 52.3%]	0.04	0.0731	49.40% [46.8 - 52%]	0.07	0.0498	61.00% [58.2 - 63.8%]	0.27
16CS0847-4	0.0297	74.80% [71.8 - 77.6%]	0.01	0.0724	49.70% [47 - 52.3%]	0.01	0.0730	49.40% [46.8 - 52%]	0.11	0.0498	61.10% [58.2 - 63.9%]	0.23
16CS0847-5	0.0298	74.70% [71.7 - 77.5%]	0.05	0.0722	49.70% [47.1 - 52.4%]	0.04	0.0727	49.60% [46.9 - 52.2%]	0.16	0.0496	61.20% [58.3 - 64%]	0.19
16CS0847-6	0.0297	74.80% [71.8 - 77.6%]	0.03	0.0723	49.70% [47.1 - 52.3%]	0.04	0.0731	49.40% [46.8 - 52%]	0.07	0.0499	61.00% [58.2 - 63.8%]	0.27
16CS1043-1	0.0295	75.00% [72 - 77.8%]	0.06	0.0720	49.80% [47.2 - 52.5%]	0.05	0.0732	49.30% [46.7 - 52%]	0.17	0.0505	60.60% [57.8 - 63.4%]	0.18
16CS1285-3	0.0320	73.00% [70 - 75.9%]	0.18	0.0740	49.00% [46.4 - 51.6%]	0.19	0.0738	49.10% [46.5 - 51.7%]	0.07	0.0512	60.30% [57.4 - 63%]	0.42
16CS1285-4	0.0294	75.00% [72 - 77.8%]	0.26	0.0717	50.00% [47.3 - 52.6%]	0.25	0.0720	49.90% [47.2 - 52.5%]	0.37	0.0452	63.80% [60.9 - 66.7%]	0.02
16CS1290-1	0.0298	74.80% [71.8 - 77.6%]	0.03	0.0724	49.70% [47 - 52.3%]	0.04	0.0731	49.40% [46.8 - 52%]	0.08	0.0499	61.00% [58.2 - 63.8%]	0.27
16CS1292-3	0.0300	74.60% [71.6 - 77.4%]	0.02	0.0726	49.60% [47 - 52.2%]	0.01	0.0731	49.40% [46.8 - 52%]	0.13	0.0498	61.00% [58.2 - 63.8%]	0.22
16CS1292-4	0.0297	74.80% [71.8 - 77.6%]	0.13	0.0722	49.80% [47.1 - 52.4%]	0.11	0.0728	49.50% [46.9 - 52.1%]	0.23	0.0497	61.10% [58.2 - 63.9%]	0.11
17CS0830-1	0.0298	74.70% [71.7 - 77.5%]	0.07	0.0737	49.10% [46.5 - 51.7%]	0.06	0.0745	48.80% [46.2 - 51.4%]	0.18	0.0516	60.00% [57.2 - 62.8%]	0.17
17CS0996-A	0.0296	74.90% [71.9 - 77.7%]	0.05	0.0718	49.90% [47.3 - 52.6%]	0.04	0.0730	49.40% [46.8 - 52%]	0.16	0.0501	60.90% [58 - 63.7%]	0.19
17CS0996-B	0.0296	74.90% [71.9 - 77.7%]	0.05	0.0718	49.90% [47.3 - 52.6%]	0.04	0.0730	49.40% [46.8 - 52%]	0.15	0.0501	60.90% [58 - 63.7%]	0.19
17CS1055-A	0.0301	74.50% [71.5 - 77.3%]	0.02	0.0722	49.80% [47.2 - 52.4%]	0.03	0.0738	49.10% [46.5 - 51.7%]	0.09	0.0504	60.70% [57.8 - 63.5%]	0.26
17CS1055-B	0.0318	73.20% [70.2 - 76%]	0.08	0.0748	48.70% [46.1 - 51.3%]	0.09	0.0743	48.90% [46.3 - 51.5%]	0.03	0.0507	60.50% [57.7 - 63.3%]	0.32
17CS1061	0.0269	77.00% [74 - 79.8%]	0.08	0.0698	50.80% [48.2 - 53.4%]	0.07	0.0706	50.50% [47.8 - 53.1%]	0.19	0.0450	64.00% [61.1 - 66.8%]	0.16
17CS1201-1	0.0298	74.70% [71.7 - 77.5%]	0.24	0.0719	49.90% [47.3 - 52.5%]	0.23	0.0722	49.80% [47.2 - 52.4%]	0.34	0.0489	61.60% [58.7 - 64.4%]	0.00
17CS1201-2	0.0290	75.30% [72.3 - 78.1%]	0.03	0.0714	50.10% [47.5 - 52.7%]	0.05	0.0709	50.30% [47.7 - 53%]	0.07	0.0447	64.20% [61.2 - 67%]	0.27

Table S3 - Results of the MLST analysis of all 44 *A. cryaerophilus* genomes

Strain	Cluster	*aspA*	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST
16CS0366-1-AR-1	I	-	233	-	173	-	345	99	-
16CS0366-1-AR-2	I	-	233	-	173	-	345	99	-
16CS0366-1-AR-3	I	-	233	-	173	-	345	99	-
16CS0366-1-AR-4	I	-	233	-	173	-	345	99	-
16CS0369-1-AR-1	I	-	233	-	173	-	345	99	-
16CS0369-1-AR-4	I	-	233	-	173	-	345	99	-
16CS0814-1	I	288	81	-	93	-	-	-	-
16CS0830-1	I	251	79	64	133	-	384	203	-
16CS0847-1	I	96	78	70	72	-	-	-	-
16CS0847-2	I	96	78	70	72	-	-	-	-
16CS0847-4	I	96	78	70	72	-	-	-	-
16CS0847-5	I	96	78	70	72	-	-	-	-
16CS0847-6	I	96	78	70	72	-	-	-	-
16CS1043-1	I	-	232	-	72	-	-	-	-
16CS1285-3	I	-	81	-	73	-	-	-	-
16CS1285-4	I	-	-	-	-	-	-	-	-
16CS1290-1	I	96	78	70	72	-	-	-	-
16CS1292-3	I	96	78	70	72	-	-	-	-
16CS1292-4	I	96	78	70	72	-	-	-	-
17CS0830-1	I	-	-	64	-	-	-	144	-
17CS0996-A	I	-	-	62	212	-	284	115	-
17CS0996-B	I	-	-	62	212	-	284	115	-
17CS1055-A	I	-	79	104	72	-	-	-	-
17CS1055-B	I	-	81	-	71	273, 273	163	119	-
17CS1061	I	-	-	67	87	-	143	-	-
17CS1201-1	I	96	78	70	72	-	-	-	-
17CS1201-2	I	-	-	-	-	-	-	-	-
AZT-1	I	-	-	66	n.p.	-	-	115	-
L397	I	-	190	140	-	n.p.	-	259	-
L398	I	228	167	145	153	-	258	211	-
L399	I	-	-	-	-	-	-	-	-
L400	I	-	92	-	-	-	-	279	-
L401	I	220	179	-	153	501, 501, 501, 501	-	265	-
L406	I	251	81	61	114	-	-	115	-
123	I	-	88	194	93	-	-	145	-
151	I	252	84	104	93	-	222	232	-
382	I	215	152	59	149	224	133	-	-
938	I	-	-	-	n.p.	-	-	-	-
LMG 10228	I	-	90	155	-	-	-	-	-
LMG 10229	I	110	85	67	87	-	158	120	-
LMG 9861	I	100	74	90	80	-	149	111	-
ATCC 49615	I	86	79	61	71	21, 265	136	98	-
G18RTA	I	-	-	-	-	-	-	-	-
M830A	I	-	-	61	193	-	133	151	-

Table S4 - Summary of mobile elements proteins and CRISPR for all tested genomes of cluster I

					Insertion sequences																				
					Insertion sequences families																				
Strain	Clusters	subgroups (German strains)	Circularized genome	Genome Length	IS1182	IS21	IS256	IS5	IS1380	IS1634	IS3	IS630	IS701	IS200/IS605	IS481	IS110	IS1595	IS4	IS982	IS1	new	Total number	Total length	Percentage
17CS1201-2	I			2,194,174		1				1	1	1				2						6	8,816	0.4
16CS1285-4	I		○	2,136,856		12	5		16	11		4		1		11					3	63	103,425	4.63
LMG 9861	I			2,023,111		3	1		1	4		3				1						13	20,669	1.02
938	I			2,146,078	2	4				1	3		1	1							2	14	18,273	0.85
17CS1061	I			1,964,531		1					1											2	3,894	0.2
LMG 10229	I			2,066,440		1	4			1	2		1	2								11	1,432	0.84
G18RTA	I			2,222,529		1															2	3	7,957	0.35
L399	I			2,100,483		2	6				3		3			1						15	22,006	1.05
L398	I			2,034,191		1	11						1									13	18,265	0.9
L401	I			2,173,179		1	14				1											16	17,568	0.81
16CS1285-3	I			2,354,421		3				3	1	1	1	1								10	18,127	0.77
LMG 10228	I			2,165,012		9	8	4	2	1	1	2				2					1	30	39,644	1.83
17CS1055-B	I			2,115,336		1	3															4	7,862	0.37
16CS0814-1	I			2,064,630		1	2		1	1	1	1	1									8	14,315	0.69
L397	I			2,312,134		1	1	4		2	1					7					6	22	53,021	2.29
AZT-1	I			1,850,477		5	2	2		2			1				1	1			1	15	26,123	1.41
16CS1043-1	I			2,086,934		4	1	1	1	1	3				1							12	19,973	0.95
L400	I			2,207,754		3					2											5	7,095	0.32
16CS0830-1	I		○	2,054,162		2		3	1	3	3			1								13	19,276	0.88
382	I			2,256,512		1	12	2		1	5					1	1					23	30,079	1.33
17CS0830-1	I			2,089,083		1		3	1		1				1							7	7,961	0.38
L406	I			2,019,452		2	1	1													1	5	9,302	0.46
123	I			1,987,555		1	1															2	2,875	0.14
M830A	I			2,281,561		4	8	1	1	3						1						18	21,075	0.89
17CS1055-A	I			2,156,610		1																1	1,889	0.09
17CS0996-A	I	iii		2,020,856		1																1	1,358	0.07
17CS0996-B	I	iii		2,019,245		1																1	1,358	0.07
16CS0366-1-AR-1	I	i		2,146,246	1	3	2	1														7	8,210	0.38
16CS0366-1-AR-2	I	i		2,145,280	1	3	2	1														7	11,092	0.52
16CS0366-1-AR-3	I	i		2,146,099	1	3	2	1														7	10,257	0.48
16CS0366-1-AR-4	I	i		2,144,077	1	3	2	1														7	8,660	0.4
16CS0369-1-AR-1	I	i		2,145,030	1	3	2	1														7	7,693	0.36
16CS0369-1-AR-4	I	i	○	2,021,348	1	3	2	2														8	12,365	0.57
151	I			2,043,164		1																1	1,889	0.09
ATCC 49615	I		○	2,055,914		1		1														2	2,884	0.14
17CS1201-1	I	ii		1,997,506		3		1			2				1							7	10,949	0.55
16CS0847-5	I	ii		2,083,468		3		1			2				1							7	13,571	0.65
16CS0847-1	I	ii		2,201,539		3		1			2				1						1	8	12,654	0.57
16CS0847-2	I	ii		2,110,837		3		1			2				1						1	8	12,927	0.61
16CS0847-4	I	ii		2,112,744		3		1			2				1	1					1	9	14,361	0.68
16CS0847-6	I	ii		2,110,907		3		1			2				1						1	8	13,275	0.63
16CS1290-1	I	ii		2,112,169		3		1			2				1						1	8	12,541	0.59
16CS1292-3	I	ii		2,143,554	1	3	3	1			2				1	1					1	13	22,063	1.03
16CS1292-4	I	ii	○	2,020,388		7		1			3		1		1						1	14	29,831	1.38

Fortsetzung **Table S4**

	Genomic Islands					
Strain	**Nr. Genomic Islands (GI)**	**Total size of the genomic islands GIs**	**Minimum genomic islands (GI) size per genome**	**Maximum genomic islands (GI) size per genome**	**Average genomic islands (GI) size per genome**	**Percentage**
17CS1201-2	9	131,979	4,115	34,114	14,664.33	60.15
16CS1285-4	15	223,420	4,108	33,005	14,894.67	104.56
LMG 9861	5	130,489	22,150	30,565	26,097.8	64.49
938	7	108,322	4,037	64,312	15,474.57	50.47
17CS1061	4	70,851	13,988	20,850	17,712.75	36.06
LMG 10229	4	80,308	5,400	38,198	20,077	38.86
G18RTA	4	71,189	14,326	21,758	17,797.25	32.03
L399	4	90,956	10,428	36,110	22,739	43.30
L398	4	78,097	11,828	30,212	19,524.25	38.39
L401	4	168,446	5,646	142,785	42,111.5	77.51
16CS1285-3	6	116,481	5,726	40,480	19,413.5	49.47
LMG 10228	10	205,340	6,334	75,665	20,534	94.84
17CS1055-B	3	29,348	4,019	18,345	9,782.67	13.87
16CS0814-1	3	30,088	4,745	13,124	110,029.33	14.57
L397	4	238,201	7,503	200,610	59,550.25	103.02
AZT-1	2	90,654	8,461	82,193	45,327	48.98
16CS1043-1	1	50,725				24.30
L400	6	239,194	10,513	79,934	39,865.67	108.34
16CS0830-1	8	136,276	4,282	32,462	17,034.5	66.34
382	10	201,281	5,330	39,046	20,128.1	89.20
17CS0830-1	4	75,686	5,760	35,528	18,921.5	36.22
L406	3	64,738	9,698	29,600	21,579.33	32.05
123	2	35,701	12,601	23,100	17,850.5	17.96
M830A	6	154,882	5,353	52,770	25,813.67	67.88
17CS1055-A	4	61,498	10,347	25,371	15,374.5	28.51
17CS0996-A	2	32,829	4,033	28,796	16,414.5	16.24
17CS0996-B	2	32,829	4,033	28,796	16,414.5	16.25
16CS0366-1-AR-1	6	63,451	8,249	35,521	10,575	29.56
16CS0366-1-AR-2	5	95,838	8,249	28,762	19,167.6	44.67
16CS0366-1-AR-3	5	105,320	8,249	48,419	21,064	49.07
16CS0366-1-AR-4	4	148,607	8,249	34,436	37,151.75	69.31
16CS0369-1-AR-1	5	159,486	8,249	75,684	31,897.2	74.35
16CS0369-1-AR-4	4	140,209	8,249	99,655	35,052.25	69.36
151	3	66,199	9,346	40,745	22,066.33	32.4
ATCC 49615	6	77,561	7,077	20,189	12,926.83	37.72
17CS1201-1	6	266,216	4,096	228,419	44,369.33	133.27
16CS0847-5	8	192,158	4,517	98,338	24,019.75	92.22
16CS0847-1	6	176,559	7,350	84,022	29,426.5	80.19
16CS0847-2	7	138,240	4,637	41,243	19,748.57	65.49
16CS0847-4	6	149,019	7,350	56,717	24,836.5	70.53
16CS0847-6	7	122,186	4,637	44,653	17,455.14	57.88
16CS1290-1	9	177,702	5,362	52,968	19,744.67	84.13
16CS1292-3	7	151,869	8,231	43,535	21,695.57	70.84
16CS1292-4	6	124,266	4,537	65,506	20,711	61.5

Fortsetzung **Table S4**

Strain	Integrated prophages: Active phages: Clostridium phage phi3626	Flavobacterium phage FpV4	Acinetobacter phage LZ35	Aeromonas phage 44RR2.8t.2	Sphingomonas phage PAU	Flavoibacterium phage FCV-20	Clostridium phage	Lactobacillus phage phiadh	Aureococcus anophagefferens	Idiomarinaceae phage Phi1M2-	Escherichia phage	Thermus phage phiYS40	Thermus phage phi OH2	Salicola phage SCTP-2	Enterococcus phage EFRM31	Enterobacteria phage HK630	Salmonella phage SJ46	Helicobacter phage UKEN32U	Pseudomonas phage JBD44	Erwinia phage	Wolbachia phage WO	Clostridium phage phiCD505	Clostridium phage CDSH1	Listeria phage LMTA-34	Length of predicted phages	Total number of predicted phages (prophage_hunter)
17CS1201-2										1	1														16,752; 23,685	2
16CS1285-4							1																		14,294	1
LMG 9861																										0
938												1													19,897	1
17CS1061																										0
LMG 10229													1	1											1,5679; 15,526	2
G18RTA															1	1									15,633; 10,007	2
L399																										0
L398											1						1								21,141; 23,006	2
L401									1									1							12,377; 17,708	2
16CS1285-3					1	1																			21,988; 25,960	2
LMG 10228																										0
17CS1055-B									1																16,617	1
16CS0814-1																										0
L397																										0
AZT-1																										0
16CS1043-1				1																					18,114	1
L400																			1						17,542	1
16CS0830-1		1																							21,317	1
382												1													17,572	1
17CS0830-1																										0
L406																				1					11,789	1
123																										0
M830A																1									22,631	1
17CS1055-A								1																	27,709	1
17CS0996-A																										0
17CS0996-B																										0
16CS0366-1-AR-1	1																								41,104	1
16CS0366-1-AR-2	1																								32,759	1
16CS0366-1-AR-3	1																								41,104	1
16CS0366-1-AR-4	1																								32,759	1
16CS0369-1-AR-1	1																								41,104	1
16CS0369-1-AR-4	1																								32,758	1
151																					2				28,675; 29,029	2
ATCC 49615																										0
17CS1201-1																										0
16CS0847-5																										0
16CS0847-1																										0
16CS0847-2			1																						20,896	1
16CS0847-4																										0
16CS0847-6			1																						11,204	1
16CS1290-1			1																						11,204	1
16CS1292-3			1																						11,204	1
16CS1292-4																										0

Fortsetzung **Table S4**

	CRISPR		
Strain	**Nr. of CRISPR**	**Nr. of spacers**	**CRISPR repeat sequence**
17CS1201-2	0		
16CS1285-4	0		
LMG 9861	0		
938	3	2; 5; 27	AATAATCGGGTCAATGTTGTAAT; GTCTTAATCCCCTTAAATCGGGTCATATGTATTGAAT; CCCCTACTCTAAAGCAAAATACGATACAAT
17CS1061	1	4	GTCTTAATCCCCTTAAATCGGGTCATATGTATTGAAT
LMG 10229	0		
G18RTA	1	22	ATTATATCAAATGGGGATTTGAGAGTAGATTAAAAC
L399	0		
L398	0		
L401	0		
16CS1285-3	1	51	ATTGTATCGTATTTTGCTTTAGAGTAGGGGTCTAAT
LMG 10228	0		
17CS1055-B	1	12	GCGACACTTTACAACAATATCGCTTAGCTAATGAAAC
16CS0814-1	1	17	GCGACACTTTACAACAATATCGCTTAGCTAATGAAAC
L397	0		
AZT-1	0		
16CS1043-1	0		
L400	0		
16CS0830-1	1	3	GTCTTAATCCCCTTAAATCGGGTCATATGTATTGAAT
382	0		
17CS0830-1	1	4	TTACATTTCCAGTAGCATATGAGTTTTCAATTGTTCCA
L406	0		
123	0		
M830A	1	20	GTCTTAATCCCCTTAAATCGGGTCATATGTATTGAAT
17CS1055-A	0		
17CS0996-A	0		
17CS0996-B	0		
16CS0366-1-AR-1	1	10	GCGACACTTTACAACAATATCGCTTAGCTAATGAAAC
16CS0366-1-AR-2	1	10	GCGACACTTTACAACAATATCGCTTAGCTAATGAAAC
16CS0366-1-AR-3	1	10	GTTTCATTAGCTAAGCGATATTGTTGTAAAGTGTCGC
16CS0366-1-AR-4	1	10	GCGACACTTTACAACAATATCGCTTAGCTAATGAAAC
16CS0369-1-AR-1	1	10	GTTTCATTAGCTAAGCGATATTGTTGTAAAGTGTCGC
16CS0369-1-AR-4	1	10	GTTTCATTAGCTAAGCGATATTGTTGTAAAGTGTCGC
151	0		
ATCC 49615	0		
17CS1201-1	0		
16CS0847-5	0		
16CS0847-1	0		
16CS0847-2	0		
16CS0847-4	0		
16CS0847-6	0		
16CS1290-1	0		
16CS1292-3	0		
16CS1292-4	0		

Table S5 - Part of the *gyr*A-gene showing the point mutation (Thr-85-Ile)

	***gyr*A-sequence**	
strain	**nucleotide sequence***	**amino acid sequence°**
16CS0366-1-AR-1	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0366-1-AR-2	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0366-1-AR-3	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0366-1-AR-4	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0369-1-AR-1	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0369-1-AR-4	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0814-1	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
16CS0830-1	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0847-1	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0847-2	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0847-4	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0847-5[#]	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS0847-6	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS1043-1	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
16CS1285-3	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
16CS1285-4	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
16CS1290-1	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS1292-3	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
16CS1292-4	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
17CS0830-1	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
17CS0996-A	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
17CS0996-B	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
17CS1055-A	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
17CS1055-B	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
17CS1061	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val
17CS1201-1[#]	TACCATCCACATGGAGATATTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp **Ile** Ser Val Tyr Asp Ala Leu Val
17CS1201-2	TACCATCCACATGGAGATACTTCTGTGTATGATGCACTTGTA	Tyr His Pro His Gly Asp Thr Ser Val Tyr Asp Ala Leu Val

* from position 235 to 276; the red-colored 'T' indicates the mutation at position 254

° from positon 79 to 92; the green-colored 'Ile' indicates the result of the mutation at position 85

[#] phenotypically susceptible to ciprofloxacin

Table S6 - Distribution of the genes with known function found in the four plasmids

Product	Distribution			
	16CS0369-1-AR-4	16CS0830-1	16CS1285-4	16CS1292-4
AAA ATPase, central region			x	
ADP-ribose pyrophosphatase (EC 3.6.1.13)			x	
Alkyl hydroperoxide reductase protein C (EC 1.11.1.15)			x	
ATP-dependent Clp protease proteolytic subunit ClpP (EC 3.4.21.92)			x	
ATP-dependent DNA helicase pcrA (EC 3.6.1.-)				x
ATP-dependent DNA helicase RecG-related protein			x	
ATPase (AAA+ superfamily)			x	
ATPase component BioM of energizing module of biotin ECF transporter			x	
Bacteriocin/lantibiotic efflux ABC transporter, permease/ATP-binding protein	x			
Chromosome (plasmid) partitioning protein ParA / Sporulation initiation inhibitor protein Soj	x	x	x	x
Chromosome (plasmid) partitioning protein ParB / Stage 0 sporulation protein J	x	x	x	x
CopG protein			x	
Deoxyguanosinetriphosphate triphosphohydrolase (EC 3.1.5.1), subgroup 1	x	x		
DNA-invertase			x	
DNA-binding protein HU				x
Ethidium bromide-methyl viologen resistance protein EmrE		x		
Exonuclease SbcC	x	x		x
Exonuclease SbcD	x	x		x
Fic domain protein, PA0574 type	x	x		x
Fic family protein			x	
FIG000605: protein co-occurring with transport systems (COG1739)		x		
FIG061771: ATP-dependent nuclease subunit A			x	
General secretion pathway protein D / Type II secretion outermembrane pore forming protein (PulD)	x			
General secretion pathway protein G	x			
GTP pyrophosphokinase (EC 2.7.6.5)	x	x		
Helicase, C-terminal:Type III restriction enzyme, res subunit:DEAD/DEAH box helicase, N-terminal	x			
Helix-turn-helix domain protein	x		x	
HipA domain-containing protein			x	
HipB protein @ Antitoxin HigA			x	
Integrase-recombinase protein XERCD family			x	
ISMca7, transposase		x		
IS1478 transposase	x			
Mobile element protein	x	x	x	x
Multidrug efflux system, inner membrane proton/drug antiporter (RND type) => CmeB		x		
Multidrug efflux system, inner membrane proton/drug antiporter (RND type) => CmeF	x			
Multidrug efflux system, membrane fusion component => CmeA		x		
Multidrug efflux system, membrane fusion component => CmeE	x			
Multidrug efflux system, outer membrane factor lipoprotein => CmeC		x		
Multidrug efflux system, outer membrane factor lipoprotein => CmeD	x			
nucleotidyltransferase family protein				x
Patatin				x
pathogenesis related protein				x
Phage integrase	x			
Phage integrase, site-specific serine recombinase			x	
Phage protein		x		x
Predicted transcriptional regulator containing an HTH domain and an uncharacterized domain shared with the mammalian protein Schlafen		x		x
Programmed cell death toxin YdcE			x	
Protein ygiW precursor			x	
putative ATP-dependent helicase	x			
Putative DNA helicase	x			
Putative DNA-binding protein in cluster with Type I restriction-modification system			x	
Putative predicted metal-dependent hydrolase			x	
Putative two-component response regulator	x			
Replicative DNA helicase (DnaB) (EC 3.6.4.12)	x	x	x	x
Resolvase	x	x		x
Sensory histidine kinase QseC			x	
Serine hydroxymethyltransferase (EC 2.1.2.1) / H4MPt-dependent SHMT	x			
Site-specific tyrosine recombinase	x	x		x

Fortsetzung **Table S6**

Product	Distribution			
	16CS0369-1-AR-4	16CS0830-1	16CS1285-4	16CS1292-4
Streptomycin 3''-O-adenylyltransferase (EC 2.7.7.47) @ Spectinomycin 9-O-adenylyltransferase				x
Transcriptional regulator, YafY family			x	
Transposase	x	x		x
Transposase, IS4 family	x			
tRNA-Leu-GAG	x			
tRNA-Leu-GAG	x			
Two-component system sensor histidine kinase	x	x		x
Two-component system response regulator QseB			x	
Type I restriction-modification system, DNA-methyltransferase subunit M (EC 2.1.1.72)		x	x	x
Type I restriction-modification system, restriction subunit R (EC 3.1.21.3)		x	x	x
Type I restriction-modification system, specificity subunit S		x	x	x
Type II restriction enzyme, methylase subunits	x			
Type IV fimbrial assembly, ATPase PilB	x			
Uncharacterized protein BT3327			x	
Uncharacterized protein yjiK			x	
UvrD/REP helicase			x	

Table S7 - Appearance and coverage of the virulence genes extracted from the plasmid of an *A. cryaerophilus* strain from New Zealand (BankIt2207814 M830MA_plasmid MK715471) in all strains tested in this study

Product	Strain	Coverage (%)	Identity (%)
WP_105918093.1 2-component system response regulator	G18RTA	56.57	97.96
7TMR-DISM-7TM/7TMR-DISMED2 domain-containing signal transduction protein	16CS0366-1-AR-1	55.83; 31.36	97.84; 99.30
	16CS0366-1-AR-3	31.36; 31.36	97.91; 99.30
	16CS0369-1-AR-4	55.83; 31.36	98.72; 97.91
WP_105918342.1 ATP-binding protein A_cry	M830A	46.60	100.00
WP_066152788.1 DUF2779 domain-containing protein A_cry	16CS0366-1-AR-1	98.31	90.97
	16CS0366-1-AR-2	98.31	90.97
	16CS0366-1-AR-3	98.31	90.97
	16CS0366-1-AR-4	98.31	90.97
	16CS0369-1-AR-1	98.31	90.97
	16CS0369-1-AR-4	98.31	90.97
	17CS1061	99.39	89.66
	L397	98.31	90.97
	L400	99.53	89.11
	G18RTA	98.31	91.80
WP_105917898.1 filamentous hemagglutinin domain-containing protein	16CS0830-1	72.09	89.48
	16CS0847-1	72.01	88.22
	16CS0847-2	72.01	88.22
	16CS0847-4	72.01	88.22
	16CS0847-5	72.01	88.22
	16CS0847-6	72.01	88.22
	16CS1043-1	43.02	93.04
	16CS1290-1	72.01	88.22
	16CS1292-3	72.01	88.22
	16CS1292-4	72.01	88.22
	17CS0830-1	53.37	90.61
	17CS0996-A	48.55	86.82
	17CS0996-B	48.55	86.82
	ATCC 49615	70.46	88.73
WP_105918336.1 integrase A_cry	L397	100.00	96.17

8.2 Supplemente zur Publikation 2

Table S1 - Genomes used in this study

WGS	Strain	Species	Accession	BioSample	BioProject
SRX6935305	16CS0817-2	*Aliarcobacter butzleri*	GCA_009761295.1	SAMN12886735	PRJNA575341
SRX6935306	16CS0821-2	*Aliarcobacter butzleri*	GCA_009761305.1	SAMN12886737	PRJNA575341
NC_009850.1	RM4018	*Aliarcobacter butzleri*	GCF_000014025.1	SAMN02604276	PRJNA224116
NZ_CABVQY010000001.1	Ab_4511	*Aliarcobacter butzleri*	GCF_902500435.1	SAMEA5956920	PRJEB34441
NZ_CP041386.1	ED-1	*Aliarcobacter butzleri*	GCF_007005625.1	SAMN12228411	PRJNA553057
NZ_CP040507.1	JV22	*Aliarcobacter butzleri*	GCF_005886055.1	SAMN00210774	PRJNA543187
NZ_QXNB01000010.1	6V	*Aliarcobacter butzleri*	GCF_004283115.1	SAMN09983781	PRJNA489609
NZ_QXMK01000010.1	55	*Aliarcobacter butzleri*	GCF_004283125.1	SAMN09982548	PRJNA489574
NC_021878.1	7h1h	*Aliarcobacter butzleri*	GCF_000215345.2	SAMN02604193	PRJNA67167
NZ_JAIX01000001.1	L350	*Aliarcobacter butzleri*	GCF_001010665.1	SAMN02581418	PRJNA233527
NZ_JAIS01000001.1	L351	*Aliarcobacter butzleri*	GCF_001010615.1	SAMN02581419	PRJNA233527
NZ_JAIT01000001.1	L352	*Aliarcobacter butzleri*	GCF_001010675.1	SAMN02581420	PRJNA233527
NZ_JAIV01000001.1	L354	*Aliarcobacter butzleri*	GCF_001010595.1	SAMN02581422	PRJNA233527
NZ_CP032823.1	ATCC 43158	*Aliarcobacter cryaerophilus*	GCF_003660105.1	SAMN03737948	PRJNA224116
NZ_CP031367.1	LMG 25534	*Aliarcobacter trophiarum*	GCF_003355515.1	SAMN03737959	PRJNA224116
NC_002163.1	NCTC 11168	*Campylobacter jejuni* subsp. *jejuni*	GCF_000009085.1	SAMEA1705929	PRJNA8
NZ_LS483488.1	NCTC 11637	*Helicobacter pylori*	GCF_900478295.1	SAMEA3178013	PRJNA224116

Table S2 - Results of the ANI analysis

Strain	NCTC 11168	NCTC 11637	ATCC 43158	LMG 25534	16CS0817-2	6V	L351	ED-1	L352	L350	Ab_4511	JV22	7h1h	RM4018	55	16CS0821-2	L354
***C. jejuni* NCTC 11168**	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***H. pylori* NCTC 11637**	64.58	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***A. cryaerophilus* ATCC 43158**	66.09	63.73	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
***A. trophiarum* LMG 25534**	66.00	63.78	85.34	100	-	-	-	-	-	-	-	-	-	-	-	-	-
***A. butzleri* 16CS0817-2**	65.97	63.31	78.17	77.22	100	-	-	-	-	-	-	-	-	-	-	-	-
***A. butzleri* 6V**	65.89	63.26	77.91	77.15	97.22	100	-	-	-	-	-	-	-	-	-	-	-
***A. butzleri* l351**	66.11	63.59	78.01	77.21	97.16	97.58	100	-	-	-	-	-	-	-	-	-	-
***A. butzleri* ED-1**	66.12	63.84	78.05	77.29	97.16	97.63	98.36	100	-	-	-	-	-	-	-	-	-
***A. butzleri* l352**	66.19	63.78	78.23	77.38	98.25	97.27	97.17	97.28	100	-	-	-	-	-	-	-	-
***A. butzleri* l350**	65.87	63.01	77.99	77.19	97.14	97.55	99.99	98.36	97.18	100	-	-	-	-	-	-	-
***A. butzleri* Ab_4511**	65.87	63.98	78.05	77.25	97.64	97.41	97.21	97.31	97.65	97.22	100	-	-	-	-	-	-
***A. butzleri* JV22**	66.25	63.87	78.11	77.32	97.61	97.57	97.29	97.41	97.72	97.28	97.70	100	-	-	-	-	-
***A. butzleri* 7h1h**	66.33	63.76	78.13	77.31	98.28	97.26	97.07	97.23	98.33	97.10	97.58	97.67	100	-	-	-	-
***A. butzleri* RM4018**	66.03	63.70	78.18	77.23	97.40	97.76	97.37	97.53	97.46	97.37	97.54	97.79	97.43	100	-	-	-
***A. butzleri* 55**	65.75	63.08	77.88	77.05	97.32	98.05	97.46	97.44	97.36	97.47	97.33	97.58	97.29	97.71	100	-	-
***A. butzleri* 16CS0821-2**	65.97	63.32	78.14	77.31	97.81	97.29	96.97	97.13	97.89	96.97	97.79	97.84	97.79	97.53	97.28	100	-
***A. butzleri* l354**	65.92	63.11	78.07	77.24	98.19	97.37	97.23	97.33	98.23	97.22	97.68	97.51	98.42	97.39	97.43	97.96	100

Table S3 - Presence and absence of AMR genes in the *A. butzleri* strains 16CS0817-2 and 16CS0821-2

	gene	alias*	Prokka locus	Prokka annotation	RefSeq locus	RefSeq annotation	16CS0817-2	16CS0821-2
Efflux pumps-related genes	*mdtB1*	EP1	RM4018p_04610	Multidrug resistance protein MdtB	ABU_RS02300	efflux RND transporter permease subunit		
	czcB		RM4018p_04620	Cobalt-zinc-cadmium resistance protein CzcB	ABU_RS02305	Transporter		
	-		RM4018p_04630	Hypothetical protein	ABU_RS02310	TolC family protein		
	fadR		RM4018p_04640	Fatty acid metabolism regulator protein	ABU_RS02315	TetR/AcrR family transcriptional regulator		
	yajR	EP2	RM4018p_05880	Inner membrane transport protein YajR	ABU_RS02930	MFS transporter		
	ohrR	-	RM4018p_06000	Organic hydroperoxide resistance transcriptional regulator ohrR	ABU_RS02990	MarR family transcriptional regulator		
	-	EP3	RM4018p_07090	Hypothetical protein	ABU_RS03530	TolC family protein		
	macA1		RM4018p_07100	Macrolide export protein MacA	ABU_RS03535	Efflux RND transporter periplasmic adaptor subunit		
	yknY		RM4018p_07110	putative ABC transporter ATP-binding protein YknY	ABU_RS03540	ABC transporter ATP-binding protein		
	macB2		RM4018p_07120	Macrolide export ATP-binding/permease protein MacB	ABU_RS03545	FtsX-like permease family protein		
	kstR2	-	RM4018p_08180	HTH-type transcriptional repressor KstR2	ABU_RS04070	TetR/AcrR family transcriptional regulator		
	bepE	EP4	RM4018p_08190	Efflux pump membrane transporter BepE	ABU_RS04075	Multidrug efflux RND transporter permease subunit		
	bepD		RM4018p_08200	Efflux pump periplasmic linker BepD	ABU_RS04080	Efflux RND transporter periplasmic adaptor subunit		
	-		RM4018p_08210	Hypothetical protein	ABU_RS04085	TolC family protein		
	mprA3		RM4018p_08220	Transcriptional repressor MprA	ABU_RS04090	MarR family transcriptional regulator		
	msbA	EP5	RM4018p_08810	Lipid A export ATP-binding/permease protein MsbA	ABU_RS04390	ABC transporter ATP-binding protein		
	uup	EP6	RM4018p_09130	ABC transporter ATP-binding protein uup	ABU_RS04550	ABC-F family ATP-binding cassette domain-containing protein		
	ttgB	EP7	RM4018p_09960	Toluene efflux pump membrane transporter TtgB	ABU_RS04960	efflux RND transporter permease subunit		
	mdtB2		RM4018p_09970	Multidrug resistance protein MdtB	ABU_RS04965	efflux RND transporter permease subunit		
	mdtE		RM4018p_09980	Multidrug resistance protein MdtE	ABU_RS04970	efflux RND transporter periplasmic adaptor subunit		
	-		RM4018p_09990	Hypothetical protein	ABU_RS04975	TolC family protein		
	bvgS	T1SS	RM4018p_10320	Virulence sensor protein BvgS	ABU_RS05140	transporter substrate-binding domain-containing protein		
	sphR		RM4018p_10330	Alkaline phosphatase synthesis transcriptional regulatory protein SphR	ABU_RS05145	response regulator transcription factor		
	-		RM4018p_10340	Hypothetical protein	ABU_RS05150	TolC family protein		
	ttgD		RM4018p_10350	Toluene efflux pump periplasmic linker protein TtgD	ABU_RS05155	Efflux RND transporter periplasmic adaptor subunit		
	-		RM4018p_10360	Hypothetical protein	ABU_RS05160	DUF4347 domain-containing protein		
	macA2		RM4018p_10370	Macrolide export protein MacA	ABU_RS05165	Efflux RND transporter periplasmic adaptor subunit		
	-		RM4018p_10380	Hypothetical protein	ABU_RS05170	HlyD family efflux transporter periplasmic adaptor subunit		
	sugE	EP8	RM4018p_10550	Quaternary ammonium compound-resistance protein SugE	ABU_RS05255	Quaternary ammonium compound efflux SMR transporter SugE		
	fsr	EP9	RM4018p_11960	Fosmidomycin resistance protein	ABU_RS05950	MFS transporter		
	hspR	EP10	RM4018p_13670	Putative heat shock protein HspR	ABU_RS06795	helix-turn-helix transcriptional regulator		
	macA3		RM4018p_13680	Macrolide export protein MacA	ABU_RS06800	HlyD family efflux transporter periplasmic adaptor subunit		
	ybhF		RM4018p_13690	putative ABC transporter ATP-binding protein YbhF	ABU_RS06805	ABC transporter ATP-binding protein		
	ybhS		RM4018p_13700	Inner membrane transport permease YbhS	ABU_RS06810	ABC transporter permease		
	ybhR		RM4018p_13710	Inner membrane transport permease YbhR	ABU_RS06815	ABC transporter permease		
	mexB1	EP11	RM4018p_18760	Multidrug resistance protein MexB	ABU_RS09310	efflux RND transporter permease subunit		
	-		RM4018p_18770	Hypothetical protein	ABU_RS09315	Hypothetical protein		
	-		RM4018p_18780	Hypothetical protein	ABU_RS09320	TolC family protein		
	-	EP12	RM4018p_19480	Hypothetical protein	ABU_RS09670	MFS transporter		
	ydhP	EP13	RM4018p_20020	Inner membrane transport protein YdhP	ABU_RS09940	MFS transporter		

Fortsetzung **Table S3**

	gene	alias*	Prokka locus	Prokka annotation	RefSeq locus	RefSeq annotation	16CS0817-2	16CS0821-2
Efflux pumps-related genes	*bcr1*	EP14	RM4018p_21670	Bicyclomycin resistance protein	ABU_RS10755	multidrug efflux MFS transporter		
	pleD5	EP15	RM4018p_21690	Response regulator PleD	ABU_RS10765	Diguanylate cyclase		
	mexA1		RM4018p_21700	Multidrug resistance protein MexA	ABU_RS10770	Efflux RND transporter periplasmic adaptor subunit		
	mexB2		RM4018p_21710	Multidrug resistance protein MexB	ABU_RS10775	Multidrug efflux RND transporter permease subunit		
	ttgC		RM4018p_21720	putative efflux pump outer membrane protein TtgC / OprM	ABU_RS10780	efflux transporter outer membrane subunit		
	oprM	EP16	RM4018p_22330	Outer membrane protein OprM	ABU_RS11085	efflux transporter outer membrane subunit		
	mexB3		RM4018p_22340	Multidrug resistance protein MexB	ABU_RS11090	Multidrug efflux RND transporter permease subunit		
	mexA2		RM4018p_22350	Multidrug resistance protein MexA	ABU_RS11095	Efflux RND transporter periplasmic adaptor subunit		
	-		RM4018p_22360	Hypothetical protein	ABU_RS11100	TetR/AcrR family transcriptional regulator		
	bcr2	EP17	RM4018p_22370	Bicyclomycin resistance protein	ABU_RS11105	multidrug efflux MFS transporter		
	cdhR	EP18	BMH_AB_233Bp_03110	HTH-type transcriptional regulator CdhR	-	-		
	bepF		BMH_AB_233Bp_03120	Efflux pump periplasmic linker BepF	-	-		
	bepE1		BMH_AB_233Bp_03130	Efflux pump membrane transporter BepE	-	-		
	oprM1		BMH_AB_233Bp_03140	Outer membrane protein OprM	-	-		
	cnrA	EP19	BMH_AB_246Bp_07320	Nickel and cobalt resistance protein CnrA	-	-		
	mdtA		BMH_AB_246Bp_07330	Multidrug resistance protein MdtA	-	-		
	-		BMH_AB_246Bp_07340	Putative HTH-type transcriptional regulator	-	-		
	oprM1		BMH_AB_246Bp_07350	Outer membrane protein OprM	-	-		
	tolC	-	Ab_2211p_17880	Outer membrane protein TolC	-	-		
Other antibiotic determinants	-	-	RM4018p_09950	Hypothetical protein	ABU_RS04955	Antibiotic resistance protein		
	-	-	RM4018p_03390	putative multidrug export ATP-binding/permease protein	ABU_RS01690	ABC transporter ATP-binding protein		
	-	-	RM4018p_11130	putative multidrug export ATP-binding/permease protein	ABU_RS05540	ABC transporter ATP-binding protein		
	-	-	RM4018p_04700	putative multidrug export ATP-binding/permease protein	ABU_RS02345	ABC transporter ATP-binding protein		
	acrB	*acrB*	RM4018p_03960	multidrug efflux pump subunit AcrB	ABU_RS01975	protein translocase subunit SecD		
	arnA	*arnA*	BMH_AB_233Bp_21330	Bifunctional polymyxin resistance protein	-	-		
	arnB	*arnB*	RM4018p_12550	UDP-4-amino-4-deoxy-L-arabinose--oxoglutarate aminotransferase	ABU_RS06230	DegT/DnrJ/EryC1/StrS family aminotransferase		
	bla1	*bla1*	RM4018p_05810	Metallo-beta-lactamase type 2	ABU_RS02895	MBL fold metallo-hydrolase		
	bla2	*bla2*	RM4018p_13040	Putative metallo-hydrolase	ABU_RS06485	MBL fold metallo-hydrolase		
	bla3	*bla3*	RM4018p_14830	Beta-lactamase OXA-15	ABU_RS07375	Class D beta-lactamase		
	cat3	*cat*	RM4018p_07870	Chloramphenicol acetyltransferase 3	ABU_RS03915	Type A chloramphenicol O-acetyltransferase		
	eptA	*eptA*	RM4018p_23280	phosphoethanolamine transferase	ABU_RS11565	phosphoethanolamine--lipid A transferase		
	gyrA	-	RM4018p_18010	DNA gyrase subunit A	ABU_RS08945	DNA gyrase subunit A		
	hcpC	*hcpC*	RM4018p_05610	putative beta-lactamase	ABU_RS02795	sel 1 repeat family protein		
	hipA1	*hlpA*	D4963p_05780	Serine/threonine-protein kinase	-	-		
	hipA2	*hlpA*	D4963p_10590	Serine/threonine-protein kinase	-	-		
	hipA3	*hlpA*	D4963p_10610	Serine/threonine-protein kinase	-	-		
	hipA4	*hlpA*	D4963p_10620	Serine/threonine-protein kinase	-	-		
	macB1	*macB*	RM4018p_04420	Macrolide export ATP-binding/permease protein MacB	ABU_RS02205	FtsX-like permease family protein		
	mrdA	-	RM4018p_10020	Penicillin-binding protein 2	ABU_RS04990	Penicillin-binding protein 2		
	pbpB	-	RM4018p_19240	Penicillin-binding protein	ABU_RS09545	Penicillin-binding protein 2		
	pbpF	-	RM4018p_19150	Penicillin-binding protein 1F	ABU_RS09500	PBP 1A family penicillin-binding protein		

Fortsetzung **Table S3**

	gene	alias*	Prokka locus	Prokka annotation	RefSeq locus	RefSeq annotation	16CS0817-2	16CS0821-2
Other antibiotic determinants	*relE*	*relE*	RM4018p_13910	Hypothetical protein	ABU_RS06915	type II toxin-antitoxin system RelE/ParE family toxin	■	■
	rlmN	*rlmN*	RM4018p_00580	putative dual specificity RNA methyltransferase	ABU_RS00285	23S rRNA (adenine (2503)-C(2))-methyltransferase RlmN	■	■
	rplD	-	RM4018p_07580	50S ribosomal protein L4	ABU_RS03770	50S ribosomal protein L4	■	■
	rplV	-	RM4018p_07620	50S ribosomal protein L22	ABU_RS03790	50S ribosomal protein L22	■	■
	sttH	*sttH*	RM4018p_02650	Streptothricin hydrolase	ABU_RS01320	cysteine hydrolase		
	tetA	*tetA*	RM4018p_03370	Tetracycline resistance protein, Class C	ABU_RS01680	MFS transporter		
	tolC	*tolC*	RM4018p_00650	Hypothetical protein	ABU_RS00315	TolC family protein	■	■
	oprF3	-	RM4018p_11260	Outer membrane porin F	ABU_RS05605	TolC family outer membrane protein		■
	vatD	-	RM4018p_06870	Streptogramin A acetyltransferase	ABU_RS03420	acyltransferase		
	wbpD	*wbpD*	RM4018p_06670	UDP-2-acetamido-3-amino-2,3-dideoxy-D-glucuronate N-acetyltransferase	ABU_RS03320	N-acetyltransferase		■
	ybiT1	-	RM4018p_10700	putative ABC transporter ATP-binding protein	ABU_RS05330	ABC-F family ATP-binding cassette domain-containing protein	■	■
	ybiT2	-	RM4018p_11470	putative ABC transporter ATP-binding protein	ABU_RS05710	ABC-F family ATP-binding cassette domain-containing protein		
	ylmA	-	RM4018p_10450	putative ABC transporter ATP-binding protein YlmA	ABU_RS05205	ATP-binding cassette domain-containing protein	■	■
Heavy metal resistance genes	-	-	RM4018p_05620	Hypothetical protein	ABU_RS02800	arcsenic resistance protein	■	■
	arsB	*arsB*	RM4018p_19420	Arsenical pump membrane protein	ABU_RS09640	arsenic transporter	■	■
	arsC1	*arsC*	RM4018p_13890	Arsenate reductase	ABU_RS06905	arsenate reductase (glutaredoxin)	■	■
	arsC2	*arsC*	RM4018p_19460	Glutaredoxin arsenate reductase	ABU_RS09660	arsenate reductase ArsC	■	■
	cadA	*cadA*	RM4018p_18720	Cadmium, zinc and cobalt-transporting ATPase	ABU_RS09290	cadmium-translocating P-type ATPase	■	■
	cnrA	-	BMH_AB_246Bp_07320	Nickel and cobalt resistance protein CnrA	-	-		
	copA1	*copA_1*	RM4018p_04810	Copper-exporting P-type ATPase A	ABU_RS02400	heavy metal translocating P-type ATPase	■	■
	copA2	*copA_2*	RM4018p_05490	putative copper-importing P-type ATPase A	ABU_RS02735	heavy metal translocating P-type ATPase	■	■
	copR	-	RM4018p_07970	transcriptional activator protein CopR	ABU_RS03965	response regulator transcription factor	■	■
	copZ	*copZ*	RM4018p_15130	copper chaperone CopZ	ABU_RS07520	heavy-metal-associated domain-containing protein	■	■
	corC	*corC*	RM4018p_11540	magnesium and cobalt efflux protein CorC	ABU_RS05745	HlyC/CorC family transporter	■	■
	csoR	*csoR*	RM4018p_04800	copper sensing transcriptional repressor	ABU_RS02395	metal-sensing transcriptional repressor	■	■
	ctpC	-	RM4018p_07160	putative manganese/ zinc-exporting P-type ATPase	ABU_RS03560	heavy metal translocating P-type ATPase	■	■
	cusS	*cusS*	Ab_4511p_10200	Sensor kinase CusS	-	-	■	■
	czcA	-	Ab_2211p_12350	Cobalt-zinc-cadmium resistance protein CzcA	-	-		
	czcB	*czcB*	RM4018p_04620	Cobalt-zinc-cadmium resistance protein CzcB	ABU_RS02305	Transporter		
	czcD	*czcD*	RM4018p_03450	cadmium, cobalt and zinc/ H+-K+ antiporter	ABU_RS01720	cation diffusion facilitator family transporter	■	■
	czcR1	-	RM4018p_04350	Transcriptional activator protein CzcR	ABU_RS02170	response regulator transcription factor	■	■
	czcR2	-	RM4018p_09080	Transcriptional activator protein CzcR	ABU_RS04525	response regulator transcription factor	■	■
	czcR3	-	RM4018p_10900	Transcriptional activator protein CzcR	ABU_RS05425	response regulator transcription factor	■	■
	czcR4	-	RM4018p_11730	Transcriptional activator protein CzcR	ABU_RS05840	response regulator transcription factor		
	czcR5	-	Ab_2211p_22630	Transcriptional activator protein CzcR	-	-		
	merT	*merT*	RM4018p_15140	Hypothetical protein	ABU_RS07525	Transporter	■	■
	modA	*modA*	RM4018p_00120	Molybdate-binding periplasmatic protein	ABU_RS00060	molybdate ABC transporter substrate-binding protein	■	■
	modB	*modB*	RM4018p_00100	Molybdenum transport system permease protein	ABU_RS00050	molybdate ABC transporter permease subunit	■	■
	mopA	*mopA*	RM4018p_00130	Molybdenum-pterin-binding protein	ABU_RS00065	LysR family transcriptional regulator	■	■
	zntB	*zntB*	RM4018p_22910	Zinc transport protein	ABU_RS11380	zinc transporter ZntB	■	■

* if mentioned in previous publications; black box = present; white box = absent

Table S4 - Presence and absence of virulence genes in the *A. butzleri* strains 16CS0817-2 and 16CS0821-2

	gene	alias*	Prokka locus	Prokka annotation	RefSeq locus	RefSeq annotation	16CS0817-2	16CS0821-2
Flagellar genes	*flaA*	*flaA*	RM4018p_22650	Flagellar filament 33 kDa core protein	ABU_RS11245	Flagellin		
	flaB	*flaB*	RM4018p_22660	Flagellar filament 33 kDa core protein	ABU_RS11250	Flagellin		
	flgB	*flgB*	RM4018p_19750	Flagellar basal body rod protein FlgB	ABU_RS09805	Flagellar basal body rod protein FlgB		
	flgC	*flgC*	RM4018p_19560	Flagellar basal-body rod protein FlgC	ABU_RS09710	Flagellar basal body rod protein FlgC		
	flgD	*flgD*	RM4018p_19700	Hypothetical protein	ABU_RS09780	Flagellar basal body rod modification protein FlgD		
	flgE1	*flgE1*	RM4018p_19680	Flagellar hook protein FlgE	ABU_RS09770	Flagellar hook basal body complex protein		
	flgE2	*flgE2*	RM4018p_19690	Flagellar hook protein FlgE	ABU_RS09775	Flagellar hook protein FlgE		
	flgG1	*flgG1*	RM4018p_19760	Flagellar basal-body rod protein FlgG	ABU_RS09810	Flagellar hook-basal body protein		
	flgG2	*flgG2*	RM4018p_19790	Flagellar basal-body rod protein FlgG	ABU_RS09825	Flagellar basal-body rod protein FlgG		
	flgH	*flgH*	RM4018p_02100	Flagellar L-ring protein	ABU_RS01045	Flagellar basal body L-ring protein FlgH		
	flgI	*flgI*	RM4018p_02030	Flagellar P-ring protein	ABU_RS01010	Flagellar basal body P-ring protein FlgI		
	flgK	*flgK*	RM4018p_02110	Flagellar hook-associated protein 1	ABU_RS01050	Flagellar hook-associated protein FlgK		
	flgL	*flgL*	RM4018p_19500	Hypothetical protein	ABU_RS09680	Flagellar hook-associated protein 3		
	flhA	*flhA*	RM4018p_19490	Flagellar biosynthesis protein FlhA	ABU_RS09675	Flagellar biosynthesis protein FlhA		
	flhB1	-	RM4018p_13940	Flagellar biosynthetic protein FlhB	ABU_RS06930	Hypothetical protein		
	flhB2	*flhB*	RM4018p_19530	Flagellar biosynthetic protein FlhB	ABU_RS09695	Flagellar biosynthesis protein FlhB		
	flhF	*flhF*	RM4018p_19600	Flagellar biosynthesis protein FlhF	ABU_RS09730	Flagellar biosynthesis protein FlhF		
	fliD	*fliD*	RM4018p_02120	Flagellar hook-associated protein 2	ABU_RS01055	Flagellar hook protein FliD		
	fliE	*fliE*	RM4018p_19570	Flagellar hook-basal body complex protein FliE	ABU_RS09715	Flagellar hook-basal body complex protein FliE		
	fliF	*fliF*	RM4018p_19740	Flagellar M-ring protein	ABU_RS09800	Flagellar M-ring protein FliF		
	fliG	*fliG*	RM4018p_19730	Flagellar motor switch protein FliG	ABU_RS09795	Flagellar motor switch protein FliG		
	fliH	*fliH*	RM4018p_19720	Hypothetical protein	ABU_RS09790	Flagellar assembly protein FliH		
	fliI	*fliI*	RM4018p_19510	Flagellum-specific ATP synthase	ABU_RS09685	Flagellar protein export ATPase FliI		
	fliK	*fliK*	RM4018p_19550	Hypothetical protein	ABU_RS09705	Flagellar hook-length control protein FliK		
	fliL	*fliL*	RM4018p_02080	Hypothetical protein	ABU_RS01035	Flagellar basal body-associated FliL family protein		
	fliM	*fliM*	RM4018p_02020	Flagellar motor switch protein FliM	ABU_RS01005	Flagellar motor switch protein FliM		
	fliN1	*fliY*	RM4018p_19640	Flagellar motor switch protein FliN	ABU_RS09750	Flagellar motor switch protein FliY		
	fliN2	*fliN*	RM4018p_19710	Flagellar motor switch protein FliN	ABU_RS09785	Flagellar motor switch protein FliN		
	fliP	*fliP*	RM4018p_10010	Flagellar biosynthetic protein FliP	ABU_RS04985	Flagellar type III secretion system pore protein FliP		
	fliQ	*fliQ*	RM4018p_02050	Flagellar biosynthetic protein FliQ	ABU_RS01020	Flagellar biosynthesis protein FliQ		
	fliR	*fliR*	RM4018p_19540	Hypothetical protein	ABU_RS09700	Flagellar biosynthetic protein FliR		
	fliS	*fliS*	RM4018p_02130	Flagellar protein FliS	ABU_RS01060	Flagellar export chaperone FliS		
	fliW2	-	RM4018p_02410	Flagellar assembly factor FliW2	ABU_RS01200	FliW protein		
	hag	*hag*	RM4018p_11190	Flagellin	ABU_RS05570	Flagellin		
	pomA	*motA*	RM4018p_04010	Chemotaxis protein PomA	ABU_RS02000	Flagellar motor stator protein MotA		
	motB	*motB*	RM4018p_04000	Motility protein B	ABU_RS01995	Flagellar motor protein MotB		

Fortsetzung **Table S4**

	gene	alias*	Prokka locus	Prokka annotation	RefSeq locus	RefSeq annotation	16CS0817-2	16CS0821-2
Chemotaxis system	*cheW*	*cheW*	RM4018p_04270	Chemotaxis protein CheW	ABU_RS02130	chemotaxis protein CheW		
	cheV	*cheV*	RM4018p_06200	Chemotaxis protein CheV	ABU_RS03090	response regulator		
	cheY1	*cheY1*	RM4018p_11900	Chemotaxis protein CheY	ABU_RS05920	response regulator		
	cheA	*cheA*	RM4018p_11910	Chemotaxis protein CheA	ABU_RS05925	chemotaxis protein CheA		
	cheR	*cheR*	RM4018p_11920	Chemotaxis protein methyltransferase	ABU_RS05930	protein-glutamate O-methyltransferase CheR		
	cheB	*cheB*	RM4018p_11940	Chemotaxis response regulator protein-glutamate methylesterase	ABU_RS05940	chemotaxis response regulator protein-glutamate methylesterase		
	cheY2	*cheY2*	RM4018p_13630	Chemotaxis protein CheY	ABU_RS06775	response regulator		
	cheY3	*cheY3*	RM4018p_19780	Chemotaxis protein CheY	ABU_RS09820	response regulator		
Urease cluster	*ureD*	*ureD*	RM4018p_08100	Urease accessory protein UreD	ABU_RS04030	Urease accessory protein UreD		
	ureA	*ureAB*	RM4018p_08110	Urease subunit alpha	ABU_RS04035	Urease subunit gamma		
	ureC	*ureC*	RM4018p_08120	Urease subunit alpha	ABU_RS04040	Urease subunit alpha		
	ureE	*ureE*	RM4018p_08130	Urease accessory protein UreE	ABU_RS04045	Urease accessory protein UreE		
	ureF	*ureF*	RM4018p_08140	Urease accessory protein UreF	ABU_RS04050	Urease		
	ureG	*ureG*	RM4018p_08150	Urease accessory protein UreG	ABU_RS04055	Urease accessory protein UreG		
Other virulence determinants	*besA*	*iroE*	RM4018p_07320	Ferri-bacillibactin esterase BesA	ABU_RS03640	alpha/beta hydrolase		
	bvgS	-	RM4018p_10320	Virulence sensor protein BvgS	ABU_RS05140	transporter substrate-binding domain-containing protein		
	ccp	*docA*	RM4018p_08900	Cytochrome c551 peroxidase	ABU_RS04435	c-type cytochrome		
	cdiA1	*hecA*	RM4018p_09450	16S rRNA endonuclease CdiA	ABU_RS04710	filamentous hemagglutinin N-terminal domain-containing protein		
	ciaB	*ciaB*	RM4018p_15560	Hypothetical protein	ABU_RS07735	invasion protein CiaB		
	cirA1	*irgA*	RM4018p_07310	Colicin I receptor	ABU_RS03635	TonB-dependent receptor		
	cirA2	-	RM4018p_20030	Colicin I receptor	ABU_RS09945	TonB-dependent receptor		
	cirA3	*cfrB*	L353p_13840	Colicin I receptor	-	-		
	cirA4	-	L353p_19620	Colicin I receptor	-	-		
	cj1349	*cj1349*	RM4018p_00690	Hypothetical protein	ABU_RS00335	DUF814 domain-containing protein		
	cvfB	*cvfB*	RM4018p_05590	conserved virulence factor B	ABU_RS02785	DNA-binding protein		
	degP	*htrA*	RM4018p_21080	Periplasmic serine endoprotease DegP	ABU_RS10475	Do family serine endopeptidase		
	fur	*fur*	RM4018p_17830	Ferric uptake regulation protein	ABU_RS08850	transcriptional repressor		
	iamA	*iamA*	RM4018p_01090	putative ribonucleotide transport ATP-binding protein (mkl)	ABU_RS00540	ATP-binding cassette domain-containing protein		
	luxS	*luxS*	RM4018p_01130	S-ribosylhomocysteine lyase	ABU_RS00560	S-ribosylhomocysteine lyase		
	murJ	*mviN*	RM4018p_08820	Lipid II flippase MurJ	ABU_RS04395	murein biosynthesis integral membrane protein MurJ		
	oprF2	*cadF*	RM4018p_04820	Outer membrane porin F	ABU_RS02405	OmpA family protein		
	pldA	*pldA*	RM4018p_08650	Putative phospholipase A1	ABU_RS04310	phospholipase A		
	rfaC	*waaC*	RM4018p_18340	Lipopolysaccharide heptosyltransferase 1 (rfaC)	ABU_RS09110	lipopolysaccharide heptosyltransferase I (waaC)		
	rfaF	*waaF*	RM4018p_18120	ADP-heptose--LPS heptosyltransferase 2 (rfaF)	ABU_RS09000	glycosyltransferase family 9 protein (waaF)		
	shlB	*hecB*	RM4018p_09440	Hemolysin transporter protein ShlB	ABU_RS04705	ShlB/FhaC/HecB family hemolysin secretion/activation protein		
	tlyA	*tlyA*	RM4018p_18480	16S/23S rRNA (cytidine-2'-O)-methyltransferase TlyA	ABU_RS09170	TlyA family rRNA methyltransferase		
	virF	*virF*	BMH_AB_233Bp_11740	Virulence regulon transcriptional activator VirF	ABU_RS07855	AraC family transcriptional regulator		
	voc	*VOC*	RM4018p_20810	Virulence protein	ABU_RS10345	VOC family protein		
Capsule cluster	*fcl*	-	Ab_4511p_20030	GDP-L-fucose synthase	-	-		
	glmM2	-	Ab_4511p_19970	Phosphoglucosamine mutase	-	-		
	gmhA	*gmhA2*	Ab_4511p_20000	Phosphoheptose_isomerase	-	-		

Fortsetzung **Table S4**

	gene	alias*	Prokka locus	Prokka annotation	RefSeq locus	RefSeq annotation	16CS0817-2	16CS0821-2
Capsule cluster	*gmhB2*	-	Ab_4511p_20020	D-glycero-alpha-D-manno-heptose-1,7-bisphosphate 7-phosphatase	-	-		
	hddA	-	Ab_4511p_19990	D-glycero-alpha-D-manno-heptose 7-phosphate kinase	-	-		
	hddC	-	Ab_4511p_20010	D-glycero-alpha-D-manno-heptose 1-phosphate guanylyltransferase	-	-		
	rmd	-	Ab_4511p_19980	GDP-6-deoxy-D-mannose reductase	-	-		
Lipid A cluster	*waaA*	-	RM4018p_19870	3-deoxy-D-manno-octulosonic acid transferase	ABU_RS09865	3-deoxy-D-manno-octulosonic acid transferase		
	lpxA	-	RM4018p_22260	Acyl-(acyl-carrier-protein)--UDP-N-acetylglucosamine O-acetyltransferase	ABU_RS11050	acyl-ACP--UDP-N-acetylglucosamine O-acyltransferase		
	lpxB	-	RM4018p_22280	Lipid-A-disaccharide synthase	ABU_RS11060	Lipid-A-disaccharide synthase		
	lpxC	-	RM4018p_20490	UDP-3-O-acyl-N-acetylglucosamine deacetylase	ABU_RS10180	UDP-3-O-acyl-N-acetylglucosamine deacetylase		
	lpxD	-	RM4018p_11650	UDP-3-O-acylglucosamine-N-acyltransferase	ABU_RS05800	UDP-3-O-(3-hydroxymyristoyl) glucosamine N-acyltransferase		
	lpxH	-	RM4018p_06230	UDP-2,3-diacylglucosamine hydrolase	ABU_RS03105	Hypothetical protein		
	lpxK	-	RM4018p_12540	Tetraacyldisaccharide 4'-kinase	ABU_RS06225	tetraacyldisaccharide 4'-kinase		
	lpxP	-	RM4018p_18330	Lipid A biosynthesis palmitoleoyltransferase	ABU_RS09105	lipid A biosynthesis acyltransferase		
	phoP1	-	RM4018p_06330	Transcriptional regulatory protein PhoP	ABU_RS03155	response regulator transcription factor		
	phoP2	-	RM4018p_15910	Transcriptional regulatory protein PhoP	ABU_RS07910	response regulator transcription factor		
	phoP3	-	RM4018p_18810	Virulence transcriptional regulatory protein PhoP	ABU_RS09330	response regulator transcription factor		
	phoQ	*phoQ*	RM4018p_10420	sensor protein	ABU_RS05190	sensor histidine kinase		
Type IV secretion system region (T4SS)	*cssS*	-	D4963p_10540	Sensor histidine kinase CssS	-	-		
	cusR	-	D4963p_10550	Transcriptional regulatory protein CusR	-	-		
	-	-	D4963p_10560	3'3'-cGAMP-specific phosphodiesterase 2	-	-		
	tap4	-	D4963p_10570	Methyl-accepting chemotaxis protein IV	-	-		
	-	-	D4963p_10580	Hypothetical protein	-	-		
	hipA2	*hlpA*	D4963p_10590	Serine/threonine-protein kinase HipA	-	-		
	-	-	D4963p_10600	Hypothetical protein	-	-		
	hipA3	*hlpA*	D4963p_10610	Serine/threonine-protein kinase HipA	-	-		
	hipA4	*hlpA*	D4963p_10620	Serine/threonine-protein kinase HipA	-	-		
	-	-	D4963p_10630	Hypothetical protein	-	-		
	-	-	D4963p_10640	Hypothetical protein	-	-		
	-	-	D4963p_10650	Hypothetical protein	-	-		
	-	-	D4963p_10660	Hypothetical protein	-	-		
	-	-	D4963p_10670	Hypothetical protein	-	-		
	-	-	D4963p_10680	Hypothetical protein	-	-		
	-	-	D4963p_10690	Hypothetical protein	-	-		
	-	-	D4963p_10700	Hypothetical protein	-	-		
	-	-	D4963p_10710	Hypothetical protein	-	-		
	-	-	D4963p_10720	Hypothetical protein	-	-		
	-	-	D4963p_10730	Hypothetical protein	-	-		
	-	-	D4963p_10740	Hypothetical protein	-	-		
	topB	-	D4963p_10750	DNA topoisomerase 3	-	-		
	-	-	D4963p_10760	Hypothetical protein	-	-		
	virB1	*virB1*	D4963p_10770	Type IV secretion system protein virB1	-	-		
	-	-	D4963p_10780	Hypothetical protein	-	-		

Fortsetzung **Table S4**

	gene	alias*	Prokka locus	Prokka annotation	RefSeq locus	RefSeq annotation	16CS0817-2	16CS0821-2
Type IV secretion system region (T4SS)	-	-	D4963p_10790	Hypothetical protein	-	-		
	-	-	D4963p_10800	Hypothetical protein	-	-		
	-	-	D4963p_10810	Hypothetical protein	-	-		
	dnaG1	-	D4963p_10820	DNA primase	-	-		
	-	-	D4963p_10830	Hypothetical protein	-	-		
	-	-	D4963p_10840	Hypothetical protein	-	-		
	-	-	D4963p_10850	Hypothetical protein	-	-		
	traG	*virD4*	D4963p_10860	Conjugal transfer protein TraG	-	-		
	virB11	*virB11*	D4963p_10870	Type IV secretion system protein VirB11	-	-		
	virB10	*virB10*	D4963p_10880	Type IV secretion system protein virB10	-	-		
	virB9	*virB9*	D4963p_10890	Type IV secretion system protein virB9	-	-		
	virB8	*virB8*	D4963p_10900	Type IV secretion system protein virB8	-	-		
	-	-	D4963p_10910	Hypothetical protein	-	-		
	-	-	D4963p_10920	Hypothetical protein	-	-		
	-	*virB6*	D4963p_10930	Hypothetical protein	-	-		
	-	-	D4963p_10940	Hypothetical protein	-	-		
	-	*virB5*	D4963p_10950	Hypothetical protein	-	-	■	
	-	*virB7*	D4963p_10960	Hypothetical protein	-	-		
	-	-	D4963p_10970	Hypothetical protein	-	-		
	virB4	*virB4*	D4963p_10980	Type IV secretion system protein virB4	-	-		
	-	*virB2*	D4963p_10990	Hypothetical protein	-	-		
	-	-	D4963p_11000	Hypothetical protein	-	-		
	-	-	D4963p_11010	Hypothetical protein	-	-		
	-	-	D4963p_11020	Hypothetical protein	-	-		
	-	-	D4963p_11030	Hypothetical protein	-	-		
	-	-	D4963p_11040	Hypothetical protein	-	-		
	-	-	D4963p_11050	Hypothetical protein	-	-		
	-	-	D4963p_11060	Hypothetical protein	-	-		
	-	-	D4963p_11070	Hypothetical protein	-	-		
	intA1	-	D4963p_11080	Prophage CP4-57 integrase	-	-		

* if mentioned in previous publications; black box = present; white box = absent

8.3 Supplemente zur Publikation 3

Table S1 - Additional metadata of the 75 *Aliarcobacter butzleri* strains used in this study

WGS	Strain	BioProject	Isolation source	Year of isolation	Country	Antimicrobial susceptibility (MIC)																				Urease assay
						ERY		GEN		CIP		DC		TC		STX		AMP		CTX			
						mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		mg/L			
SRR12709624	16CS0847-3	PRJNA665330	fecal sample (goose)	2016	Germany	4	S	2	S	0.12	S	1.5	S	2	S	8	R	8	S	32	R	+
SRR12709623	17CS0824	PRJNA665330	fecal sample (goose)	2017	Germany	8	R	4	I	0.12	S	4	R	4	R	12	R	3	S	32	R	+
SRR12709612	17CS1193-1	PRJNA665330	fecal sample (mulard duck)	2017	Germany	4	S	1	S	0.12	S	1	S	1	S	8	R	24	R	32	R	+
SRR12709601	17CS1193-2	PRJNA665330	fecal sample (mulard duck)	2017	Germany	4	S	1	S	0.25	S	1.5	S	1	S	8	R	16	R	32	R	+
SRR12709635	17CS1193-3	PRJNA665330	fecal sample (mulard duck)	2017	Germany	8	R	4	I	0.25	S	2	S	2	S	12	R	1,5	S	32	R	+
SRR12709629	17CS1197	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	16	R	4	I	0.5	S	6	R	4	R	12	R	4	S	32	R	+
SRR12709628	17CS1197-2	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	4	S	4	I	0.12	S	6	R	4	R	12	R	8	S	32	R	+
SRR12709627	17CS1197-3	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	32	R	2	S	0.5	S	6	R	4	R	12	R	4	S	32	R	+
SRR12709626	17CS1198-1	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	2	S	4	I	0.25	S	2	S	2	S	48	R	1	S	32	R	+
SRR12709625	17CS1198-2	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	4	S	4	I	0.25	S	2	S	2	S	48	R	1	S	32	R	+
SRR12709622	17CS1198-3	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	4	S	4	I	0.25	S	2	S	2	S	48	R	1	S	32	R	Ø
SRR12709621	16CS0367-AR	PRJNA665330	fecal sample (goose)	2016	Germany	16	R	3	I	0.25	S	4	R	3	R	10	R	6	S	32	R	+
SRR12709620	16CS0367-1-AR	PRJNA665330	fecal sample (goose)	2016	Germany	16	R	2	S	0.25	S	3	R	4	R	8	R	8	S	32	R	+
SRR12709619	16CS0370-1	PRJNA665330	fecal sample (goose)	2016	Germany	16	R	3	I	0.5	S	4	R	3	R	12	R	8	S	32	R	+
SRR12709618	16CS0375-1	PRJNA665330	fecal sample (goose)	2016	Germany	16	R	4	I	0.25	S	6	R	4	R	8	R	4	S	32	R	+
SRR12709617	16CS0375-2	PRJNA665330	fecal sample (goose)	2016	Germany	12	R	3	I	0.25	S	5	R	3	R	6	R	4	S	32	R	+
SRR12709616	16CS0831-1-1	PRJNA665330	fecal sample (goose)	2016	Germany	8	R	2	S	0.12	S	3	R	4	R	8	R	12	R	32	R	+
SRR12709615	16CS0831-4-1	PRJNA665330	fecal sample (goose)	2016	Germany	8	R	1	S	0.25	S	6	R	8	R	4	S	256	R	32	R	Ø
SRR12709614	17CS1200-1	PRJNA665330	fecal sample (Pekin duck)	2017	Germany	8	R	4	I	0.12	S	1.5	S	2	S	12	R	3	S	32	R	+
SRR12709613	17CS1201	PRJNA665330	fecal sample (Pekin duck)	2017	Germany	4	S	2	S	0.06	S	1	S	2	S	4	S	2	S	32	R	+
SRR12709611	17CS1205-1	PRJNA665330	fecal sample (Pekin duck)	2017	Germany	8	R	2	S	0.12	S	2	S	2	S	12	R	32	R	32	R	+
SRR12709610	17CS1205-2	PRJNA665330	fecal sample (Pekin duck)	2017	Germany	8	R	2	S	0.12	S	3	R	2	S	8	R	32	R	32	R	+
SRR12709609	17CS1206-1	PRJNA665330	fecal sample (Pekin duck)	2017	Germany	16	R	1	S	0.25	S	3	R	2	S	4	S	256	R	32	R	+
SRR12709608	17CS1208-3	PRJNA665330	fecal sample (Pekin duck)	2017	Germany	8	R	4	I	0.12	S	3	R	2	S	12	R	32	R	32	R	+
SRR12709607	17CS1167	PRJNA665330	fecal sample (goose)	2017	Germany	8	R	4	I	0.12	S	3	R	2	S	12	R	2	S	32	R	+
SRR12709606	17CS1168	PRJNA665330	fecal sample (goose)	2017	Germany	6	R	3	I	0.06	S	2	S	2	S	4	S	2	S	32	R	+
SRR12709605	17CS1056	PRJNA665330	fecal sample (goose)	2017	Germany	16	R	4	I	0.25	S	2	S	2	S	4	S	1.5	S	32	R	+
SRR12709604	17CS1066	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	8	R	4	I	0.12	S	2	S	2	S	12	R	12	R	32	R	+
SRR12709603	17CS1067	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	8	R	4	I	0.25	S	2	S	2	S	12	R	256	R	32	R	+
SRR12709602	17CS1068-1	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	32	R	2	S	0.25	S	4	R	4	R	8	R	256	R	32	R	+
SRR12709600	17CS1068-2	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	4	S	2	S	0.12	S	3	R	4	R	6	R	256	R	32	R	+
SRR12709599	17CS0916-1	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	16	R	2	S	32	R	2	S	3	R	6	R	256	R	32	R	+

Fortsetzung **Table S1**

WGS	Strain	BioProject	Isolation source	Year of isolation	Country	Antimicrobial susceptibility (MIC)																Urease assay
						ERY		GEN		CIP		DC		TC		STX		AMP		CTX		
						mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		mg/		
SRR12709598	17CS0916-2	PRJNA665330	fecal sample (Muscovy duck)	2017	Germany	32	R	2	S	32	R	3	R	5	R	8	R	256	R	32	R	+
SRR12709597	16CS0857-1	PRJNA665330	fecal sample (goose)	2016	Germany	24	R	4	I	0.25	S	6	R	8	R	12	R	256	R	32	R	+
SRR12709596	16CS0861-1	PRJNA665330	fecal sample (goose)	2016	Germany	12	R	3	I	0.12	S	3	R	2	S	12	R	3	S	32	R	+
SRR12709595	17CS1092-1	PRJNA665330	fecal sample (goose)	2017	Germany	4	S	4	I	0.25	S	3	R	4	R	12	R	6	S	32	R	+
SRR12709594	17CS1092-2	PRJNA665330	fecal sample (goose)	2017	Germany	8	R	4	I	0.25	S	1.5	S	2	S	16	R	6	S	32	R	+
SRR12709593	17CS1095	PRJNA665330	fecal sample (goose)	2017	Germany	8	R	2	S	0.12	S	1.5	S	2	S	8	R	4	S	32	R	+
SRR12709637	16CS0815-2	PRJNA665330	fecal sample (goose)	2016	Germany	2	S	2	S	0.5	S	2	S	2	S	8	R	2	S	32	R	+
SRR10215626	16CS0817-2	PRJNA575341	fecal sample (Muscovy duck)	2016	Germany	2	S	1.25	S	0.06	S	1.75	S	1.75	S	4	S	6	S	32	R	+
SRR12709636	16CS0820-1	PRJNA665330	fecal sample (Muscovy duck)	2016	Germany	16	R	2.5	I	0.25	S	2.5	R	2,5	R	8	R	256	R	32	R	+
SRR12709634	16CS0820-2	PRJNA665330	fecal sample (Muscovy duck)	2016	Germany	16	R	1.5	S	0.25	S	6	R	5	R	4	S	3	S	32	R	+
SRR10215625	16CS0821-2	PRJNA575341	fecal sample (Muscovy duck)	2016	Germany	32	R	1	S	0.5	S	4	R	3	R	12	R	4	S	32	R	+
SRR12709633	16CS0823-1	PRJNA665330	fecal sample (Muscovy duck)	2016	Germany	32	R	4	I	0.25	S	6	R	4	R	12	R	256	R	32	R	+
SRR12709632	16CS0823-2	PRJNA665330	fecal sample (Muscovy duck)	2016	Germany	12	R	3	I	0.25	S	3	R	2	S	12	R	1	S	32	R	+
SRR12709631	17CS0965-1	PRJNA665330	fecal sample (goose)	2017	Germany	4	S	4	I	0.12	S	2	S	2	S	12	R	3	S	32	R	+
SRR12709630	17CS0965-B	PRJNA665330	fecal sample (goose)	2017	Germany	4	S	2	S	0.25	S	3	R	2	S	12	R	6	S	32	R	+
GCF_900187115.1	NCTC 12481	PRJEB6403	diarrheic stool (human)	1992	USA	NA	I	NA	S	NA	S	NA		NA	S	NA	S	NA	R	NA	R	+
GCF_004283125.1	55	PRJNA489574	mussel	2014	Italy	NA	R*	NA	S*	NA	S*	NA		NA	R*	NA	S*	256	R	128	R	NA
GCF_004283115.1	6V	PRJNA489609	clams	2014	Italy	NA	R*	NA	S*	NA	S*	NA		NA	R*	NA	S*	64	R	64	R	NA
GCF_013363865.1	RMI	PRJNA637480	raw milk	2018	Germany	NA		NA		NA		NA		NA		NA		NA		NA		NA
GCF_013363855.1	RMIII	PRJNA637480	raw milk	2018	Germany	NA		NA		NA		NA		NA		NA		NA		NA		NA
GCF_000215345.2	7h1h	PRJNA67167	fecal sample (beef)	2007	UK	NA		NA		NA		NA		NA		NA		NA		NA		NA
ERR3523212	Ab_1426_2003	PRJEB34441	diarrheic stool (human)	2003	France	1	S	0.125	S	0.06	S	NA		NA		NA		16-32	S/R	8	R	Ø
ERR3523199	Ab_1711	PRJEB34441	poultry slaughterhouse	2011	Portugal	2	S	0.125	S	8	R	NA		NA		NA		4	S	8	R	+
ERR3523202	Ab_2211	PRJEB34441	slaughterhouse surface	2011	Portugal	2	S	0.5	S	0.125	S	NA		NA		NA		32	R	16	R	Ø
ERR3523203	Ab_2811	PRJEB34441	carcass neck skin (poultry)	2011	Portugal	32	R	0.25	S	16	R	NA		NA		NA		64	R	32	R	Ø
ERR3523197	Ab_3711	PRJEB34441	caecum (poultry)	2011	Portugal	1	S	0.25	S	4	R	NA		NA		NA		16-32	S/R	4	R	+
ERR3523210	Ab_4211	PRJEB34441	carcass drippings (poultry)	2011	Portugal	2	S	0.25	S	16	R	NA		NA		NA		32	R	8	R	Ø
ERR3523201	Ab_4511	PRJEB34441	carcass drippings (flock)	2011	Portugal	2	S	0.25	S	0.25	S	NA		NA		NA		32	R	16	R	Ø
ERR3523204	Ab_A103	PRJEB34441	river water	2016	Portugal	2	S	0.5	S	16	R	NA		NA		NA		16	S	16	R	Ø
ERR3523214	Ab_A111	PRJEB34441	river water	2016	Portugal	2	S	0.25	S	0.06	S	NA		NA		NA		8	S	8	R	+
ERR3523198	Ab_CR1132	PRJEB34441	ready-to-eat vegetables	2016	Portugal	1	S	0.25	S	0.06	S	NA		NA		NA		8	S	16	R	+
ERR3523196	Ab_CR1143	PRJEB34441	meat (poultry)	2016	Portugal	4	S	0.25	S	8	R	NA		NA		NA		64	R	16	R	Ø

Fortsetzung **Table S1**

WGS	Strain	BioProject	Isolation source	Year of isolation	Country	Antimicrobial susceptibility (MIC)																Urease assay
						ERY		GEN		CIP		DC		TC		STX		AMP		CTX		
						mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		mg/L		
ERR3523205	Ab_CR424	PRJEB34441	meat (poultry)	2015	Portugal	32	R	0.5	S	0.25	S	NA		NA		NA		32	R	8	R	Ø
ERR3523200	Ab_CR461	PRJEB34441	meat (fish)	2015	Portugal	1	S	0.125	S	0.06	S	NA		NA		NA		32	R	16	R	Ø
ERR3523213	Ab_CR502	PRJEB34441	meat (poultry)	2015	Portugal	32	R	0.25	S	32	R	NA		NA		NA		4	S	4	R	+
ERR3523209	Ab_CR604	PRJEB34441	meat (beef)	2015	Portugal	0.125	S	0.25	S	0.06	S	NA		NA		NA		8	S	8	R	+
ERR3523206	Ab_CR641	PRJEB34441	meat (poultry)	2015	Portugal	32	R	0.25	S	0.5	S	NA		NA		NA		8	S	8	R	Ø
ERR3523216	Ab_CR891	PRJEB34441	meat (poultry)	2016	Portugal	2	S	0.25	S	0.125	S	NA		NA		NA		2	S	16	R	Ø
ERR3523215	Ab_CR892	PRJEB34441	meat (poultry)	2016	Portugal	2	S	0.25	S	8	R	NA		NA		NA		32	R	8	R	+
ERR3523207	Ab_DQ20dA1	PRJEB34441	milk (goat)	2015	Portugal	2	S	0.25	S	16	R	NA		NA		NA		64	R	32	R	Ø
ERR3523217	Ab_DQ31A1	PRJEB34441	milk (sheep)	2015	Portugal	4	S	0.25	S	0.06	S	NA		NA		NA		32	R	32	R	Ø
ERR3523211	Ab_DQ40A1	PRJEB34441	dairy plant equipment surface	2015	Portugal	2	S	0.25	S	0.03	S	NA		NA		NA		4	S	4	R	+
ERR3523208	Ab_DQ64A1	PRJEB34441	dairy plant equipment surface	2015	Portugal	1	S	0.25	S	0.06	S	NA		NA		NA		4	S	2	S	+

Fortsetzung **Table S1**

WGS	MLST							Reference
	aspA	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST	
SRR12709624	4	12	1	19	346	~103	24	new	this study
SRR12709623	4	58	44	19	150	4	89	new	this study
SRR12709612	4	4	44	12	376	4	160	new	this study
SRR12709601	4	4	44	12	376	4	160	new	this study
SRR12709635	4	4	51	126	471	20	89	new	this study
SRR12709629	~15	4	40	19	~677	63	9	new	this study
SRR12709628	~15	4	40	19	~677	63	9	new	this study
SRR12709627	~15	4	40	19	~677	63	9	new	this study
SRR12709626	4	4	4	19	351	219	158	new	this study
SRR12709625	4	4	4	19	351	219	158	new	this study
SRR12709622	4	4	4	19	351	219	158	new	this study
SRR12709621	4	4	40	19	471, 534	194	158	new	this study
SRR12709620	4	4	40	19	471, 534	194	158	new	this study
SRR12709619	4	4	40	19	471, 534	194	158	new	this study
SRR12709618	4	4	40	19	471, 534	194	158	new	this study
SRR12709617	4	4	40	19	471, 534	194	158	new	this study
SRR12709616	4	4	40	19	471, 534	194	158	new	this study
SRR12709615	81	69	26	66	182	124	86	205	this study
SRR12709614	41	133	51	19	510	4	160	new	this study
SRR12709613	41	133	51	19	510	4	160	new	this study
SRR12709611	37	23	7	~165	659	26	new	new	this study
SRR12709610	37	23	7	~165	659	26	new	new	this study
SRR12709609	37	23	7	~165	659	26	new	new	this study
SRR12709608	37	23	7	~165	659	26	new	new	this study
SRR12709607	4	4	4	4	139	4	89	18	this study
SRR12709606	~69	12	9	19	410	290	165	new	this study
SRR12709605	37	133	44	12	~379	278	160	new	this study
SRR12709604	4	4	4	4	~139	4	89	new	this study
SRR12709603	4	4	4	4	3	4	89	17	this study
SRR12709602	4	4	4	4	139	4	89	18	this study
SRR12709600	4	4	4	4	~139	4	89	new	this study
SRR12709599	20	7	20	23	186?	45	37	new	this study

WGS	MLST							Reference
	aspA	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST	
SRR12709598	20	7	20	23	186?	45	37	new	this study
SRR12709597	20	7	20	7	186	8	7	new	this study
SRR12709596	4	12	1	19	~418	213	160	new	this study
SRR12709595	153	4	40	19	497?	98	158	new	this study
SRR12709594	153	4	40	19	489?	98	158	new	this study
SRR12709593	153	4	40	19	528?	98	158	new	this study
SRR12709637	182	4	40	136	413?	211	87	new	this study
SRR10215626	177	39	40	123	~511	194	24	new	[1], this study
SRR12709636	47	217	4	129	~694	123	37	new	this study
SRR12709634	47	217	9	129	~615	123	37	new	this study
SRR10215625	47	217	4	129	694?	123	37	new	[1], this study
SRR12709633	47	217	9	129	~632	123	37	new	this study
SRR12709632	~229	4	34	4	488	102	63	new	this study
SRR12709631	153	39	1	19	184	11	~9	new	this study
SRR12709630	20	39	11	11	513	26	55	new	this study
GCF_900187115.1	1	1	1	1	1, 142	1	1	new	NA, [9][†], [10][§]
GCF_004283125.1	236	161	1	183	521	296	207	537	[2]
GCF_004283115.1	332	8	1	166	685	367	292	675	[2]
GCF_013363865.1	NA	NA	NA	NA	NA	NA	NA	NA	NA
GCF_013363855.1	NA	NA	NA	NA	NA	NA	NA	NA	NA
GCF_000215345.2	150	4	1	122	~370	194	52	-	[3,6]
ERR3523212	8	8	8	8	9	9	8	47	[4,6]
ERR3523199	13	4	40	15	545	102	158	-	[5,6]
ERR3523202	234	15	26	164	375	260	176	460	[5,6]
ERR3523203	23	44	24	2	137	62	20	107	[5,6]
ERR3523197	20	2	1	~15	28	71	165	-	[5,6]
ERR3523210	20	25	7	2	~465	32	2	-	[5,6]
ERR3523201	23	44	24	44	112	35	20	510	[5,6]
ERR3523204	20	~39	11	11	98	87	178	-	[6]
ERR3523214	20	12	11	19	~692	127	88	-	[6]
ERR3523198	240	133	40	12	~376	278	160	-	[6,7]
ERR3523196	23	5	24	44	112	35	55	-	[6,7]

Fortsetzung **Table S1**

WGS	MLST								References
	aspA	*atpA*	*glnA*	*gltA*	*glyA*	*pgm*	*tkt*	ST	
ERR3523205	23	7	33	38	~745	~120	174	-	[6, 7]
ERR3523200	5	5	24	27	80	62	20	-	[6, 7]
ERR3523213	20	39	11	15	~666	~26	2	-	[6, 7]
ERR3523209	26	22	4	44	~24	55	165	-	[6, 7]
ERR3523206	23	44	24	15	124	55	20	108	[6, 7]
ERR3523216	21	22	21	24	48	27	25	94	[6, 7]
ERR3523215	10	14	41	30	~602	~263	~31	-	[6, 7]
ERR3523207	59	47	26	53	~205	88	60	-	[6, 8]
ERR3523217	55	45	26	48	113	85	59	172	[6, 8]
ERR3523211	4	164	40	19	528	102	4	-	[6, 8]
ERR3523208	10	20	11	19	192	134	11	-	[6, 8]

MIC- minimal inhibitory concentration; ERY - erythromycin; GEN - gentamicin, CIP - ciprofloxacin; DC - doxycycline; TC - tetracycline; STX - streptomycin; AMP - ampicillin; CTX - cefotaxime; ST - sequence type; NA - not available/ not applicable; + - positive; Ø – negative; # - antimicrobial susceptibility values and result of the urease assay were taken from reference [9]; § - results of the MLST analysis were taken from reference [10] * - no specific MIC values available only phenotype

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Table S2 - Results of the ANI analysis

	NCTC_11168	ATCC_43158	LMG_25534	NCTC_12481	17CS1068-1	17CS1095	16CS0861-1	RMI	Ab_CR1132	Ab_DQ40A1	Ab_4211	Ab_1711	Ab_2211	16CS0367-1-AR	16CS0817-2
C.jejuni_NCTC_11168	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.cryaerophilus_ATCC_43158	66.09	100	-	-	-	-	-	-	-	-	-	-	-	-	-
A.trophiarum_LMG_25534	66.00	85.34	100	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_NCTC_12481	66.54	78.17	77.22	100	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1068-1	65.95	78.15	77.27	97.53	100	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1095	65.95	78.27	77.29	97.56	98.47	100	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0861-1	65.90	78.20	76.21	97.48	98.40	98.48	100	-	-	-	-	-	-	-	-
A.butzleri_RMI	65.83	78.03	77.19	97.40	97.91	98.18	97.85	100	-	-	-	-	-	-	-
A.butzleri_Ab_CR1132	65.79	78.06	77.22	97.46	98.53	98.45	98.52	97.81	100	-	-	-	-	-	-
A.butzleri_Ab_DQ40A1	65.84	78.16	77.24	97.23	98.14	98.16	98.10	97.55	98.05	100	-	-	-	-	-
A.butzleri_Ab_4211	65.98	78.08	77.30	97.41	97.60	97.68	97.66	97.43	97.61	97.52	100	-	-	-	-
A.butzleri_Ab_1711	65.81	78.21	77.22	97.37	97.94	98.37	98.00	97.84	98.03	97.79	97.61	100	-	-	-
A.butzleri_Ab_2211	65.79	77.96	77.17	97.35	97.16	97.08	97.08	96.75	96.96	96.98	97.07	97.00	100	-	-
A.butzleri_16CS0367-1-AR	65.82	78.03	77.27	97.40	98.69	98.52	98.39	97.92	98.35	98.17	97.61	97.98	97.01	100	-
A.butzleri_16CS0817-2	65.81	78.24	77.31	97.37	98.25	98.64	98.39	97.92	98.34	97.91	97.61	98.08	96.95	98.34	100
A.butzleri_16CS0831-4-1	65.59	77.99	77.14	97.15	96.97	97.01	97.00	96.92	97.10	96.90	97.13	96.98	97.65	97.12	97.01
A.butzleri_16CS0820-2	65.88	77.96	77.27	97.48	97.76	97.88	97.73	97.77	97.76	97.58	97.74	97.78	96.89	97.78	97.70
A.butzleri_Ab_DQ20dA1	65.79	77.93	77.11	97.52	97.16	97.32	97.20	97.13	97.19	96.97	97.25	97.11	98.14	97.20	97.15
A.butzleri_Ab_CR424	65.91	78.13	77.24	97.46	97.51	97.58	97.53	97.40	97.51	97.39	97.57	97.28	97.57	97.38	97.26
A.butzleri_17CS0916-1	65.76	78.08	77.27	97.66	98.13	98.07	98.22	98.00	98.12	97.92	97.98	97.92	97.36	98.14	97.97
A.butzleri_17CS1193-1	65.84	78.08	77.20	97.56	98.58	98.42	98.66	97.92	99.55	98.11	97.63	98.05	97.04	98.49	98.35
A.butzleri_16CS0823-1	65.96	78.02	77.19	97.50	97.82	97.81	97.79	97.77	97.76	97.59	97.75	97.77	96.92	97.77	97.69
A.butzleri_17CS1206-1	65.81	78.12	77.27	97.71	98.05	98.01	98.01	97.93	97.92	97.86	97.72	97.93	97.07	98.05	97.90
A.butzleri_16CS0823-2	65.90	78.12	77.20	97.50	98.32	98.32	98.20	98.06	98.14	98.02	97.62	97.97	97.05	98.28	98.19
A.butzleri_17CS1205-1	65.84	78.18	77.21	97.70	98.05	98.01	98.00	97.95	97.95	97.89	97.72	97.93	97.05	98.08	97.88
A.butzleri_17CS1200-1	65.83	78.22	77.24	97.56	98.36	98.48	98.65	97.96	98.63	98.10	97.75	98.16	97.14	98.43	98.28
A.butzleri_17CS1208-3	65.83	78.12	77.24	97.71	98.04	98.08	97.97	97.95	97.93	98.21	97.72	97.95	97.01	98.05	97.89
A.butzleri_Ab_CR892	65.90	78.17	77.23	97.55	97.60	97.64	97.46	97.40	97.48	97.32	97.61	97.51	97.22	97.52	97.46
A.butzleri_16CS0375-1	65.72	78.04	77.27	97.41	98.69	98.53	98.40	97.94	98.35	98.16	97.62	98.00	97.00	100	98.33

Fortsetzung **Table S2**

	16CS0831-4-1	16CS0820-2	Ab_DQ20dA1	Ab_CR424	17CS0916-1	17CS1193-1	16CS0823-1	17CS1206-1	16CS0823-2	17CS1205-1	17CS1200-1	17CS1208-3	Ab_CR892	16CS0375-1	6V
C.jejuni_NCTC_11168	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.cryaerophilus_ATCC_43158	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.trophiarum_LMG_25534	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_NCTC_12481	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1068-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1095	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0861-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_RMI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR1132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ40A1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_4211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_1711	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_2211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0367-1-AR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0817-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0831-4-1	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0820-2	96.91	100	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ20dA1	97.90	96.99	100	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR424	97.03	97.42	97.47	100	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0916-1	97.18	98.05	97.32	97.69	100	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1193-1	96.96	97.81	97.27	97.57	98.20	100	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0823-1	96.93	100	97.00	97.42	98.05	97.82	100	-	-	-	-	-	-	-	-
A.butzleri_17CS1206-1	96.96	97.94	97.31	97.60	98.20	97.97	97.95	100	-	-	-	-	-	-	-
A.butzleri_16CS0823-2	97.05	97.87	97.22	97.47	98.07	98.27	97.85	98.03	100	-	-	-	-	-	-
A.butzleri_17CS1205-1	96.94	97.96	97.25	97.61	98.19	97.97	97.96	100	98.06	100	-	-	-	-	-
A.butzleri_17CS1200-1	97.07	97.75	97.28	97.54	98.20	98.98	97.75	98.09	98.19	98.11	100	-	-	-	-
A.butzleri_17CS1208-3	96.92	97.98	97.30	97.59	98.19	97.95	97.97	100	98.06	99.99	98.10	100	-	-	-
A.butzleri_Ab_CR892	97.03	97.62	97.13	97.48	97.87	97.62	97.64	97.73	97.56	97.70	97.62	97.72	100	-	-
A.butzleri_16CS0375-1	97.13	97.76	97.28	97.36	98.13	98.48	97.75	98.03	98.30	98.05	98.42	98.02	97.53	100	-

Fortsetzung **Table S2**

	17CS1092-1	Ab_DQ64A1	Ab_A111	Ab_CR502	16CS0857-1	Ab_A103	17CS1067	17CS0824	17CS1197-2	16CS0370-1	16CS0375-2	16CS0815-2	17CS0916-2	17CS0965-1	17CS1167	Ab_4511
C.jejuni_NCTC_11168	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.cryaerophilus_ATCC_43158	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.trophiarum_LMG_25534	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_NCTC_12481	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1068-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1095	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0861-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_RMI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR1132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ40A1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_4211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_1711	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_2211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0367-1-AR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0817-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0831-4-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0820-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ20dA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR424	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0916-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1193-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0823-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1206-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0823-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1205-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1200-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1208-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR892	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0375-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fortsetzung **Table S2**

	17CS1168	17CS1205-2	17CS1197	Ab_CR1143	16CS0847-3	17CS1193-3	Ab_2811	17CS1068-2	Ab_3711	17CS1198-2	16CS0367-AR	17CS1193-2	17CS1198-1	7h1h	Ab_CR604	RMIII
C.jejuni_NCTC_11168	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.cryaerophilus_ATCC_43158	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.trophiarum_LMG_25534	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_NCTC_12481	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1068-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1095	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0861-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_RMI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR1132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ40A1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_4211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_1711	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_2211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0367-1-AR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0817-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0831-4-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0820-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ20dA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR424	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0916-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1193-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0823-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1206-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0823-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1205-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1200-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1208-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR892	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0375-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fortsetzung **Table S2**

	17CS1056	Ab_CR461	17CS1092-2	Ab_CR891	55	17CS1197-3	16CS0821-2	Ab_CR641	16CS0820-1	17CS1201	17CS1198-3	17CS1066	17CS0965-B	Ab_DQ31A1	Ab_1426_2003	16CS0831-1-1
C.jejuni_NCTC_11168	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.cryaerophilus_ATCC_43158	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.trophiarum_LMG_25534	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_NCTC_12481	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_17CS1068-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_17CS1095	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_16CS0861-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_RMI	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_Ab_CR1132	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_Ab_DQ40A1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_Ab_4211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_Ab_1711	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_Ab_2211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_16CS0367-1-AR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_16CS0817-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_16CS0831-4-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_16CS0820-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_Ab_DQ20dA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_Ab_CR424	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_17CS0916-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_17CS1193-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_16CS0823-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_17CS1206-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_16CS0823-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_17CS1205-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_17CS1200-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_17CS1208-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_Ab_CR892	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butleri_16CS0375-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fortsetzung **Table S2**

	NCTC_11168	ATCC_43158	LMG_25534	NCTC_12481	17CS1068-1	17CS1095	16CS0861-1	RMI	Ab_CR1132	Ab_DQ40A1	Ab_4211	Ab_1711	Ab_2211	16CS0367-1-AR	16CS0817-2
A.butzleri_6V	65.89	77.91	77.15	97.76	97.39	97.42	97.38	97.06	97.24	97.10	97.38	97.18	97.53	97.24	97.18
A.butzleri_17CS1092-1	65.91	78.13	77.31	97.38	98.35	99.26	98.42	98.04	98.37	98.02	97.68	98.24	96.96	98.42	98.61
A.butzleri_Ab_DQ64A1	65.82	78.02	77.33	97.24	97.61	97.84	97.60	97.62	97.58	97.40	97.46	97.56	96.85	97.60	97.56
A.butzleri_Ab_A111	65.88	78.07	77.18	97.47	98.01	98.07	98.00	98.02	97.98	97.74	97.59	97.92	96.90	97.92	97.82
A.butzleri_Ab_CR502	65.89	78.11	77.30	97.39	97.85	97.94	97.79	97.70	97.90	97.55	97.47	97.85	96.95	97.70	97.69
A.butzleri_16CS0857-1	65.83	78.15	77.25	97.70	98.12	98.19	98.12	97.95	98.02	97.95	97.84	98.04	97.38	98.15	97.87
A.butzleri_Ab_A103	65.97	78.12	77.20	97.41	98.05	98.16	98.04	97.87	98.05	97.80	97.63	97.92	97.20	97.96	97.86
A.butzleri_17CS1067	65.90	78.13	77.23	97.56	99.99	98.44	98.43	97.90	98.52	98.16	97.62	97.95	97.20	98.70	98.29
A.butzleri_17CS0824	65.83	78.04	77.13	97.57	98.79	98.46	98.44	97.99	98.44	98.11	97.54	98.03	97.08	98.52	98.26
A.butzleri_17CS1197-2	65.87	78.11	77.31	97.44	98.42	98.69	98.45	98.00	98.38	98.21	97.63	98.20	96.84	98.50	98.42
A.butzleri_16CS0370-1	65.85	78.03	77.23	97.40	98.68	98.54	98.40	97.95	98.36	98.19	97.61	98.03	97.01	100	98.36
A.butzleri_16CS0375-2	65.89	78.05	77.22	97.42	98.66	98.50	98.37	97.88	98.34	98.15	97.58	97.95	96.99	99.98	98.30
A.butzleri_16CS0815-2	65.89	78.14	77.27	97.42	98.36	98.48	98.24	97.91	98.24	98.16	97.60	98.04	97.05	98.44	98.27
A.butzleri_17CS0916-2	65.71	78.08	77.26	97.64	98.18	98.07	98.18	97.99	98.11	97.92	97.92	97.94	97.36	98.13	97.98
A.butzleri_17CS0965-1	65.82	78.10	77.20	97.40	98.38	99.05	98.18	98.01	98.34	98.11	97.64	98.13	96.93	98.40	98.74
A.butzleri_17CS1167	65.85	78.11	77.22	97.54	100	98.45	98.44	97.89	98.51	98.15	97.60	97.96	97.17	98.69	98.26
A.butzleri_Ab_4511	65.82	78.06	77.28	97.56	97.75	97.77	97.65	97.15	97.58	97.56	97.78	97.53	97.19	97.52	97.54
A.butzleri_17CS1168	65.77	78.16	77.20	97.41	98.24	98.53	98.34	97.84	98.33	98.11	97.76	98.03	96.97	98.42	98.32
A.butzleri_17CS1205-2	65.81	78.10	77.23	97.71	98.04	98.10	97.96	97.96	97.97	97.91	97.75	97.95	98.04	98.07	97.92
A.butzleri_17CS1197	65.87	78.08	77.29	97.45	98.40	98.68	98.41	98.00	98.35	98.19	97.59	98.18	96.86	98.50	98.40
A.butzleri_Ab_CR1143	65.86	78.06	77.18	97.70	97.90	97.90	97.87	97.71	97.79	97.66	97.61	97.60	97.29	97.72	97.54
A.butzleri_16CS0847-3	65.82	78.09	77.25	97.50	98.35	98.47	98.34	97.95	98.45	98.25	97.69	98.13	97.01	98.40	98.34
A.butzleri_17CS1193-3	66.03	78.01	77.17	97.54	98.72	98.50	98.56	97.87	98.42	98.12	97.61	98.14	96.99	98.97	98.39
A.butzleri_Ab_2811	66.08	78.32	77.56	97.35	97.50	97.47	97.61	97.21	97.43	97.35	97.59	97.30	97.26	97.38	97.29
A.butzleri_17CS1068-2	65.69	78.08	77.24	97.55	100	98.45	98.44	97.88	98.53	98.15	97.66	97.97	97.14	98.71	98.27
A.butzleri_Ab_3711	66.06	78.14	77.21	97.43	97.71	97.88	97.78	97.77	97.69	97.43	97.61	97.76	97.04	97.77	97.52
A.butzleri_17CS1198-2	65.83	77.99	77.17	97.52	98.64	98.46	98.38	97.98	98.52	98.10	97.61	98.12	97.16	98.54	98.36
A.butzleri_16CS0367-AR	65.96	78.03	77.21	97.39	98.68	98.55	98.40	97.91	98.46	98.19	97.58	98.02	97.02	99.99	98.36
A.butzleri_17CS1193-2	65.78	78.12	77.23	97.59	98.60	98.44	98.65	97.96	99.56	98.14	97.64	97.07	97.06	98.50	98.35

Fortsetzung **Table S2**

	16CS0831-4-1	16CS0820-2	Ab_DQ20dA1	Ab_CR424	17CS0916-1	17CS1193-1	16CS0823-1	17CS1206-1	16CS0823-2	17CS1205-1	17CS1200-1	17CS1208-3	Ab_CR892	16CS0375-1	6V
A.butzleri_6V	97.09	97.19	97.49	97.35	97.41	97.34	97.22	97.41	97.28	97.42	97.36	97.40	97.62	97.25	100
A.butzleri_17CS1092-1	97.00	97.79	97.09	97.45	97.99	98.46	97.80	97.80	98.42	97.92	98.38	97.90	97.46	98.40	97.30
A.butzleri_Ab_DQ64A1	96.81	97.72	96.94	97.37	97.84	97.73	97.72	97.82	97.66	97.81	97.66	97.83	97.54	97.64	97.07
A.butzleri_Ab_A111	97.02	97.81	97.17	97.44	98.04	98.12	97.82	97.93	97.95	97.94	98.05	97.90	97.54	97.93	97.17
A.butzleri_Ab_CR502	96.91	97.97	97.13	97.42	98.01	97.93	97.96	97.90	97.85	97.94	97.91	97.92	97.35	97.70	97.17
A.butzleri_16CS0857-1	97.20	97.96	97.43	97.82	98.67	98.14	97.97	98.17	98.15	98.17	98.14	98.18	97.95	98.14	97.57
A.butzleri_Ab_A103	97.11	97.75	97.34	97.65	98.13	98.09	97.76	97.89	98.05	97.91	98.08	97.89	97.70	97.98	97.36
A.butzleri_17CS1067	96.97	97.76	97.19	97.58	98.22	98.58	97.76	98.03	98.21	98.02	98.39	98.04	97.59	98.69	97.41
A.butzleri_17CS0824	97.06	97.73	97.32	97.59	98.09	98.58	97.72	98.09	98.25	98.06	98.42	98.07	97.60	98.51	97.41
A.butzleri_17CS1197-2	97.10	97.80	97.17	97.36	98.17	98.48	97.80	97.97	98.45	98.01	98.47	97.98	97.53	98.49	97.21
A.butzleri_16CS0370-1	97.12	97.78	97.20	97.36	98.14	98.49	97.77	98.06	98.29	98.08	98.43	98.06	97.52	100	97.21
A.butzleri_16CS0375-2	97.09	97.75	97.17	97.30	98.11	98.45	97.75	98.01	98.22	98.03	98.39	98.00	97.50	99.98	97.20
A.butzleri_16CS0815-2	97.02	97.75	97.19	97.52	98.09	98.38	97.75	98.01	98.34	98.05	98.29	98.01	97.52	98.47	97.31
A.butzleri_17CS0916-2	97.19	98.05	97.36	97.71	100	98.20	98.04	98.20	98.07	98.20	98.19	98.20	97.87	98.13	97.39
A.butzleri_17CS0965-1	96.98	97.77	97.06	97.44	98.11	98.50	97.80	97.92	98.45	97.93	98.43	97.92	97.64	98.41	97.24
A.butzleri_17CS1167	96.99	97.77	97.19	97.55	98.19	98.59	97.76	98.07	98.23	98.05	98.38	98.07	97.59	98.69	97.41
A.butzleri_Ab_4511	97.07	97.81	97.36	97.73	97.89	97.74	97.84	97.61	97.59	97.72	97.75	97.61	97.84	97.52	97.42
A.butzleri_17CS1168	97.01	97.79	97.07	97.47	98.08	98.46	97.79	98.06	98.25	98.04	98.33	98.05	97.39	98.45	97.25
A.butzleri_17CS1205-2	96.98	97.98	97.30	97.62	98.21	97.94	97.98	100	98.08	100	98.13	100	97.71	98.05	97.43
A.butzleri_17CS1197	97.07	97.77	97.15	97.32	98.13	98.44	97.77	97.98	98.43	98.04	98.44	98.00	97.51	98.49	97.20
A.butzleri_Ab_CR1143	97.18	97.73	97.42	97.68	98.07	97.87	97.72	97.97	97.75	97.97	97.87	97.95	97.92	97.71	97.58
A.butzleri_16CS0847-3	97.03	97.75	97.19	97.47	98.15	98.49	97.77	98.03	98.28	98.04	98.40	97.03	97.57	98.41	97.34
A.butzleri_17CS1193-3	97.02	97.76	97.19	97.60	98.28	98.65	97.76	98.04	98.21	98.05	98.59	98.02	97.62	98.98	97.29
A.butzleri_Ab_2811	96.96	97.50	97.22	97.52	97.75	97.60	97.52	97.59	97.38	97.60	97.66	97.60	97.70	97.37	97.39
A.butzleri_17CS1068-2	97.97	97.79	97.19	97.54	98.20	98.62	97.78	98.03	98.25	98.01	98.39	98.04	97.56	98.70	97.37
A.butzleri_Ab_3711	97.14	97.85	97.26	97.50	97.94	97.91	97.88	97.80	97.87	97.81	97.79	97.77	97.58	97.76	97.32
A.butzleri_17CS1198-2	96.96	97.78	97.24	97.58	98.19	98.63	97.77	98.07	98.28	98.05	98.43	98.05	97.66	98.52	97.34
A.butzleri_16CS0367-AR	97.09	97.76	97.18	97.34	98.16	98.51	97.75	98.05	98.28	98.08	98.47	98.05	97.57	99.99	97.22
A.butzleri_17CS1193-2	96.98	97.81	97.30	97.58	98.20	100	97.83	97.98	98.29	97.98	98.97	97.98	97.66	98.49	97.36

Fortsetzung **Table S2**

	17CS1092-1	Ab_DQ64A1	Ab_A111	Ab_CR502	16CS0857-1	Ab_A103	17CS1067	17CS0824	17CS1197-2	16CS0370-1	16CS0375-2	16CS0815-2	17CS0916-2	17CS0965-1	17CS1167	Ab_4511
A.butzleri_6V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1092-1	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ64A1	97.72	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_A111	98.09	97.70	100	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR502	97.83	97.58	98.30	100	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0857-1	98.01	97.91	98.01	98.01	100	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_A103	98.06	97.70	98.81	98.27	98.00	100	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1067	98.37	97.59	98.00	97.85	98.14	98.04	100	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0824	98.28	97.69	98.00	97.81	98.16	98.06	98.78	100	-	-	-	-	-	-	-	-
A.butzleri_17CS1197-2	98.73	97.62	97.96	97.78	98.06	97.98	98.37	98.39	100	-	-	-	-	-	-	-
A.butzleri_16CS0370-1	98.42	97.61	97.94	97.70	98.13	97.99	98.69	98.54	98.49	100	-	-	-	-	-	-
A.butzleri_16CS0375-2	98.38	97.53	97.89	97.68	98.11	97.95	98.67	98.51	98.44	99.98	100	-	-	-	-	-
A.butzleri_16CS0815-2	98.44	97.59	97.86	97.85	98.10	97.97	98.35	98.36	98.30	98.42	98.40	100	-	-	-	-
A.butzleri_17CS0916-2	97.99	97.83	98.03	98.01	98.67	98.13	98.21	98.09	98.18	98.14	98.10	98.08	100	-	-	-
A.butzleri_17CS0965-1	99.05	97.74	98.02	97.72	98.11	98.15	98.33	98.37	98.60	98.41	98.40	98.37	98.11	100	-	-
A.butzleri_17CS1167	98.34	97.60	98.01	97.85	98.11	98.06	99.99	98.80	98.40	98.69	98.66	98.36	98.19	98.39	100	-
A.butzleri_Ab_4511	97.69	97.48	97.49	97.60	98.05	97.65	97.75	97.72	97.54	97.51	97.48	97.68	97.90	97.54	97.75	100
A.butzleri_17CS1168	98.47	97.68	97.88	97.81	98.09	97.98	98.25	98.35	98.42	98.43	98.38	98.56	98.09	98.37	98.24	97.65
A.butzleri_17CS1205-2	97.94	97.83	97.94	97.92	98.19	97.93	98.04	98.09	98.02	98.09	98.02	98.06	98.21	97.96	98.08	97.64
A.butzleri_17CS1197	98.70	97.61	97.93	97.87	98.05	97.97	98.35	98.36	100	98.49	98.43	98.32	98.14	98.57	98.38	97.53
A.butzleri_Ab_CR1143	97.72	97.69	97.80	97.64	98.10	97.78	97.90	97.87	97.79	97.71	97.68	97.79	98.07	97.52	97.90	98.40
A.butzleri_16CS0847-3	98.54	97.67	97.96	97.83	98.10	98.06	98.35	98.38	98.47	98.41	98.38	98.79	98.16	98.41	98.35	97.68
A.butzleri_17CS1193-3	98.40	97.60	98.04	97.87	98.18	98.14	98.75	98.68	98.46	98.97	98.95	98.34	98.28	98.43	98.74	97.68
A.butzleri_Ab_2811	97.48	97.28	97.50	97.45	97.86	97.62	97.53	97.52	97.35	97.40	97.33	97.45	97.75	97.44	97.50	98.81
A.butzleri_17CS1068-2	98.34	97.57	98.06	97.89	98.12	98.08	99.99	98.81	98.44	98.71	98.68	98.35	98.20	98.41	100	97.76
A.butzleri_Ab_3711	97.91	97.76	97.77	97.73	97.98	97.87	97.72	97.80	97.75	97.73	97.73	97.74	97.96	97.85	97.72	97.66
A.butzleri_17CS1198-2	98.29	97.70	98.04	97.85	98.18	98.02	97.65	98.65	98.44	98.52	98.48	98.29	98.17	98.45	98.63	97.72
A.butzleri_16CS0367-AR	98.44	97.64	97.92	97.72	98.14	97.98	98.68	98.52	98.50	99.99	99.97	98.43	98.16	98.43	98.68	97.51
A.butzleri_17CS1193-2	98.49	97.75	98.12	97.96	98.15	98.13	98.59	98.60	98.49	98.50	98.46	98.31	98.20	98.51	98.60	97.77

Fortsetzung **Table S2**

	17CS1168	17CS1205-2	17CS1197	Ab_CR1143	16CS0847-3	17CS1193-3	Ab_2811	17CS1068-2	Ab_3711	17CS1198-2	16CS0367-AR	17CS1193-2	17CS1198-1	7h1h	Ab_CR604	RMIII
A.butzleri_6V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1092-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ64A1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_A111	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR502	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0857-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_A103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1067	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0824	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1197-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0370-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0375-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0815-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0916-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0965-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1167	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_4511	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1168	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1205-2	98.07	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1197	98.37	98.05	100	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR1143	97.76	97.97	97.67	100	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0847-3	98.67	98.08	98.46	97.84	100	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1193-3	98.27	98.07	98.44	97.88	98.44	100	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_2811	97.53	97.60	97.37	98.26	97.56	97.59	100	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1068-2	98.26	97.54	98.42	97.89	98.37	98.76	97.53	100	-	-	-	-	-	-	-	-
A.butzleri_Ab_3711	97.71	97.80	97.67	97.81	97.89	97.83	97.57	97.75	100	-	-	-	-	-	-	-
A.butzleri_17CS1198-2	98.33	98.07	98.41	97.85	98.47	98.77	97.47	98.65	97.78	100	-	-	-	-	-	-
A.butzleri_16CS0367-AR	98.46	98.06	98.50	97.70	98.41	98.94	97.43	98.70	97.76	98.50	100	-	-	-	-	-
A.butzleri_17CS1193-2	98.47	98.00	98.45	97.89	98.54	98.65	97.63	98.63	97.91	98.65	98.50	100	-	-	-	-

Fortsetzung **Table S2**

	17CS1056	Ab_CR461	17CS1092-2	Ab_CR891	55	17CS1197-3	16CS0821-2	Ab_CR641	16CS0820-1	17CS1201	17CS1198-3	17CS1066	17CS0965-B	Ab_DQ31A1	Ab_1426_2003	16CS0831-1-1
A.butzleri_6V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1092-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_DQ64A1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_A111	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR502	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0857-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_A103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1067	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0824	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1197-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0370-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0375-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0815-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0916-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS0965-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1167	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_4511	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1168	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1205-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR1143	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0847-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1193-3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_2811	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1068-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_3711	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1198-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0367-AR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1193-2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fortsetzung **Table S2**

	NCTC_11168	ATCC_43158	LMG_25534	NCTC_12481	17CS1068-1	17CS1095	16CS0861-1	RMI	Ab_CR1132	Ab_DQ40A1	Ab_4211	Ab_1711	Ab_2211	16CS0367-1-AR	16CS0817-2
A.butzleri_17CS1198-1	65.85	78.05	77.17	97.52	98.64	98.46	98.37	97.97	98.51	98.11	97.59	98.13	97.15	98.54	98.36
A.butzleri_7h1h	66.83	78.13	77.31	97.44	98.27	98.37	98.35	97.95	98.33	98.17	97.60	98.00	97.05	98.28	98.25
A.butzleri_Ab_CR604	65.81	78.09	77.21	97.56	98.04	98.13	98.09	97.82	98.04	97.87	97.67	97.76	97.25	97.96	97.85
A.butzleri_RMIII	65.77	78.04	77.14	97.38	97.91	98.19	97.90	99.97	97.90	97.61	97.46	97.82	96.78	97.91	97.96
A.butzleri_17CS1056	65.82	78.22	77.30	97.53	98.39	98.37	98.59	97.89	99.27	98.19	97.64	98.01	97.09	98.36	98.33
A.butzleri_Ab_CR461	65.80	78.13	77.30	97.44	97.64	97.67	97.56	97.31	97.39	97.33	97.49	97.49	96.98	97.42	97.23
A.butzleri_17CS1092-2	65.95	78.08	77.28	97.39	98.35	99.24	98.41	98.04	98.38	98.05	97.70	98.26	96.98	98.41	98.61
A.butzleri_Ab_CR891	65.69	78.05	77.18	97.36	97.23	97.43	97.36	97.01	97.23	97.13	97.33	97.12	97.66	97.21	97.13
A.butzleri_55	65.75	77.88	76.95	97.72	97.46	97.47	97.42	97.19	97.27	97.09	97.23	97.23	97.32	97.20	97.29
A.butzleri_17CS1197-3	65.84	78.11	77.33	97.43	98.42	98.69	98.46	98.01	98.38	98.22	97.63	98.19	96.86	98.46	98.44
A.butzleri_16CS0821-2	65.82	78.14	77.24	97.48	97.84	97.96	97.82	97.89	97.74	97.56	97.86	97.80	97.00	97.85	97.77
A.butzleri_Ab_CR641	65.78	78.08	77.20	97.59	97.79	97.82	97.84	97.56	97.74	97.53	97.79	97.69	97.32	97.60	97.63
A.butzleri_16CS0820-1	65.89	78.09	77.25	97.49	97.86	97.98	97.84	97.93	97.78	97.58	97.89	97.81	97.03	97.86	97.81
A.butzleri_17CS1201	65.84	78.16	77.19	97.58	98.37	98.47	98.64	97.91	98.65	98.15	97.74	98.17	97.17	98.54	98.25
A.butzleri_17CS1198-3	65.83	78.01	77.25	97.50	98.66	98.48	98.40	97.98	98.54	98.13	97.62	98.14	97.13	98.54	98.36
A.butzleri_17CS1066	65.81	78.09	77.31	97.55	100	98.44	98.43	97.92	98.52	98.16	97.59	97.97	97.18	98.69	98.26
A.butzleri_17CS0965-B	65.87	78.14	77.20	97.40	98.06	98.15	98.02	98.10	98.04	97.79	97.79	98.04	96.96	97.96	97.86
A.butzleri_Ab_DQ31A1	65.70	77.95	77.17	97.41	97.25	97.28	97.08	97.04	97.07	96.93	97.22	97.11	98.03	97.10	97.08
A.butzleri_Ab_1426_2003	65.93	78.07	77.33	97.82	97.40	97.46	97.39	97.24	97.39	97.14	97.31	97.29	97.36	97.21	97.30
A.butzleri_16CS0831-1-1	65.75	78.04	77.22	97.44	98.68	98.54	98.40	97.96	98.40	98.21	97.62	98.04	97.03	99.99	98.37

Fortsetzung **Table S2**

	16CS0831-4-1	16CS0820-2	Ab_DQ20dA1	Ab_CR424	17CS0916-1	17CS1193-1	16CS0823-1	17CS1206-1	16CS0823-2	17CS1205-1	17CS1200-1	17CS1208-3	Ab_CR892	16CS0375-1	6V
A.butzleri_17CS1198-1	96.94	97.77	97.25	97.57	98.17	98.62	97.76	98.08	98.29	98.06	98.42	98.06	97.65	98.53	97.40
A.butzleri_7h1h	96.94	97.74	97.13	97.46	98.07	98.40	97.74	97.98	98.20	97.98	98.40	97.99	97.48	98.26	97.26
A.butzleri_Ab_CR604	97.19	97.81	97.35	97.58	98.30	98.09	97.81	98.09	98.08	98.08	98.11	98.07	97.77	97.99	97.47
A.butzleri_RMIII	96.88	97.80	97.08	97.39	97.98	97.93	97.80	97.92	98.09	97.91	97.99	97.92	97.44	97.92	97.06
A.butzleri_17CS1056	97.07	97.74	97.27	97.56	98.04	99.23	97.76	97.96	98.24	98.00	98.83	98.00	97.58	98.37	97.31
A.butzleri_Ab_CR461	96.99	97.56	97.18	97.49	97.85	97.57	97.58	97.65	97.59	97.64	97.59	97.63	97.69	97.41	97.32
A.butzleri_17CS1092-2	97.01	97.77	97.11	97.43	98.00	98.44	97.78	97.91	98.40	97.93	98.38	97.90	97.47	98.40	97.30
A.butzleri_Ab_CR891	97.41	97.08	97.59	97.37	97.47	97.30	97.08	97.52	97.27	97.49	97.37	97.52	97.38	97.21	97.36
A.butzleri_55	97.15	97.25	97.43	97.14	97.41	97.38	97.26	97.49	97.37	97.48	97.36	97.48	97.43	97.20	98.05
A.butzleri_17CS1197-3	97.10	97.80	97.17	97.34	98.15	98.48	97.81	97.98	98.45	98.03	98.47	97.98	97.52	98.45	97.22
A.butzleri_16CS0821-2	96.96	98.83	97.08	97.44	98.02	97.90	98.86	97.89	97.79	97.91	97.87	97.91	97.56	97.84	97.28
A.butzleri_Ab_CR641	97.19	97.80	97.39	97.68	98.09	97.80	97.80	97.94	97.73	97.94	97.84	97.93	97.84	97.61	97.58
A.butzleri_16CS0820-1	96.99	98.85	97.10	97.45	98.04	97.93	98.87	97.93	97.81	97.95	97.92	97.96	97.59	97.85	97.28
A.butzleri_17CS1201	97.07	97.74	97.29	97.52	98.18	98.99	97.74	98.08	98.18	98.11	100	98.09	97.63	98.43	97.38
A.butzleri_17CS1198-3	96.96	97.81	97.24	97.60	98.17	98.65	97.80	98.09	98.31	98.07	98.44	98.07	97.64	98.53	97.36
A.butzleri_17CS1066	96.96	97.75	97.17	97.55	98.20	98.60	97.75	98.06	98.21	97.04	98.40	98.06	97.60	98.69	97.41
A.butzleri_17CS0965-B	97.13	97.92	97.21	97.56	98.14	98.09	97.93	97.99	98.11	97.99	98.12	97.99	97.71	98.03	97.25
A.butzleri_Ab_DQ31A1	97.76	97.01	98.18	97.35	97.30	97.22	97.07	97.33	97.09	97.31	97.17	97.30	97.34	97.12	97.41
A.butzleri_Ab_1426_2003	97.07	97.37	97.41	97.37	97.52	97.45	97.39	97.55	97.38	97.54	97.41	97.53	97.53	97.22	97.97
A.butzleri_16CS0831-1-1	97.12	97.75	97.21	97.38	98.18	98.52	97.75	98.07	98.31	98.09	98.47	98.06	97.51	100	97.27

Fortsetzung **Table S2**

	17CS1092-1	Ab_DQ64A1	Ab_A111	Ab_CR502	16CS0857-1	Ab_A103	17CS1067	17CS0824	17CS1197-2	16CS0370-1	16CS0375-2	16CS0815-2	17CS0916-2	17CS0965-1	17CS1167	Ab_4511
A.butzleri_17CS1198-1	98.33	97.71	98.06	97.86	98.17	98.03	98.64	98.64	98.45	98.53	98.48	98.29	98.17	98.45	98.62	97.70
A.butzleri_7h1h	98.29	97.56	97.97	97.78	98.06	97.93	98.31	98.31	98.27	98.28	98.28	98.31	98.08	98.29	98.39	97.52
A.butzleri_Ab_CR604	97.96	97.75	97.82	97.84	98.28	97.97	98.04	98.06	97.83	97.99	97.92	98.09	98.30	98.03	98.06	97.73
A.butzleri_RMIII	98.01	97.73	98.14	97.73	97.93	97.83	97.92	97.96	98.00	97.93	97.88	97.91	97.97	98.14	97.89	97.35
A.butzleri_17CS1056	98.45	97.60	98.02	97.83	98.10	97.05	98.40	98.47	98.34	98.35	98.35	98.30	98.14	98.37	98.37	97.75
A.butzleri_Ab_CR461	97.61	97.62	97.62	97.52	97.88	97.70	97.65	97.61	97.47	97.40	97.37	97.51	97.88	97.62	97.64	98.39
A.butzleri_17CS1092-2	100	97.72	98.09	97.86	98.00	98.06	98.36	98.29	98.74	98.41	98.38	98.43	98.00	99.03	98.33	97.70
A.butzleri_Ab_CR891	97.31	97.11	97.27	97.16	97.55	97.38	97.22	97.35	97.24	97.21	97.18	97.30	97.47	97.34	97.25	97.35
A.butzleri_55	97.34	97.11	97.27	97.20	97.56	97.45	97.46	97.45	97.21	97.16	97.16	97.27	97.40	97.39	97.50	97.36
A.butzleri_17CS1197-3	98.72	97.62	97.95	97.87	98.04	97.97	98.37	98.39	100	98.46	98.41	98.32	98.15	98.59	98.40	97.53
A.butzleri_16CS0821-2	97.86	97.62	97.94	97.98	97.96	97.88	97.83	97.82	97.94	97.85	97.83	97.84	98.02	97.90	97.84	97.79
A.butzleri_Ab_CR641	97.71	97.62	97.81	97.78	98.03	97.86	97.82	97.75	97.67	97.60	97.56	97.74	98.09	97.87	97.80	98.73
A.butzleri_16CS0820-1	97.87	97.65	97.95	98.01	97.99	97.94	97.85	97.84	97.96	97.87	97.84	97.83	98.03	97.90	97.86	97.82
A.butzleri_17CS1201	98.40	97.65	98.01	97.92	98.15	98.11	98.40	98.48	98.51	98.45	98.40	98.30	98.18	98.39	98.40	97.73
A.butzleri_17CS1198-3	98.35	97.67	98.06	97.89	98.16	98.04	98.66	98.64	98.47	98.53	98.49	98.28	98.17	98.48	98.64	97.71
A.butzleri_17CS1066	98.36	97.63	98.01	97.84	98.12	98.06	99.99	98.79	98.40	98.68	98.65	98.37	98.20	98.38	99.99	97.76
A.butzleri_17CS0965-B	98.13	97.76	99.11	98.46	98.00	98.55	98.05	98.02	98.00	98.05	97.99	97.90	98.13	98.21	98.06	97.66
A.butzleri_Ab_DQ31A1	97.08	96.76	97.13	97.04	97.36	97.30	97.23	97.27	97.10	97.11	97.09	97.08	97.30	97.05	97.26	97.21
A.butzleri_Ab_1426_2003	97.33	97.25	97.33	97.31	97.67	97.44	97.44	97.43	97.32	97.22	97.15	97.31	97.52	97.39	97.42	97.49
A.butzleri_16CS0831-1-1	98.45	97.66	97.96	97.74	98.16	98.00	98.79	98.55	98.50	100	99.98	98.49	98.17	98.45	98.69	97.55

Fortsetzung **Table S2**

	17CS1168	17CS1205-2	17CS1197	Ab_CR1143	16CS0847-3	17CS1193-3	Ab_2811	17CS1068-2	Ab_3711	17CS1198-2	16CS0367-AR	17CS1193-2	17CS1198-1	7h1h	Ab_CR604	RMIII
A.butzleri_17CS1198-1	98.36	98.09	98.42	97.84	98.47	98.68	97.46	98.65	97.80	99.99	98.51	98.63	100	-	-	-
A.butzleri_7h1h	98.40	98.00	98.23	97.72	98.34	98.17	97.44	98.30	97.78	98.37	98.29	98.43	98.37	100	-	-
A.butzleri_Ab_CR604	98.06	98.07	97.83	97.87	98.08	98.14	97.57	98.06	97.85	98.05	97.97	98.12	98.05	97.99	100	-
A.butzleri_RMIII	97.93	97.93	97.99	97.62	97.98	97.95	97.22	97.90	97.77	97.94	97.91	97.97	97.93	97.95	97.80	100
A.butzleri_17CS1056	98.45	98.01	98.30	97.90	98.37	98.43	97.60	98.39	97.83	98.56	98.36	99.24	98.56	98.36	98.08	97.92
A.butzleri_Ab_CR461	97.54	97.64	97.47	98.47	97.62	97.64	98.46	97.65	97.65	97.67	97.41	97.61	97.68	97.37	97.58	97.40
A.butzleri_17CS1092-2	98.48	97.96	98.72	97.73	98.55	98.39	97.57	98.33	97.93	98.30	98.43	98.46	98.33	98.31	97.97	98.03
A.butzleri_Ab_CR891	97.27	97.51	97.23	97.46	97.38	97.40	97.31	97.21	97.21	97.35	97.23	97.33	97.34	97.27	97.60	97.02
A.butzleri_55	97.31	97.51	97.22	97.47	97.33	97.34	97.14	97.46	97.26	97.36	97.17	97.41	97.38	97.29	97.41	97.13
A.butzleri_17CS1197-3	98.40	98.02	99.99	97.69	98.46	98.42	97.38	98.44	97.75	98.41	98.48	98.49	98.42	98.28	97.81	98.01
A.butzleri_16CS0821-2	97.90	97.91	97.92	97.85	97.87	97.83	97.47	97.87	97.89	97.82	97.84	97.91	97.81	97.78	97.83	97.92
A.butzleri_Ab_CR641	97.70	97.94	97.66	98.47	97.74	97.74	98.52	97.81	97.88	97.71	97.62	97.82	97.70	97.76	97.87	97.55
A.butzleri_16CS0820-1	97.91	97.96	97.93	97.87	97.83	97.84	97.53	97.89	97.93	97.84	97.86	97.95	97.82	97.81	97.84	97.97
A.butzleri_17CS1201	98.29	98.13	98.48	97.86	98.40	98.63	97.64	98.40	97.88	98.47	98.48	98.98	98.46	98.40	98.06	97.96
A.butzleri_17CS1198-3	98.35	98.10	98.44	97.84	98.46	98.69	97.48	98.66	97.82	99.99	98.51	98.67	99.99	98.37	98.03	97.94
A.butzleri_17CS1066	98.26	98.06	98.38	97.90	98.36	98.76	97.50	99.99	97.72	98.63	98.68	98.61	98.63	98.31	98.07	97.92
A.butzleri_17CS0965-B	97.93	97.99	97.97	97.81	98.08	98.13	97.61	98.07	97.85	98.02	98.04	98.07	98.03	97.96	97.94	98.16
A.butzleri_Ab_DQ31A1	97.13	97.31	97.09	97.30	97.13	97.11	97.11	97.23	97.19	97.19	97.11	97.27	97.19	96.94	97.29	97.02
A.butzleri_Ab_1426_2003	97.31	97.55	97.32	97.50	97.38	97.35	97.20	97.40	97.41	97.36	97.21	97.47	97.35	97.27	97.54	97.20
A.butzleri_16CS0831-1-1	98.44	98.08	98.50	97.76	98.46	98.98	97.47	98.70	97.78	98.54	99.99	98.54	98.55	98.32	98.00	97.95

Fortsetzung **Table S2**

	17CS1056	Ab_CR461	17CS1092-2	Ab_CR891	55	17CS1197-3	16CS0821-2	Ab_CR641	16CS0820-1	17CS1201	17CS1198-3	17CS1066	17CS0965-B	Ab_DQ31A1	Ab_1426_2003	16CS0831-1-1
A.butzleri_17CS1198-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_7h1h	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR604	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_RMIII	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1056	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR461	97.67	100	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1092-2	98.44	97.64	100	-	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR891	97.31	97.20	97.30	100	-	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_55	97.30	97.25	97.31	97.16	100	-	-	-	-	-	-	-	-	-	-	-
A.butzleri_17CS1197-3	98.34	97.45	98.73	97.25	97.23	100	-	-	-	-	-	-	-	-	-	-
A.butzleri_16CS0821-2	97.76	97.59	97.87	97.07	97.25	97.90	100	-	-	-	-	-	-	-	-	-
A.butzleri_Ab_CR641	97.81	98.59	97.72	97.49	97.37	97.66	97.80	100	-	-	-	-	-	-	-	-
A.butzleri_16CS0820-1	97.81	97.62	97.87	97.09	97.28	97.92	99.98	97.83	100	-	-	-	-	-	-	-
A.butzleri_17CS1201	98.86	97.60	98.40	97.37	97.39	98.51	97.86	97.84	97.91	100	-	-	-	-	-	-
A.butzleri_17CS1198-3	98.58	97.65	98.35	97.34	97.30	98.44	97.84	97.74	97.86	98.47	100	-	-	-	-	-
A.butzleri_17CS1066	98.39	97.66	98.35	97.23	97.49	98.40	97.85	97.81	97.87	98.41	98.64	100	-	-	-	-
A.butzleri_17CS0965-B	98.03	97.75	98.15	97.27	97.38	97.98	98.06	97.81	98.10	98.12	98.04	98.04	100	-	-	-
A.butzleri_Ab_DQ31A1	97.20	97.17	97.05	97.49	97.37	97.10	97.06	97.29	97.07	97.20	97.16	97.24	97.17	100	-	-
A.butzleri_Ab_1426_2003	97.35	97.33	97.35	97.27	98.08	97.32	97.39	97.59	97.39	97.40	97.38	97.43	97.44	97.31	100	-
A.butzleri_16CS0831-1-1	98.38	97.44	98.44	97.24	97.17	98.48	97.85	97.63	97.87	98.47	98.55	98.68	98.05	97.13	97.23	100

Table S3 - Results of the *in silico* DDH-Analysis

Strain	Species	*A. butzleri* NCTC 12481		
		Identities / HSP length		Difference in % G+C (≤ 1 either distinct or same species; > 1 distinct species)
		Distance	DDH estimate (GLM-based)	
16CS0367-1-AR	*A. butzleri*	0.0243	79.20% [76.2 - 81.9%]	0.11
16CS0367-AR	*A. butzleri*	0.0243	79.20% [76.2 - 81.9%]	0.10
16CS0370-1	*A. butzleri*	0.0243	79.20% [76.2 - 81.9%]	0.12
16CS0375-1	*A. butzleri*	0.0243	79.20% [76.3 - 81.9%]	0.12
16CS0375-2	*A. butzleri*	0.0243	79.20% [76.2 - 81.9%]	0.12
16CS0815-2	*A. butzleri*	0.0244	79.10% [76.2 - 81.8%]	0.20
16CS0817-2	*A. butzleri*	0.0249	78.70% [75.8 - 81.4%]	0.06
16CS0820-1	*A. butzleri*	0.0238	79.70% [76.7 - 82.3%]	0.08
16CS0820-2	*A. butzleri*	0.0242	79.30% [76.4 - 82%]	0.01
16CS0821-2	*A. butzleri*	0.0239	79.50% [76.6 - 82.2%]	0.07
16CS0823-1	*A. butzleri*	0.0242	79.30% [76.4 - 82%]	0.01
16CS0823-2	*A. butzleri*	0.0245	79.00% [76 - 81.7%]	0.02
16CS0831-1-1	*A. butzleri*	0.0243	79.20% [76.3 - 81.9%]	0.12
16CS0831-4-1	*A. butzleri*	0.0269	77.00% [74 - 79.8%]	0.15
16CS0847-3	*A. butzleri*	0.0240	79.50% [76.5 - 82.1%]	0.10
16CS0857-1	*A. butzleri*	0.0224	80.80% [77.9 - 83.4%]	0.07
16CS0861-1	*A. butzleri*	0.0241	79.40% [76.4 - 82%]	0.12
17CS0824	*A. butzleri*	0.0234	79.90% [77 - 82.6%]	0.14
17CS0916-1	*A. butzleri*	0.0216	81.50% [78.6 - 84.1%]	0.01
17CS0916-2	*A. butzleri*	0.0216	81.50% [78.6 - 84.1%]	0.02
17CS0965-1	*A. butzleri*	0.0253	78.40% [75.4 - 81.1%]	0.22
17CS0965-B	*A. butzleri*	0.0241	79.40% [76.5 - 82.1%]	0.07
17CS1056	*A. butzleri*	0.0240	79.40% [76.5 - 82.1%]	0.08
17CS1066	*A. butzleri*	0.0239	79.60% [76.6 - 82.2%]	0.13
17CS1067	*A. butzleri*	0.0239	79.50% [76.6 - 82.2%]	0.13
17CS1068-1	*A. butzleri*	0.0239	79.60% [76.6 - 82.2%]	0.15
17CS1068-2	*A. butzleri*	0.0238	79.60% [76.7 - 82.3%]	0.12
17CS1092-1	*A. butzleri*	0.0238	79.70% [76.7 - 82.3%]	0.01
17CS1095	*A. butzleri*	0.0236	79.80% [76.8 - 82.4%]	0.04
17CS1167	*A. butzleri*	0.0239	79.50% [76.6 - 82.2%]	0.15
17CS1168	*A. butzleri*	0.0246	79.00% [76 - 81.7%]	0.14
17CS1193-1	*A. butzleri*	0.0235	79.90% [76.9 - 82.5%]	0.13
17CS1193-2	*A. butzleri*	0.0235	79.90% [76.9 - 82.5%]	0.13
17CS1193-3	*A. butzleri*	0.0236	79.80% [76.9 - 82.5%]	0.17
17CS1197	*A. butzleri*	0.0244	79.10% [76.2 - 81.8%]	0.06
17CS1197-2	*A. butzleri*	0.0243	79.20% [76.2 - 81.8%]	0.07
17CS1197-3	*A. butzleri*	0.0244	79.10% [76.2 - 81.8%]	0.07
17CS1198-1	*A. butzleri*	0.0236	79.80% [76.9 - 82.5%]	0.16
17CS1198-2	*A. butzleri*	0.0236	79.80% [76.9 - 82.5%]	0.16
17CS1198-3	*A. butzleri*	0.0236	79.80% [76.9 - 82.5%]	0.16
17CS1200-1	*A. butzleri*	0.0230	80.30% [77.4 - 82.9%]	0.11
17CS1201	*A. butzleri*	0.0230	80.30% [77.4 - 83%]	0.11
17CS1205-1	*A. butzleri*	0.0225	80.70% [77.8 - 83.3%]	0.18
17CS1205-2	*A. butzleri*	0.0225	80.70% [77.8 - 83.3%]	0.18
17CS1206-1	*A. butzleri*	0.0225	80.70% [77.8 - 83.3%]	0.18
17CS1208-3	*A. butzleri*	0.0225	80.70% [77.8 - 83.3%]	0.17
55	*A. butzleri*	0.0219	81.20% [78.4 - 83.8%]	0.27
6V	*A. butzleri*	0.0222	81.00% [78.1 - 83.6%]	0.21
7h1h	*A. butzleri*	0.0242	79.30% [76.3 - 81.9%]	0.00
Ab_1426_2003	*A. butzleri*	0.0218	81.30% [78.4 - 83.9%]	0.28
Ab_1711	*A. butzleri*	0.0252	78.50% [75.5 - 81.2%]	0.02
Ab_2211	*A. butzleri*	0.0256	78.10% [75.1 - 80.8%]	0.22
Ab_2811	*A. butzleri*	0.0246	79.00% [76 - 81.7%]	0.28
Ab_3711	*A. butzleri*	0.0241	79.30% [76.4 - 82%]	0.26
Ab_4211	*A. butzleri*	0.0239	79.50% [76.6 - 82.2%]	0.12
Ab_4511	*A. butzleri*	0.0241	79.40% [76.4 - 82%]	0.02
Ab_A103	*A. butzleri*	0.0242	79.30% [76.4 - 82%]	0.00
Ab_A111	*A. butzleri*	0.0232	80.10% [77.2 - 82.7%]	0.07
Ab_CR461	*A. butzleri*	0.0240	79.40% [76.5 - 82.1%]	0.26
Ab_CR424	*A. butzleri*	0.0239	79.50% [76.6 - 82.2%]	0.22
Ab_CR502	*A. butzleri*	0.0244	79.10% [76.2 - 81.8%]	0.11
Ab_CR604	*A. butzleri*	0.0223	80.90% [78 - 83.5%]	0.03
Ab_CR641	*A. butzleri*	0.0232	80.10% [77.2 - 82.8%]	0.08
Ab_CR891	*A. butzleri*	0.0258	78.00% [75 - 80.7%]	0.09
Ab_CR892	*A. butzleri*	0.0234	79.90% [77 - 82.6%]	0.18
Ab_CR1132	*A. butzleri*	0.0239	79.50% [76.6 - 82.2%]	0.39
Ab_CR1143	*A. butzleri*	0.0232	80.10% [77.2 - 82.8%]	0.19
Ab_DQ20dA1	*A. butzleri*	0.0240	79.40% [76.5 - 82.1%]	0.21
Ab_DQ31A	*A. butzleri*	0.0254	78.30% [75.3 - 81%]	0.30
Ab_DQ40A1	*A. butzleri*	0.0237	79.70% [76.8 - 82.4%]	0.20
Ab_DQ64A1	*A. butzleri*	0.0253	78.40% [75.4 - 81.1%]	0.25
RMI	*A. butzleri*	0.0240	79.40% [76.5 - 82.1%]	0.23
RMIII	*A. butzleri*	0.0241	79.40% [76.4 - 82%]	0.18
ATCC_43158	*A. cryaerophilus*	0.2025	21.70% [19.4 - 24.1%]	0.40
LMG_25534	*A. trophiarum*	0.2101	20.90% [18.7 - 23.3%]	1.16
NCTC_11168	*C. jejuni* subsp. *jejuni*	0.2010	21.80% [19.6 - 24.3%]	3.49

Table S4 - Results of the Snippy analysis. This table shows the SNP-distances between 70 analysed *A. butzleri* strains.

Strain	16CS0367-1-AR	16CS0367-AR	16CS0370-1	16CS0375-1	16CS0375-2	16CS0815-2	16CS0817-2	16CS0820-1	16CS0820-2	16CS0821-2	16CS0823-1	16CS0823-2	16CS0831-1-1	16CS0831-4-1
16CS0367-1-AR	0	32	0	0	0	9956	9335	13464	13476	13469	13476	10778	0	19445
16CS0367-AR	32	0	32	32	32	9976	9347	13461	13477	13466	13477	10787	32	19442
16CS0370-1	0	32	0	0	0	9956	9335	13464	13476	13469	13476	10778	0	19445
16CS0375-1	0	32	0	0	0	9956	9335	13464	13476	13469	13476	10778	0	19445
16CS0375-2	0	32	0	0	0	9956	9335	13464	13476	13469	13476	10778	0	19445
16CS0815-2	9956	9976	9956	9956	9956	0	10234	13749	13817	13759	13817	10920	9956	19823
16CS0817-2	9335	9347	9335	9335	9335	10234	0	13626	13759	13630	13759	10753	9335	19571
16CS0820-1	13464	13461	13464	13464	13464	13749	13626	0	6973	12	6973	13591	13464	19827
16CS0820-2	13476	13477	13476	13476	13476	13817	13759	6973	0	6981	0	13490	13476	19930
16CS0821-2	13469	13466	13469	13469	13469	13751	13630	12	6981	0	6981	13595	13469	19833
16CS0823-1	13476	13477	13476	13476	13476	13817	13759	6973	0	6981	0	13490	13476	19930
16CS0823-2	10778	10787	10778	10778	10778	10920	10753	13591	13490	13595	13490	0	10778	19599
16CS0831-1-1	0	32	0	0	0	9956	9335	13464	13476	13469	13476	10778	0	19445
16CS0831-4-1	19445	19442	19445	19445	19445	19823	19571	19827	19930	19833	19930	19599	19445	0
16CS0847-3	9924	9938	9924	9924	9924	9066	9963	13865	13820	13869	13820	10941	9924	19791
16CS0857-1	12856	12861	12856	12856	12856	13106	13155	13014	13076	13016	13076	13058	12856	19503
16CS0861-1	9895	9914	9895	9895	9895	10363	9853	13479	13578	13483	13578	10947	9895	19551
17CS0824	8708	8731	8708	8708	8708	10176	10576	13486	13575	13490	13575	11196	8708	19422
17CS0916-1	12865	12867	12865	12865	12865	12859	12926	12651	12562	12653	12562	12741	12865	19624
17CS0916-2	12865	12867	12865	12865	12865	12859	12926	12651	12562	12653	12562	12741	12865	19624
17CS0965-1	9259	9273	9259	9259	9259	10312	8184	13425	13695	13429	13695	10511	9259	19526
17CS0965-B	12549	12549	12549	12549	12549	12844	12647	12458	12635	12462	12635	12782	12549	19423
17CS1056	9483	9497	9483	9483	9483	9685	9877	13380	13372	13382	13372	10696	9483	19561
17CS1066	8462	8475	8462	8462	8462	9688	10226	13337	13424	13341	13424	10994	8462	19469
17CS1067	8463	8476	8463	8463	8463	9689	10231	13336	13429	13340	13429	11001	8463	19476
17CS1068-1	8462	8475	8462	8462	8462	9686	10228	13335	13426	13339	13426	10996	8462	19475
17CS1068-2	8461	8474	8461	8461	8461	9687	10227	13334	13425	13338	13425	10995	8461	19474
17CS1092-1	9011	9023	9011	9011	9011	9736	7436	13444	13522	13448	13522	10362	9011	19598
17CS1092-2	9010	9022	9010	9010	9010	9735	7435	13443	13521	13447	13521	10361	9010	19597

Fortsetzung **Table S4**

Strain	16CS0847-3	16CS0857-1	16CS0861-1	17CS0824	17CS0916-1	17CS0916-2	17CS0965-1	17CS0965-B	17CS1056	17CS1066	17CS1067	17CS1068-1	17CS1068-2	17CS1092-1	17CS1092-2
16CS0367-1-AR	9924	12856	9895	8708	12865	12865	9259	12549	9483	8462	8463	8462	8461	9011	9010
16CS0367-AR	9938	12861	9914	8731	12867	12867	9273	12549	9497	8475	8476	8475	8474	9023	9022
16CS0370-1	9924	12856	9895	8708	12865	12865	9259	12549	9483	8462	8463	8462	8461	9011	9010
16CS0375-1	9924	12856	9895	8708	12865	12865	9259	12549	9483	8462	8463	8462	8461	9011	9010
16CS0375-2	9924	12856	9895	8708	12865	12865	9259	12549	9483	8462	8463	8462	8461	9011	9010
16CS0815-2	9066	13106	10363	10176	12859	12859	10312	12844	9685	9688	9689	9686	9687	9736	9735
16CS0817-2	9963	13155	9853	10576	12926	12926	8184	12647	9877	10226	10231	10228	10227	7436	7435
16CS0820-1	13865	13014	13479	13486	12651	12651	13425	12458	13380	13337	13336	13335	13334	13444	13443
16CS0820-2	13820	13076	13578	13575	12562	12562	13695	12635	13372	13424	13429	13426	13425	13522	13521
16CS0821-2	13869	13016	13483	13490	12653	12653	13429	12462	13382	13341	13340	13339	13338	13448	13447
16CS0823-1	13820	13076	13578	13575	12562	12562	13695	12635	13372	13424	13429	13426	13425	13522	13521
16CS0823-2	10941	13058	10947	11196	12741	12741	10511	12782	10696	10994	11001	10996	10995	10362	10361
16CS0831-1-1	9924	12856	9895	8708	12865	12865	9259	12549	9483	8462	8463	8462	8461	9011	9010
16CS0831-4-1	19791	19503	19551	19422	19624	19624	19526	19423	19561	19469	19476	19475	19474	19598	19597
16CS0847-3	0	12942	9809	10340	12677	12677	9995	12853	9611	9939	9944	9941	9938	9445	9444
16CS0857-1	12942	0	12771	12660	9159	9159	13032	12754	12504	12817	12820	12819	12816	12843	12844
16CS0861-1	9809	12771	0	9960	12691	12691	10032	12679	9044	9495	9500	9497	9496	9891	9890
17CS0824	10340	12660	9960	0	12637	12637	10639	12580	9860	7984	7985	7982	7983	10156	10157
17CS0916-1	12677	9159	12691	12637	0	0	12861	12580	12475	12666	12671	12668	12667	12733	12734
17CS0916-2	12677	9159	12691	12637	0	0	12861	12580	12475	12666	12671	12668	12667	12733	12734
17CS0965-1	9995	13032	10032	10639	12861	12861	0	12584	10184	10227	10234	10229	10228	6033	6032
17CS0965-B	12853	12754	12679	12580	12580	12580	12584	0	12405	12660	12667	12664	12663	12518	12519
17CS1056	9611	12504	9044	9860	12475	12475	10184	12405	0	9658	9665	9662	9659	9621	9620
17CS1066	9939	12817	9495	7984	12666	12666	10227	12660	9658	0	11	6	7	9998	9997
17CS1067	9944	12820	9500	7985	12671	12671	10234	12667	9665	11	0	7	8	10005	10004
17CS1068-1	9941	12819	9497	7982	12668	12668	10229	12664	9662	6	7	0	3	10000	9999
17CS1068-2	9938	12816	9496	7983	12667	12667	10228	12663	9659	7	8	3	0	9999	9998
17CS1092-1	9445	12843	9891	10156	12733	12733	6033	12518	9621	9998	10005	10000	9999	0	1
17CS1092-2	9444	12844	9890	10157	12734	12734	6032	12519	9620	9997	10004	9999	9998	1	0

Fortsetzung **Table S4**

Strain	17CS1095	17CS1167	17CS1168	17CS1193-1	17CS1193-2	17CS1193-3	17CS1197	17CS1197-2	17CS1197-3	17CS1198-1	17CS1198-2	17CS1198-3	17CS1200-1	17CS1201	17CS1205-1
16CS0367-1-AR	9164	8463	9883	9277	9277	6492	9445	9443	9443	9058	9058	9058	9585	9584	12581
16CS0367-AR	9182	8476	9897	9291	9291	6512	9460	9458	9458	9072	9072	9072	9594	9593	12590
16CS0370-1	9164	8463	9883	9277	9277	6492	9445	9443	9443	9058	9058	9058	9585	9584	12581
16CS0375-1	9164	8463	9883	9277	9277	6492	9445	9443	9443	9058	9058	9058	9585	9584	12581
16CS0375-2	9164	8463	9883	9277	9277	6492	9445	9443	9443	9058	9058	9058	9585	9584	12581
16CS0815-2	10087	9687	8892	9329	9329	9784	9759	9757	9757	9838	9838	9838	10257	10256	12703
16CS0817-2	8440	10229	9902	9615	9615	9947	8815	8813	8813	10147	10147	10147	10268	10267	12612
16CS0820-1	13576	13336	13589	13198	13198	13388	13459	13459	13459	13309	13309	13309	13353	13352	13203
16CS0820-2	13711	13427	13590	13316	13316	13540	13634	13634	13634	13398	13398	13398	13500	13499	13194
16CS0821-2	13580	13340	13591	13204	13204	13392	13461	13461	13461	13313	13313	13313	13357	13356	13207
16CS0823-1	13711	13427	13590	13316	13316	13540	13634	13634	13634	13398	13398	13398	13500	13499	13194
16CS0823-2	10562	10997	10958	10613	10613	10682	9911	9909	9909	11068	11068	11068	10955	10954	12765
16CS0831-1-1	9164	8463	9883	9277	9277	6492	9445	9443	9443	9058	9058	9058	9585	9584	12581
16CS0831-4-1	19756	19476	19397	19450	19450	19411	19547	19545	19545	19591	19591	19591	19489	19488	19522
16CS0847-3	9933	9942	8691	9437	9437	9336	9849	9847	9847	9924	9924	9924	9738	9737	12754
16CS0857-1	13017	12820	12849	12655	12655	12695	13040	13038	13038	12777	12777	12777	12752	12751	12393
16CS0861-1	10074	9498	9546	8718	8718	9529	10074	10072	10072	9578	9578	9578	8898	8897	12365
17CS0824	10564	7985	10042	9364	9364	8389	10532	10530	10530	8671	8671	8671	9977	9976	12287
17CS0916-1	12853	12669	12703	12513	12513	12432	12739	12737	12737	12736	12736	12736	12711	12710	12235
17CS0916-2	12853	12669	12703	12513	12513	12432	12739	12737	12737	12736	12736	12736	12711	12710	12235
17CS0965-1	5886	10230	10164	9886	9886	9936	8365	8363	8363	10375	10375	10375	10405	10404	12553
17CS0965-B	12723	12665	12635	12388	12388	12556	12746	12746	12746	12633	12633	12633	12670	12669	12810
17CS1056	10034	9663	9150	4514	4514	9235	9711	9709	9709	9378	9378	9378	7258	7257	12277
17CS1066	10355	9	9573	8569	8569	8172	10034	10032	10032	8418	8418	8418	9796	9795	12338
17CS1067	10360	10	9574	8574	8574	8173	10037	10035	10035	8421	8421	8421	9801	9800	12339
17CS1068-1	10357	5	9571	8571	8571	8172	10034	10032	10032	8418	8418	8418	9796	9795	12340
17CS1068-2	10356	6	9570	8568	8568	8171	10033	10031	10031	8417	8417	8417	9793	9792	12339
17CS1092-1	4241	10001	10034	9594	9594	9703	8263	8261	8261	10135	10135	10135	9982	9981	12419
17CS1092-2	4240	10000	10035	9593	9593	9702	8262	8260	8260	10134	10134	10134	9981	9980	12420

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Strain	17CS1205-2	17CS1206-1	17CS1208-3	ERR3523196	ERR3523197	ERR3523198	ERR3523199	ERR3523200	ERR3523201	ERR3523202	ERR3523203	ERR3523204	ERR3523205	ERR3523206	ERR3523207
16CS0367-1-AR	12577	12581	12580	13226	13708	9599	11410	13405	13395	18303	13210	12577	14784	13738	18042
16CS0367-AR	12586	12590	12589	13230	13712	9605	11426	13401	13403	18300	13218	12578	14789	13742	18039
16CS0370-1	12577	12581	12580	13226	13708	9599	11410	13405	13395	18303	13210	12577	14784	13738	18042
16CS0375-1	12577	12581	12580	13226	13708	9599	11410	13405	13395	18303	13210	12577	14784	13738	18042
16CS0375-2	12577	12581	12580	13226	13708	9599	11410	13405	13395	18303	13210	12577	14784	13738	18042
16CS0815-2	12708	12703	12711	13303	13829	9484	11826	13459	13470	18536	13230	12780	14782	13732	18508
16CS0817-2	12607	12612	12611	13403	13742	9800	10957	13441	13546	18416	13354	12752	14802	13838	18262
16CS0820-1	13198	13203	13203	12305	12711	13427	13022	12105	12164	18669	12149	12803	14321	12717	18531
16CS0820-2	13189	13194	13193	12517	12902	13519	13127	12277	12297	18810	12244	12884	14358	12789	18596
16CS0821-2	13202	13207	13207	12311	12713	13433	13026	12109	12167	18671	12156	12805	14325	12723	18539
16CS0823-1	13189	13194	13193	12517	12902	13519	13127	12277	12297	18810	12244	12884	14358	12789	18596
16CS0823-2	12758	12763	12763	13281	13751	10882	12029	13562	13406	18755	13268	12713	14996	13583	18417
16CS0831-1-1	12577	12581	12580	13226	13708	9599	11410	13405	13395	18303	13210	12577	14784	13738	18042
16CS0831-4-1	19517	19522	19516	18879	19673	19418	19696	18740	18521	14067	18103	19277	18264	18774	14335
16CS0847-3	12747	12752	12751	13329	13911	9553	11559	13394	13397	18614	13152	12663	14923	13752	18532
16CS0857-1	12398	12393	12400	12409	12878	12813	13136	12814	12348	18303	12278	12506	14249	12750	18303
16CS0861-1	12358	12363	12360	13180	13738	9060	11942	13258	13207	18456	12974	12716	14777	13487	18291
17CS0824	12279	12283	12282	13092	13514	9640	12069	13242	13182	18361	12982	12732	14700	13604	18166
17CS0916-1	12228	12233	12232	12184	12745	12687	12790	12530	12259	18414	12097	12460	14096	12765	18385
17CS0916-2	12228	12233	12232	12184	12745	12687	12790	12530	12259	18414	12097	12460	14096	12765	18385
17CS0965-1	12546	12551	12549	13333	13495	10103	11001	13591	13509	18632	13418	12707	14744	13761	18511
17CS0965-B	12805	12810	12808	12539	12720	12601	11691	12638	12387	18412	12412	7882	14169	12886	18207
17CS1056	12272	12277	12278	12982	13470	4057	11598	13179	13155	18331	12930	12359	14663	13439	18450
17CS1066	12334	12338	12339	13057	13599	8908	11869	13249	13247	18335	13054	12645	14727	13439	18170
17CS1067	12335	12339	12340	13062	13598	8913	11874	13254	13250	18344	13059	12650	14732	13442	18175
17CS1068-1	12336	12340	12341	13059	13599	8910	11871	13251	13249	18341	13056	12647	14731	13441	18174
17CS1068-2	12335	12339	12340	13060	13598	8907	11870	13250	13248	18340	13055	12646	14731	13440	18173
17CS1092-1	12412	12417	12414	13278	13527	9721	9864	13461	13463	18465	13235	12652	14602	13603	18441
17CS1092-2	12413	12418	12415	13277	13526	9722	9863	13460	13462	18464	13234	12651	14601	13602	18440

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Strain	ERR3523208	ERR3523209	ERR3523210	ERR3523211	ERR3523212	ERR3523213	ERR3523214	ERR3523215	ERR3523216	ERR3523217	GCF-900187115-1
16CS0367-1-AR	13639	12565	13918	9890	16138	13038	12432	14228	17161	18066	15466
16CS0367-AR	13639	12569	13913	9908	16129	13044	12436	14231	17163	18062	15434
16CS0370-1	13639	12565	13918	9890	16138	13038	12432	14228	17161	18066	15466
16CS0375-1	13639	12565	13918	9890	16138	13038	12432	14228	17161	18066	15466
16CS0375-2	13639	12565	13918	9890	16138	13038	12432	14228	17161	18066	15466
16CS0815-2	13704	12918	14082	10340	16378	13295	12715	14399	17371	18178	15689
16CS0817-2	13729	12996	13871	10119	16354	13121	12553	14290	17578	18117	15633
16CS0820-1	12298	13102	12843	13652	16085	12310	12508	13715	17493	18414	15139
16CS0820-2	12192	13366	13213	13618	16023	12464	12441	13591	17531	18479	15017
16CS0821-2	12304	13106	12851	13656	16092	12313	12510	13721	17498	18420	15137
16CS0823-1	12192	13366	13213	13618	16023	12464	12441	13591	17531	18479	15017
16CS0823-2	13621	12698	14170	11267	16430	13060	12545	14331	17450	18374	15687
16CS0831-1-1	13639	12565	13918	9890	16138	13038	12432	14228	17161	18066	15466
16CS0831-4-1	19865	19249	19201	19373	19247	19840	19450	19347	16833	14549	18849
16CS0847-3	13812	12960	14121	10128	16322	13091	12571	14354	17474	18326	15809
16CS0857-1	12902	12296	13418	12863	16064	12845	12667	13386	17098	18131	15228
16CS0861-1	13655	12542	13926	9924	16104	13130	12569	14224	17347	18186	15491
17CS0824	13577	12554	13795	10263	16158	13016	12392	14038	17248	18102	15471
17CS0916-1	12979	11953	13173	12728	15896	12784	12444	13062	17086	18218	15110
17CS0916-2	12979	11953	13173	12728	15896	12784	12444	13062	17086	18218	15110
17CS0965-1	13548	12945	13916	10446	16387	12869	12415	14360	17376	18259	15696
17CS0965-B	12802	12877	13027	12580	16047	9405	6408	13680	17142	18122	15247
17CS1056	13427	12558	13739	9643	16178	12699	12190	14191	17365	18111	15469
17CS1066	13578	12483	13766	9960	16101	12959	12570	14145	17253	18130	15553
17CS1067	13579	12488	13773	9965	16104	12966	12575	14152	17258	18137	15552
17CS1068-1	13576	12485	13770	9962	16105	12963	12572	14149	17259	18136	15559
17CS1068-2	13573	12484	13767	9961	16102	12962	12569	14150	17258	18135	15556
17CS1092-1	13566	12904	13972	9986	16388	12915	12381	14295	17442	18258	15751
17CS1092-2	13565	12905	13971	9985	16387	12914	12380	14294	17441	18257	15750

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Strain	16CS0367-1-AR	16CS0367-AR	16CS0370-1	16CS0375-1	16CS0375-2	16CS0815-2	16CS0817-2	16CS0820-1	16CS0820-2	16CS0821-2	16CS0823-1	16CS0823-2	16CS0831-1-1	16CS0831-4-1
17CS1095	9164	9182	9164	9164	9164	10087	8440	13576	13711	13580	13711	10562	9164	19756
17CS1167	8463	8476	8463	8463	8463	9687	10229	13336	13427	13340	13427	10997	8463	19476
17CS1168	9883	9897	9883	9883	9883	8892	9902	13589	13590	13591	13590	10958	9883	19397
17CS1193-1	9277	9291	9277	9277	9277	9329	9615	13198	13316	13204	13316	10613	9277	19450
17CS1193-2	9277	9291	9277	9277	9277	9329	9615	13198	13316	13204	13316	10613	9277	19450
17CS1193-3	6492	6512	6492	6492	6492	9784	9947	13388	13540	13392	13540	10682	6492	19411
17CS1197	9445	9460	9445	9445	9445	9759	8815	13459	13634	13461	13634	9911	9445	19547
17CS1197-2	9443	9458	9443	9443	9443	9757	8813	13459	13634	13461	13634	9909	9443	19545
17CS1197-3	9443	9458	9443	9443	9443	9757	8813	13459	13634	13461	13634	9909	9443	19545
17CS1198-1	9058	9072	9058	9058	9058	9838	10147	13309	13398	13313	13398	11068	9058	19591
17CS1198-2	9058	9072	9058	9058	9058	9838	10147	13309	13398	13313	13398	11068	9058	19591
17CS1198-3	9058	9072	9058	9058	9058	9838	10147	13309	13398	13313	13398	11068	9058	19591
17CS1200-1	9585	9594	9585	9585	9585	10257	10268	13353	13500	13357	13500	10955	9585	19489
17CS1201	9584	9593	9584	9584	9584	10256	10267	13352	13499	13356	13499	10954	9584	19488
17CS1205-1	12581	12590	12581	12581	12581	12703	12612	13203	13194	13207	13194	12765	12581	19522
17CS1205-2	12577	12586	12577	12577	12577	12708	12607	13198	13189	13202	13189	12758	12577	19517
17CS1206-1	12581	12590	12581	12581	12581	12703	12612	13203	13194	13207	13194	12763	12581	19522
17CS1208-3	12580	12589	12580	12580	12580	12711	12611	13203	13193	13207	13193	12763	12580	19516
Ab-CR1143	13226	13230	13226	13226	13226	13303	13403	12305	12517	12311	12517	13281	13226	18879
Ab-3711	13708	13712	13708	13708	13708	13829	13742	12711	12902	12713	12902	13751	13708	19673
Ab-CR1132	9599	9605	9599	9599	9599	9484	9800	13427	13519	13433	13519	10882	9599	19418
Ab-1711	11410	11426	11410	11410	11410	11826	10957	13022	13127	13026	13127	12029	11410	19696
Ab-CR461	13405	13401	13405	13405	13405	13459	13441	12105	12277	12109	12277	13562	13405	18740
Ab-4511	13395	13403	13395	13395	13395	13470	13546	12164	12297	12167	12297	13406	13395	18521
Ab-2211	18303	18300	18303	18303	18303	18536	18416	18669	18810	18671	18810	18755	18303	14067
Ab-2811	13210	13218	13210	13210	13210	13230	13354	12149	12244	12156	12244	13268	13210	18103
Ab-A103	12577	12578	12577	12577	12577	12780	12752	12803	12884	12805	12884	12713	12577	19277
Ab-CR424	14784	14789	14784	14784	14784	14782	14802	14321	14358	14325	14358	14996	14784	18264
Ab-CR641	13738	13742	13738	13738	13738	13732	13838	12717	12789	12723	12789	13583	13738	18774

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Strain	16CS0847-3	16CS0857-1	16CS0861-1	17CS0824	17CS0916-1	17CS0916-2	17CS0965-1	17CS0965-B	17CS1056	17CS1066	17CS1067	17CS1068-1	17CS1068-2	17CS1092-1	17CS1092-2
17CS1095	9933	13017	10074	10564	12853	12853	5886	12723	10034	10355	10360	10357	10356	4241	4240
17CS1167	9942	12820	9498	7985	12669	12669	10230	12665	9663	9	10	5	6	10001	10000
17CS1168	8691	12849	9546	10042	12703	12703	10164	12635	9150	9573	9574	9571	9570	10034	10035
17CS1193-1	9437	12655	8718	9364	12513	12513	9886	12388	4514	8569	8574	8571	8568	9594	9593
17CS1193-2	9437	12655	8718	9364	12513	12513	9886	12388	4514	8569	8574	8571	8568	9594	9593
17CS1193-3	9336	12695	9529	8389	12432	12432	9936	12556	9235	8172	8173	8172	8171	9703	9702
17CS1197	9849	13040	10074	10532	12739	12739	8365	12746	9711	10034	10037	10034	10033	8263	8262
17CS1197-2	9847	13038	10072	10530	12737	12737	8363	12746	9709	10032	10035	10032	10031	8261	8260
17CS1197-3	9847	13038	10072	10530	12737	12737	8363	12746	9709	10032	10035	10032	10031	8261	8260
17CS1198-1	9924	12777	9578	8671	12736	12736	10375	12633	9378	8418	8421	8418	8417	10135	10134
17CS1198-2	9924	12777	9578	8671	12736	12736	10375	12633	9378	8418	8421	8418	8417	10135	10134
17CS1198-3	9924	12777	9578	8671	12736	12736	10375	12633	9378	8418	8421	8418	8417	10135	10134
17CS1200-1	9738	12752	8898	9977	12711	12711	10405	12670	7258	9796	9801	9796	9793	9982	9981
17CS1201	9737	12751	8897	9976	12710	12710	10404	12669	7257	9795	9800	9795	9792	9981	9980
17CS1205-1	12754	12393	12365	12287	12235	12235	12553	12810	12277	12338	12339	12340	12339	12419	12420
17CS1205-2	12747	12398	12358	12279	12228	12228	12546	12805	12272	12334	12335	12336	12335	12412	12413
17CS1206-1	12752	12393	12363	12283	12233	12233	12551	12810	12277	12338	12339	12340	12339	12417	12418
17CS1208-3	12751	12400	12360	12282	12232	12232	12549	12808	12278	12339	12340	12341	12340	12414	12415
Ab-CR1143	13329	12409	13180	13092	12184	12184	13333	12539	12982	13057	13062	13059	13060	13278	13277
Ab-3711	13911	12878	13738	13514	12745	12745	13495	12720	13470	13599	13598	13599	13598	13527	13526
Ab-CR1132	9553	12813	9060	9640	12687	12687	10103	12601	4057	8908	8913	8910	8907	9721	9722
Ab-1711	11559	13136	11942	12069	12790	12790	11001	11691	11598	11869	11874	11871	11870	9864	9863
Ab-CR461	13394	12814	13258	13242	12530	12530	13591	12638	13179	13249	13254	13251	13250	13461	13460
Ab-4511	13397	12348	13207	13182	12259	12259	13509	12387	13155	13247	13250	13249	13248	13463	13462
Ab-2211	18614	18303	18456	18361	18414	18414	18632	18412	18331	18335	18344	18341	18340	18465	18464
Ab-2811	13152	12278	12974	12982	12097	12097	13418	12412	12930	13054	13059	13056	13055	13235	13234
Ab-A103	12663	12506	12716	12732	12460	12460	12707	7882	12359	12645	12650	12647	12646	12652	12651
Ab-CR424	14923	14249	14777	14700	14096	14096	14744	14169	14663	14727	14732	14731	14731	14602	14601
Ab-CR641	13752	12750	13487	13604	12765	12765	13761	12886	13439	13439	13442	13441	13440	13603	13602

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Strain	17CS1095	17CS1167	17CS1168	17CS1193-1	17CS1193-2	17CS1193-3	17CS1197	17CS1197-2	17CS1197-3	17CS1198-1	17CS1198-2	17CS1198-3	17CS1200-1	17CS1201	17CS1205-1
17CS1095	0	10358	10095	9926	9926	10159	8286	8284	8284	10303	10303	10303	10401	10400	12691
17CS1167	10358	0	9572	8572	8572	8175	10035	10033	10033	8419	8419	8419	9797	9796	12341
17CS1168	10095	9572	0	9330	9330	9940	9987	9985	9985	9670	9670	9670	9781	9780	12481
17CS1193-1	9926	8572	9330	0	0	8894	9606	9604	9604	8978	8978	8978	6766	6765	12245
17CS1193-2	9926	8572	9330	0	0	8894	9606	9604	9604	8978	8978	8978	6766	6765	12245
17CS1193-3	10159	8175	9940	8894	8894	0	9643	9641	9641	8439	8439	8439	9112	9111	12478
17CS1197	8286	10035	9987	9606	9606	9643	0	2	2	10169	10169	10169	9784	9783	12573
17CS1197-2	8284	10033	9985	9604	9604	9641	2	0	0	10167	10167	10167	9782	9781	12571
17CS1197-3	8284	10033	9985	9604	9604	9641	2	0	0	10167	10167	10167	9782	9781	12571
17CS1198-1	10303	8419	9670	8978	8978	8439	10169	10167	10167	0	0	0	9382	9381	12206
17CS1198-2	10303	8419	9670	8978	8978	8439	10169	10167	10167	0	0	0	9382	9381	12206
17CS1198-3	10303	8419	9670	8978	8978	8439	10169	10167	10167	0	0	0	9382	9381	12206
17CS1200-1	10401	9797	9781	6766	6766	9112	9784	9782	9782	9382	9382	9382	0	1	12345
17CS1201	10400	9796	9780	6765	6765	9111	9783	9781	9781	9381	9381	9381	1	0	12344
17CS1205-1	12691	12341	12481	12245	12245	12478	12573	12571	12571	12206	12206	12206	12345	12344	0
17CS1205-2	12684	12337	12486	12240	12240	12474	12566	12564	12564	12202	12202	12202	12341	12340	11
17CS1206-1	12689	12341	12481	12245	12245	12478	12571	12569	12569	12206	12206	12206	12345	12344	6
17CS1208-3	12687	12342	12491	12246	12246	12478	12567	12565	12565	12209	12209	12209	12345	12344	22
Ab-CR1143	13345	13060	13176	12987	12987	12835	13325	13325	13325	13009	13009	13009	13124	13123	12537
Ab-3711	13771	13600	13748	13516	13516	13395	13783	13781	13781	13593	13593	13593	13796	13795	13417
Ab-CR1132	10196	8911	9484	2632	2632	9105	9933	9933	9933	9094	9094	9094	7971	7970	12374
Ab-1711	10083	11872	11535	11419	11419	11569	10700	10698	10698	11619	11619	11619	11426	11425	12878
Ab-CR461	13643	13252	13301	13205	13205	12978	13586	13586	13586	13140	13140	13140	13260	13259	12842
Ab-4511	13551	13250	13222	12990	12990	13107	13480	13480	13480	13155	13155	13155	13009	13008	12795
Ab-2211	18726	18342	18304	18269	18269	18302	18533	18535	18535	18369	18369	18369	18404	18403	18447
Ab-2811	13319	13057	13070	12826	12826	12759	13309	13309	13309	12934	12934	12934	12959	12958	12621
Ab-A103	12821	12648	12451	12333	12333	12396	12796	12794	12794	12522	12522	12522	12547	12546	12823
Ab-CR424	14796	14732	14793	14677	14677	14565	14843	14841	14841	14899	14899	14899	14735	14734	14487
Ab-CR641	13874	13442	13600	13378	13378	13389	13745	13745	13745	13422	13422	13422	13454	13453	12939

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Strain	17CS1205-2	17CS1206-1	17CS1208-3	ERR3523196	ERR3523197	ERR3523198	ERR3523199	ERR3523200	ERR3523201	ERR3523202	ERR3523203	ERR3523204	ERR3523205	ERR3523206	ERR3523207
17CS1095	12684	12689	12687	13345	13771	10196	10083	13643	13551	18726	13319	12821	14796	13874	18650
17CS1167	12337	12341	12342	13060	13600	8911	11872	13252	13250	18342	13057	12648	14732	13442	18175
17CS1168	12486	12481	12491	13176	13748	9484	11535	13301	13222	18304	13070	12451	14793	13600	18177
17CS1193-1	12240	12245	12246	12987	13516	2632	11419	13205	12990	18269	12826	12333	14677	13378	18209
17CS1193-2	12240	12245	12246	12987	13516	2632	11419	13205	12990	18269	12826	12333	14677	13378	18209
17CS1193-3	12474	12478	12478	12835	13395	9105	11569	12978	13107	18302	12759	12396	14565	13389	18143
17CS1197	12566	12571	12567	13325	13783	9933	10700	13586	13480	18533	13309	12796	14843	13745	18389
17CS1197-2	12564	12569	12565	13325	13781	9933	10698	13586	13480	18535	13309	12794	14841	13745	18389
17CS1197-3	12564	12569	12565	13325	13781	9933	10698	13586	13480	18535	13309	12794	14841	13745	18389
17CS1198-1	12202	12206	12209	13009	13593	9094	11619	13140	13155	18369	12934	12522	14899	13422	18376
17CS1198-2	12202	12206	12209	13009	13593	9094	11619	13140	13155	18369	12934	12522	14899	13422	18376
17CS1198-3	12202	12206	12209	13009	13593	9094	11619	13140	13155	18369	12934	12522	14899	13422	18376
17CS1200-1	12341	12345	12345	13124	13796	7971	11426	13260	13009	18404	12959	12547	14735	13454	18312
17CS1201	12340	12344	12344	13123	13795	7970	11425	13259	13008	18403	12958	12546	14734	13453	18311
17CS1205-1	11	6	22	12537	13417	12374	12878	12842	12795	18447	12621	12823	14487	12939	18376
17CS1205-2	0	5	11	12536	13414	12369	12873	12839	12792	18444	12618	12820	14482	12934	18373
17CS1206-1	5	0	16	12541	13419	12374	12878	12844	12797	18449	12623	12825	14487	12939	18378
17CS1208-3	11	16	0	12535	13419	12375	12878	12842	12795	18442	12621	12824	14485	12936	18371
Ab-CR1143	12536	12541	12535	0	12336	13114	13081	8052	7867	17492	7412	12749	13297	8959	17554
Ab-3711	13414	13419	13419	12336	0	13634	13290	12578	12440	18483	12404	13245	14162	12665	18404
Ab-CR1132	12369	12374	12375	13114	13634	0	11513	13307	13287	18320	12995	12551	14803	13484	18308
Ab-1711	12873	12878	12878	13081	13290	11513	0	13104	13098	18605	12981	12592	14693	13537	18596
Ab-CR461	12839	12844	12842	8052	12578	13307	13104	0	7167	17462	5729	12636	13208	7434	17306
Ab-4511	12792	12797	12795	7867	12440	13287	13098	7167	0	17169	4636	12573	13032	7767	17082
Ab-2211	18444	18449	18442	17492	18483	18320	18605	17462	17169	0	16852	18063	17096	17469	13051
Ab-2811	12618	12623	12621	7412	12404	12995	12981	5729	4636	16852	0	12390	12744	7825	16690
Ab-A103	12820	12825	12824	12749	13245	12551	12592	12636	12573	18063	12390	0	14128	12981	17908
Ab-CR424	14482	14487	14485	13297	14162	14803	14693	13208	13032	17096	12744	14128	0	13292	17006
Ab-CR641	12934	12939	12936	8959	12665	13484	13537	7434	7767	17469	7825	12981	13292	0	17375

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Strain	ERR3523208	ERR3523209	ERR3523210	ERR3523211	ERR3523212	ERR3523213	ERR3523214	ERR3523215	ERR3523216	ERR3523217	GCF-900187115-1
17CS1095	13782	12992	14120	10386	16393	13179	12552	14487	17583	18467	15820
17CS1167	13577	12486	13771	9963	16106	12964	12573	14150	17260	18137	15560
17CS1168	13576	12682	13911	10013	16243	13147	12406	14270	17245	18007	15656
17CS1193-1	13398	12422	13711	9521	16082	12699	12238	14105	17228	17976	15410
17CS1193-2	13398	12422	13711	9521	16082	12699	12238	14105	17228	17976	15410
17CS1193-3	13527	12288	13605	9955	16019	12850	12235	14083	17023	17909	15376
17CS1197	13687	12878	14040	9745	16329	12994	12585	14301	17286	18269	15687
17CS1197-2	13685	12876	14038	9745	16329	12994	12585	14301	17288	18269	15689
17CS1197-3	13685	12876	14038	9745	16329	12994	12585	14301	17288	18269	15689
17CS1198-1	13610	12590	13737	9979	16149	12823	12480	14063	17321	18078	15522
17CS1198-2	13610	12590	13737	9979	16149	12823	12480	14063	17321	18078	15522
17CS1198-3	13610	12590	13737	9979	16149	12823	12480	14063	17321	18078	15522
17CS1200-1	13619	12545	13860	9839	16089	12970	12551	14135	17272	17969	15382
17CS1201	13618	12544	13859	9838	16088	12969	12550	14134	17271	17968	15381
17CS1205-1	13289	12325	13657	12458	16246	12955	12683	13777	17083	18083	15463
17CS1205-2	13282	12320	13652	12452	16245	12950	12676	13770	17076	18078	15464
17CS1206-1	13287	12325	13657	12456	16250	12955	12681	13775	17081	18083	15469
17CS1208-3	13287	12321	13654	12455	16245	12955	12678	13767	17075	18078	15460
Ab-CR1143	12646	12741	12294	13105	15689	12714	12658	12725	16251	17353	15030
Ab-3711	12846	13253	13284	13530	16522	12897	12753	13813	17297	18356	15536
Ab-CR1132	13609	12605	13833	9791	16229	12976	12354	14333	17305	18005	15535
Ab-1711	13201	13257	13497	11845	16444	10861	11625	14237	17270	18348	15717
Ab-CR461	12628	12886	11804	13219	15538	12626	12510	12691	16171	17206	14977
Ab-4511	12682	12748	11870	13199	15470	12677	12766	12519	16012	17053	14895
Ab-2211	18808	18126	18112	18203	18052	18775	18461	18191	15799	13103	17572
Ab-2811	12693	12656	11649	12995	15222	12696	12423	12312	15085	16679	14950
Ab-A103	13207	12732	13345	12608	15886	10114	7425	13644	16958	17805	15217
Ab-CR424	14535	14484	13756	14674	16028	14385	13989	13787	16718	17007	15352
Ab-CR641	12868	13020	12048	13385	15727	12962	12875	12848	16342	17309	15061

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Strain	16CS0367-1-AR	16CS0367-AR	16CS0370-1	16CS0375-1	16CS0375-2	16CS0815-2	16CS0817-2	16CS0820-1	16CS0820-2	16CS0821-2	16CS0823-1	16CS0823-2	16CS0831-1-1	16CS0831-4-1
Ab-DQ20dA1	18042	18039	18042	18042	18042	18596	18508	18262	18531	18539	18596	18417	18042	14335
Ab-DQ64A1	13639	13639	13639	13639	13639	12192	13704	13729	12298	12304	12192	13621	13639	19865
Ab-CR604	12565	12569	12565	12565	12565	13366	12918	12996	13102	13106	13366	12698	12565	19249
Ab-4211	13918	13913	13918	13918	13918	13213	14082	13871	12843	12851	13213	14170	13918	19201
Ab-DQ40A1	9890	9908	9890	9890	9890	13618	10340	10119	13652	13656	13618	11267	9890	19373
Ab-1426-2003	16138	16129	16138	16138	16138	16023	16378	16354	16085	16092	16023	16430	16138	19247
Ab-CR502	13038	13044	13038	13038	13038	12464	13295	13121	12310	12313	12464	13060	13038	19840
Ab-A111	12432	12436	12432	12432	12432	12441	12715	12553	12508	12510	12441	12545	12432	19450
Ab-CR892	14228	14231	14228	14228	14228	13591	14399	14290	13715	13721	13591	14331	14228	19347
Ab-CR891	17161	17163	17161	17161	17161	17531	17371	17578	17493	17498	17531	17450	17161	16833
Ab-DQ31A1	18066	18062	18066	18066	18066	18479	18178	18117	18414	18420	18479	18374	18066	14549
NCTC-12481	15466	15434	15466	15466	15466	15017	15689	15633	15139	15137	15017	15687	15466	18849

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Strain	16CS0847-3	16CS0857-1	16CS0861-1	17CS0824	17CS0916-1	17CS0916-2	17CS0965-1	17CS0965-B	17CS1056	17CS1066	17CS1067	17CS1068-1	17CS1068-2	17CS1092-1	17CS1092-2
Ab-DQ20dA1	18532	18303	18291	18166	18385	18385	18511	18207	18450	18170	18175	18174	18173	18441	18440
Ab-DQ64A1	13812	12902	13655	13577	12979	12979	13548	12802	13427	13578	13579	13576	13573	13566	13565
Ab-CR604	12960	12296	12542	12554	11953	11953	12945	12877	12558	12483	12488	12485	12484	12904	12905
Ab-4211	14121	13418	13926	13795	13173	13173	13916	13027	13739	13766	13773	13770	13767	13972	13971
Ab-DQ40A1	10128	12863	9924	10263	12728	12728	10446	12580	9643	9960	9965	9962	9961	9986	9985
Ab-1426-2003	16322	16064	16104	16158	15896	15896	16387	16047	16178	16101	16104	16105	16102	16388	16387
Ab-CR502	13091	12845	13130	13016	12784	12784	12869	9405	12699	12959	12966	12963	12962	12915	12914
Ab-A111	12571	12667	12569	12392	12444	12444	12415	6408	12190	12570	12575	12572	12569	12381	12380
Ab-CR892	14354	13386	14224	14038	13062	13062	14360	13680	14191	14145	14152	14149	14150	14295	14294
Ab-CR891	17474	17098	17347	17248	17086	17086	17376	17142	17365	17253	17258	17259	17258	17442	17441
Ab-DQ31A1	18326	18131	18186	18102	18218	18218	18259	18122	18111	18130	18137	18136	18135	18258	18257
NCTC-12481	15809	15228	15491	15471	15110	15110	15696	15247	15469	15553	15552	15559	15556	15751	15750

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Strain	17CS1095	17CS1167	17CS1168	17CS1193-1	17CS1193-2	17CS1193-3	17CS1197	17CS1197-2	17CS1197-3	17CS1198-1	17CS1198-2	17CS1198-3	17CS1200-1	17CS1201	17CS1205-1
Ab-DQ20dA1	18650	18175	18177	18209	18209	18143	18389	18389	18389	18376	18376	18376	18312	18311	18376
Ab-DQ64A1	13782	13577	13576	13398	13398	13527	13687	13685	13685	13610	13610	13610	13619	13618	13289
Ab-CR604	12992	12486	12682	12422	12422	12288	12878	12876	12876	12590	12590	12590	12545	12544	12325
Ab-4211	14120	13771	13911	13711	13711	13605	14040	14038	14038	13737	13737	13737	13860	13859	13657
Ab-DQ40A1	10386	9963	10013	9521	9521	9955	9745	9745	9745	9979	9979	9979	9839	9838	12458
Ab-1426-2003	16393	16106	16243	16082	16082	16019	16329	16329	16329	16149	16149	16149	16089	16088	16246
Ab-CR502	13179	12964	13147	12699	12699	12850	12994	12994	12994	12823	12823	12823	12970	12969	12955
Ab-A111	12552	12573	12406	12238	12238	12235	12585	12585	12585	12480	12480	12480	12551	12550	12683
Ab-CR892	14487	14150	14270	14105	14105	14083	14301	14301	14301	14063	14063	14063	14135	14134	13777
Ab-CR891	17583	17260	17245	17228	17228	17023	17286	17288	17288	17321	17321	17321	17272	17271	17083
Ab-DQ31A1	18467	18137	18007	17976	17976	17909	18269	18269	18269	18078	18078	18078	17969	17968	18083
NCTC-12481	15820	15560	15656	15410	15410	15376	15687	15689	15689	15522	15522	15522	15382	15381	15463

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Strain	17CS1205-2	17CS1206-1	17CS1208-3	ERR3523196	ERR3523197	ERR3523198	ERR3523199	ERR3523200	ERR3523201	ERR3523202	ERR3523203	ERR3523204	ERR3523205	ERR3523206	ERR3523207
Ab-DQ206A1	18373	18378	18371	17554	18404	18308	18596	17306	17082	13051	16690	17908	17006	17375	0
Ab-DQ64A1	13282	13287	13287	12646	12846	13609	13201	12628	12682	18808	12693	13207	14535	12868	18650
Ab-CR604	12320	12325	12321	12741	13253	12605	13257	12886	12748	18126	12656	12732	14484	13020	18054
Ab-4211	13652	13657	13654	12294	13284	13833	13497	11804	11870	18112	11649	13345	13756	12048	18078
Ab-DQ40A1	12452	12456	12455	13105	13530	9791	11845	13219	13199	18203	12995	12608	14674	13385	18187
Ab-1426-2003	16245	16250	16245	15689	16522	16229	16444	15538	15470	18052	15222	15886	16028	15727	17887
Ab-CR502	12950	12955	12955	12714	12897	12976	10861	12626	12677	18775	12696	10114	14385	12962	18533
Ab-A111	12676	12681	12678	12658	12753	12354	11625	12510	12766	18461	12423	7425	13989	12875	18176
Ab-CR892	13770	13775	13767	12725	13813	14333	14237	12691	12519	18191	12312	13644	13787	12848	17951
Ab-CR891	17076	17081	17075	16251	17297	17305	17270	16171	16012	15799	15085	16958	16718	16342	16169
Ab-DQ31A1	18078	18083	18078	17353	18356	18005	18348	17206	17053	13103	16679	17805	17007	17309	13461
NCTC-12481	15464	15469	15460	15030	15536	15535	15717	14977	14895	17572	14950	15217	15352	15061	17193

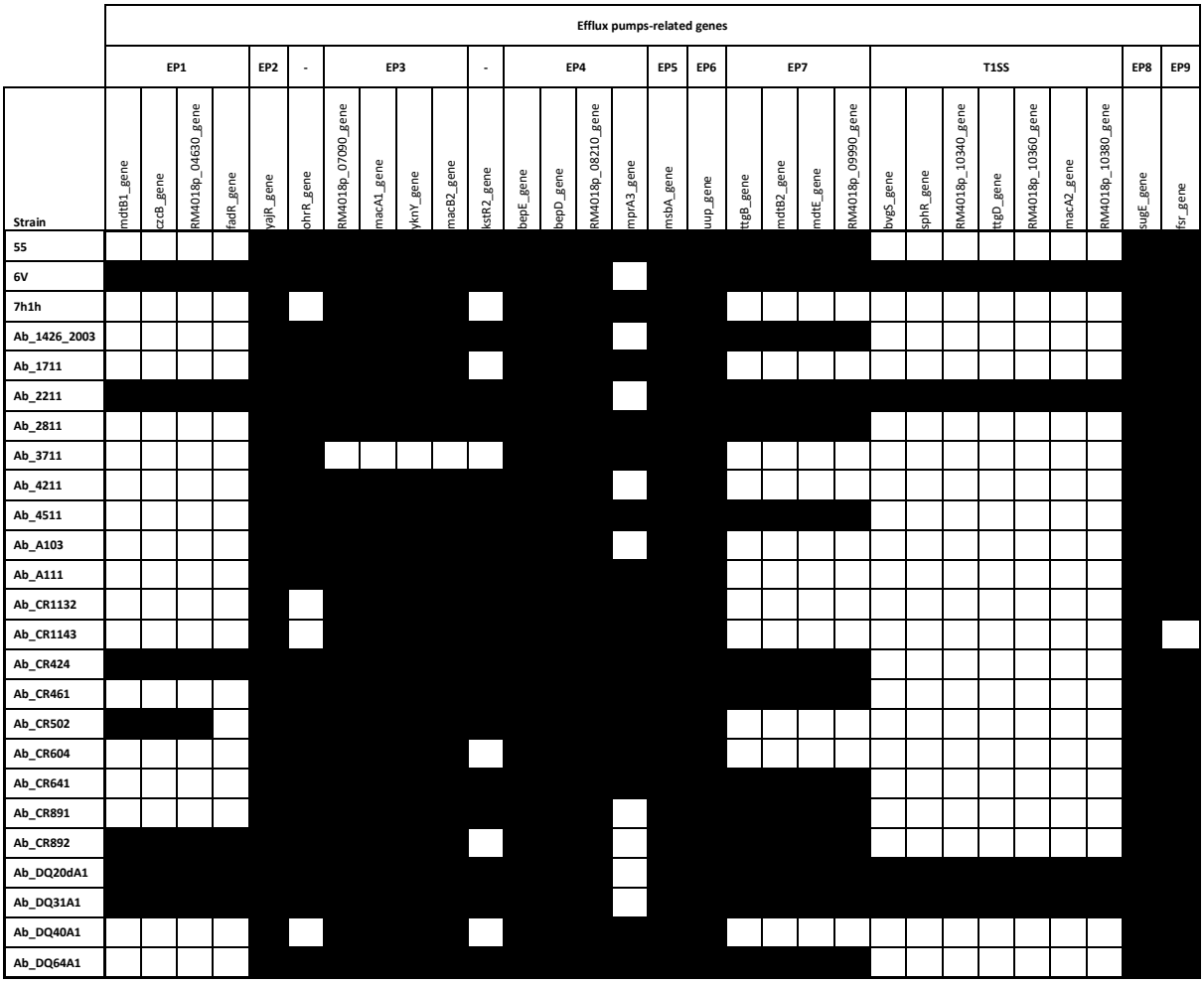
Fortsetzung **Table S4**

Strain	ERR3523208	ERR3523209	ERR3523210	ERR3523211	ERR3523212	ERR3523213	ERR3523214	ERR3523215	ERR3523216	ERR3523217	GCF-900187115-1
Ab-DQ20dA1	18650	18054	18078	18187	17887	18533	18176	17951	16169	13461	17193
Ab-DQ64A1	0	13177	13483	13672	16354	13019	12721	13696	17660	18433	15293
Ab-CR604	13177	0	13627	12665	16222	13065	12839	13799	16495	17763	15393
Ab-4211	13483	13627	0	14052	16015	13155	13322	13222	16855	17946	15127
Ab-DQ40A1	13672	12665	14052	0	16152	13049	12426	14092	17214	17989	15491
Ab-1426-2003	16354	16222	16015	16152	0	16150	16028	15910	17715	17742	14903
Ab-CR502	13019	13065	13155	13049	16150	0	9666	13759	17531	18477	15347
Ab-A111	12721	12839	13322	12426	16028	9666	0	13711	17171	18088	15349
Ab-CR892	13696	13799	13222	14092	15910	13759	13711	0	17216	17841	14901
Ab-CR891	17660	16495	16855	17214	17715	17531	17171	17216	0	15768	17004
Ab-DQ31A1	18433	17763	17946	17989	17742	18477	18088	17841	15768	0	17212
NCTC-12481	15293	15393	15127	15491	14903	15347	15349	14901	17004	17212	0

Table S5 - Presence and absence of AMR genes in the *A. butzleri* strains

	Efflux pumps-related genes																												
	EP1				EP2	-	EP3				-	EP4				EP5	EP6	EP7				T1SS							
Strain	mdtB1_gene	czcB_gene	RM4018p_04630_gene	fadR_gene	yajR_gene	ohrR_gene	RM4018p_07090_gene	macA1_gene	yknY_gene	macB2_gene	kstR2_gene	bepE_gene	bepD_gene	RM4018p_08210_gene	mpra3_gene	msbA_gene	oup_gene	ttgB_gene	mdtB2_gene	mdtE_gene	RM4018p_09990_gene	bvgS_gene	sphR_gene	RM4018p_10340_gene	ttgD_gene	RM4018p_10360_gene	macA2_gene	RM4018p_10380_gene
16CS0847-3																													
17CS0824																													
17CS1193-1																													
17CS1193-2																													
17CS1193-3																													
17CS1197-2																													
17CS1197-3																													
17CS1197																													
17CS1198-1																													
17CS1198-2																													
17CS1198-3																													
16CS0367-1-AR																													
16CS0367-AR																													
16CS0370-1																													
16CS0375-1																													
16CS0375-2																													
16CS0831-1-1																													
16CS0831-4-1																													
17CS1200-1																													
17CS1201																													
17CS1205-1																													
17CS1205-2																													
17CS1206-1																													
17CS1208-3																													
17CS1167																													
17CS1168																													
17CS1056																													
17CS1066																													
17CS1067																													
17CS1068-1																													
17CS1068-2																													
17CS0916-1																													
17CS0916-2																													
16CS0857-1																													
16CS0861-1																													
17CS1092-1																													
17CS1092-2																													
17CS1095																													
16CS0815-2																													
16CS0817-2																													
16CS0820-1																													
16CS0820-2																													
16CS0821-2																													
16CS0823-1																													
16CS0823-2																													
17CS0965-1																													
17CS0965-B																													
RMI																													
RMIII																													
NCTC_12481																													

Fortsetzung **Table S5**



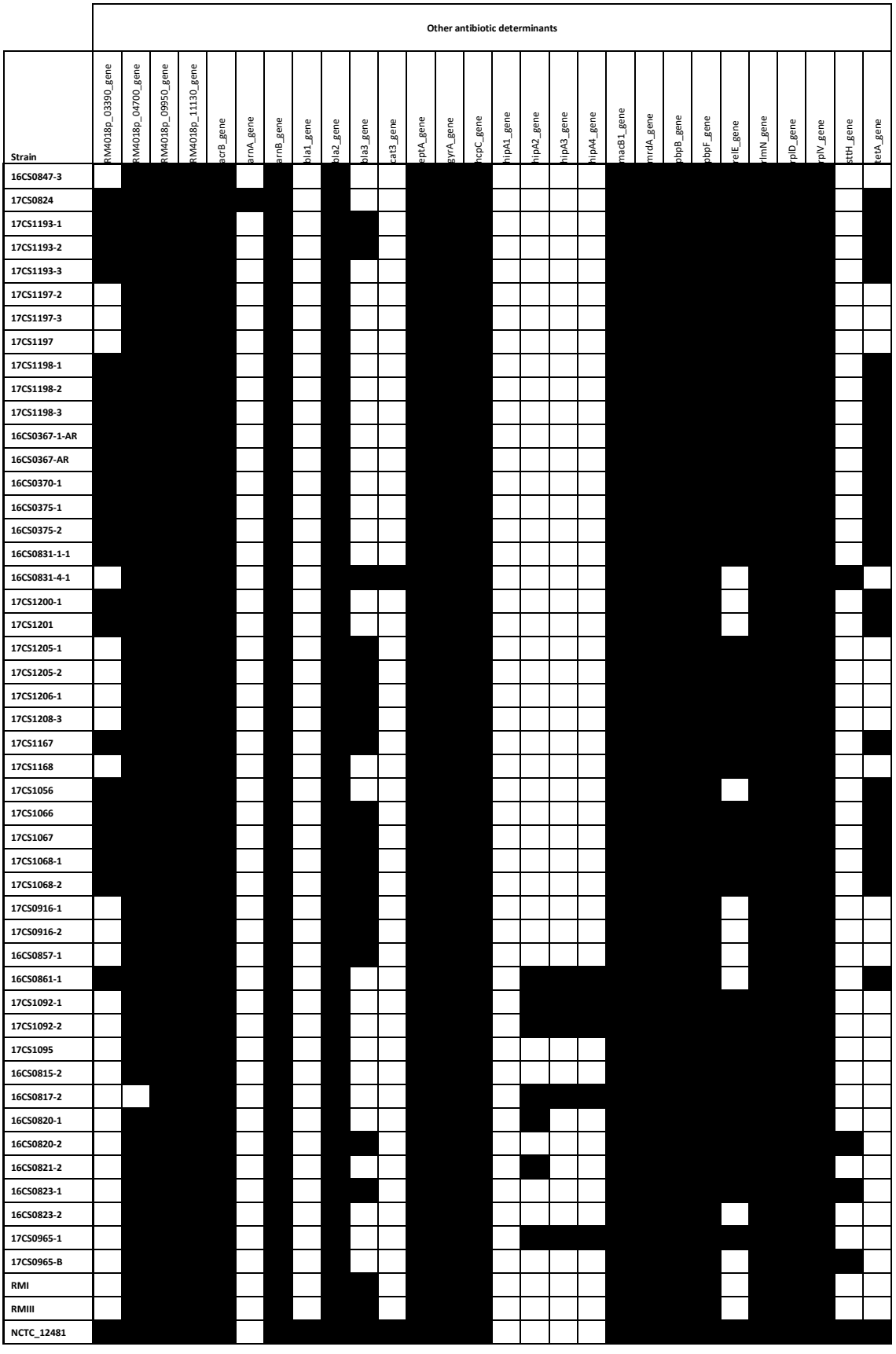
Fortsetzung **Table S5**

	Efflux pumps-related genes																													
	EP10					EP11			EP12	EP13	EP14	EP15				EP16				EP17	EP18				EP19				-
Strain	hspR_gene	macA3_gene	ybhF_gene	ybh5_gene	ybhR_gene	mexB1_gene	RM4018p_18770_gene	RM4018p_18780_gene	RM4018p_19480_gene	ydhP_gene	bcr1_gene	pleD5_gene	mexA1_gene	mexB2_gene	ttgC_gene	oprM_gene	mexB3_gene	mexA2_gene	RM4018p_22360_gene	bcr2_gene	cdhR_gene	bepF_gene	bepE1_gene	oprM1_gene	cmrA_gene	mdtA_gene	BMH_AB_2468p_07340_gene	oprM1_gene	tolC_gene
16CS0847-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										■
17CS0824	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										■
17CS1193-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										■
17CS1193-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										■
17CS1193-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1197-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1197-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1197	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1198-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1198-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1198-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0367-1-AR	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0367-AR	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0370-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0375-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0375-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0831-1-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0831-4-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1200-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1201	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1205-1	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■										
17CS1205-2	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■										
17CS1206-1	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■										
17CS1208-3	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■										
17CS1167	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1168	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1056	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1066	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1067	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1068-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS1068-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
17CS0916-1	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■										
17CS0916-2	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■										
16CS0857-1	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■										
16CS0861-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										■
17CS1092-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■															
17CS1092-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■															
17CS1095	■	■	■	■	■				■	■	■	■	■	■	■	■	■	■	■	■										
16CS0815-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0817-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0820-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0820-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0821-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0823-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
16CS0823-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■															
17CS0965-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■															
17CS0965-B	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■															
RMI	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■															
RMIII	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■															
NCTC_12481	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■										

Fortsetzung **Table S5**

	Efflux pumps-related genes																														
	EP10					EP11			EP12	EP13	EP14	EP15				EP16				EP17	EP18				EP19				-		
Strain	hspR_gene	macA3_gene	ybhF_gene	ybh5_gene	ybhR_gene	mexB1_gene	RM4018p_18770_gene	RM4018p_18780_gene	RM4018p_19480_gene	ydhP_gene	bcr1_gene	mexD5_gene	mexA1_gene	mexB2_gene	ttgC_gene	oprM_gene	mexB3_gene	mexA2_gene	RM4018p_22360_gene	bcr2_gene	cdhR_gene	bepF_gene	bepE1_gene	oprM1_gene	cmrA_gene	mdtA_gene	BMH_AB_2468p_07340_gene	oprM1_gene	tolC_gene			
55	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
6V	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
7h1h	■	■	■	■	■							■	■	■	■	■	■	■	■	■												■
Ab_1426_2003	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_1711	■	■	■	■	■			■				■	■	■	■	■	■	■	■	■												
Ab_2211	■	■	■	■	■							■	■	■	■	■	■	■	■	■												■
Ab_2811	■	■	■	■	■							■	■		■	■	■	■	■	■												
Ab_3711	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_4211	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_4511	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_A103	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_A111	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_CR1132	■	■	■	■	■							■	■	■	■	■	■	■	■	■												■
Ab_CR1143	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_CR424	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_CR461	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_CR502	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_CR604	■	■	■	■	■			■				■	■	■	■	■	■	■	■	■												
Ab_CR641	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_CR891	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_CR892	■	■	■	■	■							■	■	■	■	■	■	■	■	■												■
Ab_DQ20dA1	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_DQ31A1	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_DQ40A1	■	■	■	■	■							■	■	■	■	■	■	■	■	■												
Ab_DQ64A1	■	■	■	■	■							■	■	■	■	■	■	■	■	■												

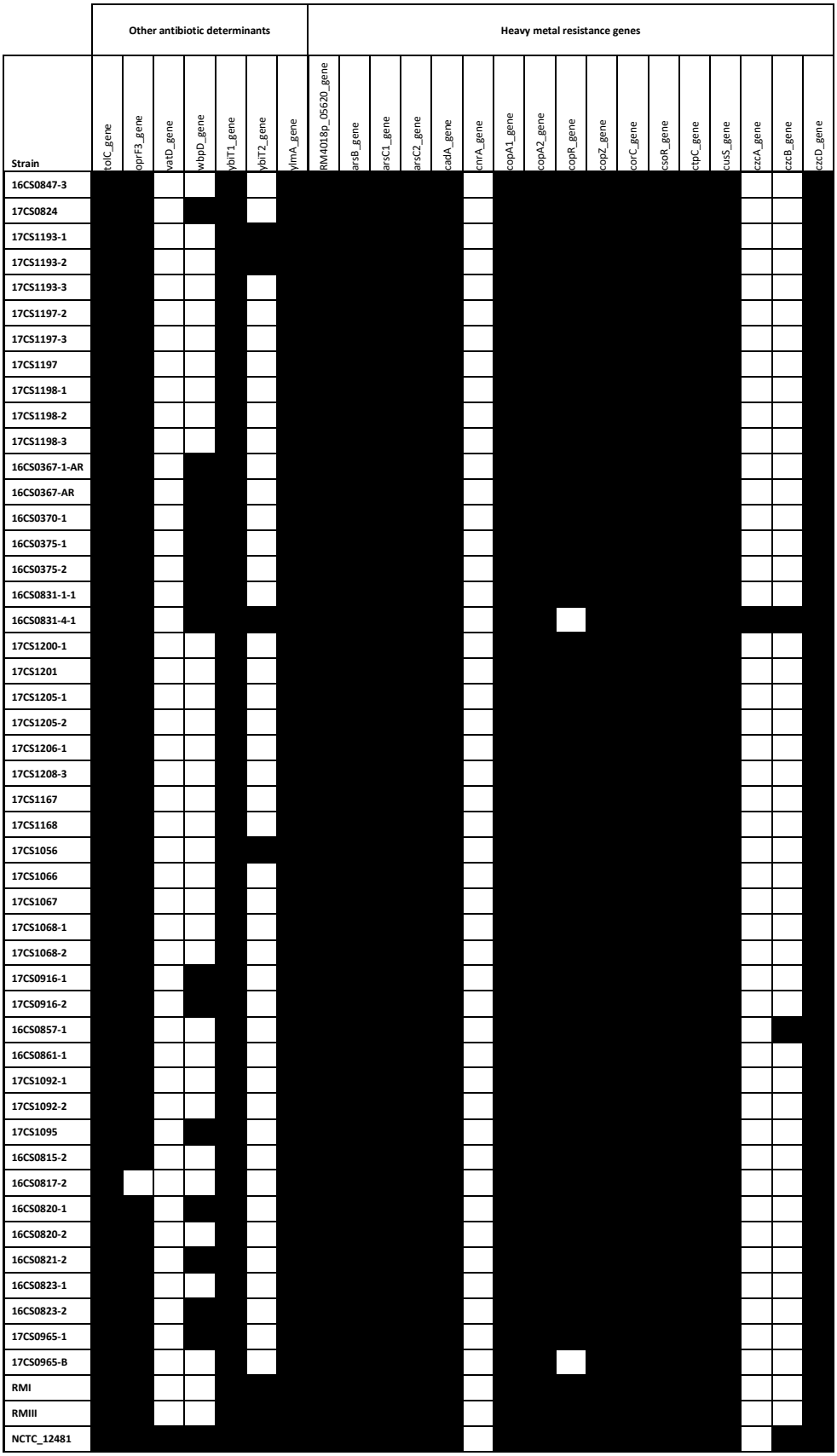
Fortsetzung **Table S5**



Fortsetzung **Table S5**

	Other antibiotic determinants																												
Strain	RM4018p_03390_gene	RM4018p_04700_gene	RM4018p_09950_gene	RM4018p_11130_gene	acrB_gene	arnA_gene	arnB_gene	bla1_gene	bla2_gene	bla3_gene	cat3_gene	eptA_gene	gyrA_gene	rcpC_gene	hipA1_gene	hipA2_gene	hipA3_gene	hipA4_gene	macB1_gene	mrdA_gene	pbpB_gene	pbpF_gene	relE_gene	rlmN_gene	rplD_gene	rplV_gene	sttH_gene	tetA_gene
55	■	■	■	■	■	□	■	■	■	■	■	■	■	■	□	□	□	□	■	■	■	■	■	■	■	■	■	■
6V	■	■	■	■	■	□	■	■	■	■	■	■	■	■	■	□	□	□	■	■	■	■	■	■	■	■	■	■
7h1h	■	■	■	■	■	□	■	□	■	■	■	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	■
Ab_1426_2003	■	■	■	■	■	□	■	■	■	■	■	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	■	■
Ab_1711	□	■	■	■	■	□	■	□	■	□	□	■	■	■	□	■	■	■	■	■	■	■	■	■	■	■	□	□
Ab_2211	■	■	■	■	■	□	■	□	■	■	■	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	■	■
Ab_2811	■	■	■	■	■	□	■	□	■	■	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	■
Ab_3711	□	■	■	■	■	□	■	□	■	■	□	■	■	■	□	■	□	□	■	■	■	■	■	■	■	■	□	□
Ab_4211	■	■	■	■	■	□	■	□	■	■	□	■	■	■	□	■	■	■	■	■	■	■	□	■	■	■	■	■
Ab_4511	□	■	■	■	■	□	■	□	■	■	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	□
Ab_A103	□	■	■	■	■	□	■	□	■	□	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	□
Ab_A111	□	■	■	■	■	□	■	□	■	□	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	□
Ab_CR1132	■	■	■	■	■	□	■	□	■	■	□	■	■	■	□	■	■	■	■	■	■	■	■	■	■	■	□	■
Ab_CR1143	□	■	■	■	■	□	■	□	■	■	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	□
Ab_CR424	■	■	■	■	■	□	■	□	■	■	■	■	■	■	□	■	■	■	■	■	■	■	□	■	■	■	■	■
Ab_CR461	□	■	■	■	■	□	■	□	■	■	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	□
Ab_CR502	□	■	■	■	■	□	■	□	■	□	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	■	□
Ab_CR604	□	■	■	■	■	□	■	□	■	□	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	■	□
Ab_CR641	□	■	■	■	■	□	■	□	■	□	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	□
Ab_CR891	□	■	■	■	■	□	■	□	■	□	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	■	□
Ab_CR892	■	■	■	■	■	□	■	□	■	■	■	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	■	■
Ab_DQ20dA1	■	■	■	■	■	□	■	□	■	■	■	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	■	■
Ab_DQ31A1	■	■	■	■	■	□	■	□	■	■	■	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	■	■
Ab_DQ40A1	■	■	■	■	■	□	■	□	■	□	□	■	■	■	□	□	□	□	■	■	■	■	■	■	■	■	□	■
Ab_DQ64A1	■	■	■	■	■	□	■	□	■	■	□	■	■	■	□	□	□	□	■	■	■	■	□	■	■	■	□	■

Fortsetzung **Table S5**



	Other antibiotic determinants							Heavy metal resistance genes																
Strain	tolC_gene	oprF3_gene	vatD_gene	wbpD_gene	ybiT1_gene	ybiT2_gene	ylmA_gene	RM4018p_05620_gene	arsB_gene	arsC1_gene	arsC2_gene	cadA_gene	cnrA_gene	copA1_gene	copA2_gene	copR_gene	copZ_gene	corC_gene	csoR_gene	ctpC_gene	cusS_gene	czcA_gene	czcB_gene	czcD_gene
16CS0847-3																								
17CS0824																								
17CS1193-1																								
17CS1193-2																								
17CS1193-3																								
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16CS0367-1-AR																								
16CS0367-AR																								
16CS0370-1																								
16CS0375-1																								
16CS0375-2																								
16CS0831-1-1																								
16CS0831-4-1																								
17CS1200-1																								
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17CS1092-1																								
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16CS0815-2																								
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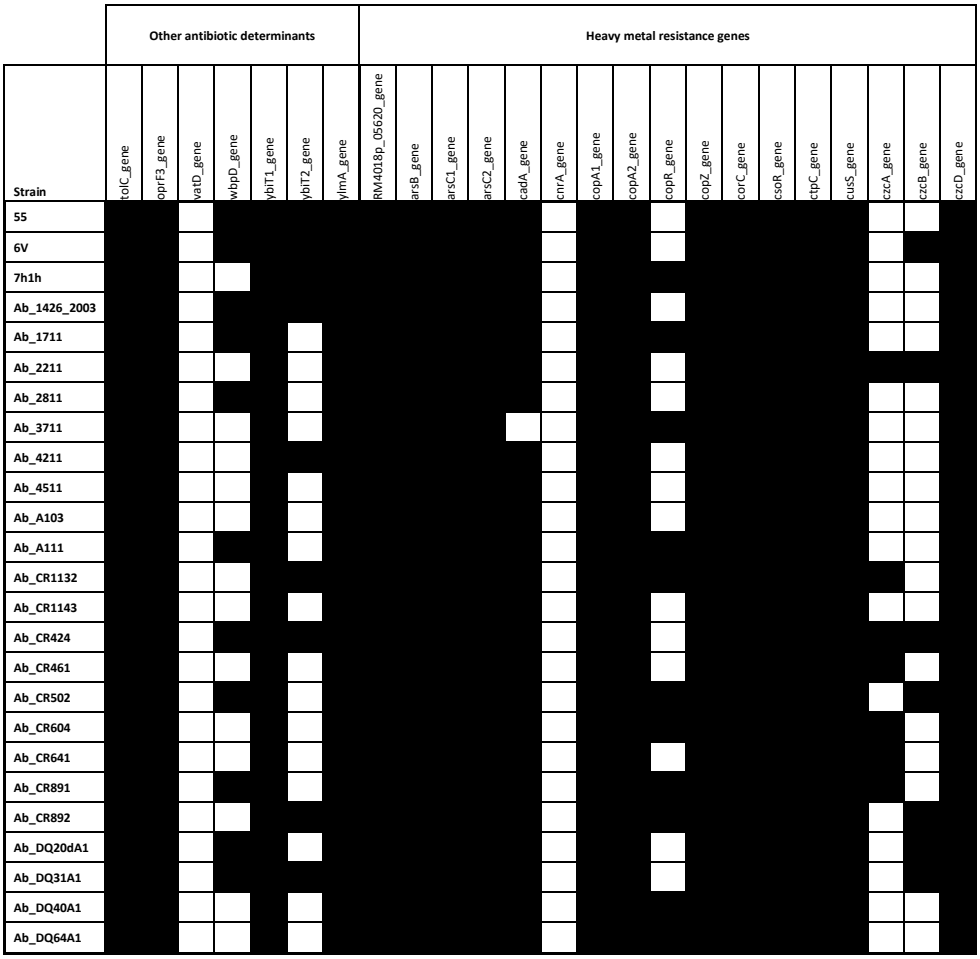
16CS0823-1																								
16CS0823-2																								
17CS0965-1																								
17CS0965-B																								
RMI																								
RMIII																								
NCTC_12481																								

Fortsetzung **Table S5**



Fortsetzung **Table S5**

	Heavy metal resistance genes										Total number of genes
Strain	czcR1_gene	czcR2_gene	czcR3_gene	czcR4_gene	czcR5_gene	merT_gene	modA_gene	modB_gene	mopA_gene	zntB_gene	
16CS0847-3	■	■	■	■		■	■	■	■	■	78
17CS0824	■	■	■	■		■	■	■	■	■	80
17CS1193-1	■	■	■	■		■	■	■	■	■	83
17CS1193-2	■	■	■	■		■	■	■	■	■	83
17CS1193-3	■	■	■	■		■	■	■	■	■	80
17CS1197-2	■	■	■			■	■	■	■	■	75
17CS1197-3	■	■	■			■	■	■	■	■	75
17CS1197	■	■	■			■	■	■	■	■	75
17CS1198-1	■	■	■	■		■	■	■	■	■	79
17CS1198-2	■	■	■	■		■	■	■	■	■	79
17CS1198-3	■	■	■	■		■	■	■	■	■	79
16CS0367-1-AR	■	■	■	■		■	■	■	■	■	81
16CS0367-AR	■	■	■	■		■	■	■	■	■	81
16CS0370-1	■	■	■	■		■	■	■	■	■	81
16CS0375-1	■	■	■	■		■	■	■	■	■	81
16CS0375-2	■	■	■	■		■	■	■	■	■	81
16CS0831-1-1	■	■	■	■		■	■	■	■	■	81
16CS0831-4-1	■	■	■			■	■	■	■	■	98
17CS1200-1	■	■	■			■	■	■	■	■	77
17CS1201	■	■	■			■	■	■	■	■	77
17CS1205-1	■	■	■			■	■	■	■	■	73
17CS1205-2	■	■	■			■	■	■	■	■	73
17CS1206-1	■	■	■			■	■	■	■	■	73
17CS1208-3	■	■	■			■	■	■	■	■	73
17CS1167	■	■	■			■	■	■	■	■	80
17CS1168	■	■	■	■		■	■	■	■	■	77
17CS1056	■	■	■	■		■	■	■	■	■	79
17CS1066	■	■	■			■	■	■	■	■	80
17CS1067	■	■	■			■	■	■	■	■	80
17CS1068-1	■	■	■			■	■	■	■	■	80
17CS1068-2	■	■	■			■	■	■	■	■	80
17CS0916-1	■	■	■			■	■	■	■	■	74
17CS0916-2	■	■	■			■	■	■	■	■	74
16CS0857-1	■	■	■			■	■	■	■	■	78
16CS0861-1	■	■	■	■		■	■	■	■	■	82
17CS1092-1	■	■	■			■	■	■	■	■	74
17CS1092-2	■	■	■			■	■	■	■	■	74
17CS1095	■	■	■			■	■	■	■	■	74
16CS0815-2	■	■	■			■	■	■	■	■	76
16CS0817-2	■	■	■			■	■	■	■	■	77
16CS0820-1	■	■	■			■	■	■	■	■	80
16CS0820-2	■	■	■			■	■	■	■	■	80
16CS0821-2	■	■	■			■	■	■	■	■	80
16CS0823-1	■	■	■			■	■	■	■	■	80
16CS0823-2	■	■	■			■	■	■	■	■	71
17CS0965-1	■	■	■			■	■	■	■	■	80
17CS0965-B	■	■	■			■	■	■	■	■	71
RMI	■	■	■			■	■	■	■	■	74
RMIII	■	■	■			■	■	■	■	■	74
NCTC_12481	■	■	■	■		■	■	■	■	■	104

Fortsetzung **Table S5**

	Heavy metal resistance genes										Total number of genes
Strain	czcR1_gene	czcR2_gene	czcR3_gene	czcR4_gene	czcR5_gene	merT_gene	modA_gene	modB_gene	mopA_gene	zntB_gene	
55	■	■	■			■	■	■	■	■	89
6V	■	■	■		■	■	■	■	■	■	102
7h1h	■	■	■	■		■	■	■	■	■	82
Ab_1426_2003	■	■	■			■	■	■	■	■	87
Ab_1711	■	■	■			■	■	■	■	■	79
Ab_2211	■	■	■	■	■	■	■	■	■	■	100
Ab_2811	■	■	■			■	■	■	■	■	73
Ab_3711	■	■	■			■	■	■	■	■	66
Ab_4211	■	■	■			■	■	■	■	■	83
Ab_4511	■	■	■			■	■	■	■	■	81
Ab_A103	■	■	■			■	■	■	■	■	70
Ab_A111	■	■	■			■	■	■	■	■	73
Ab_CR1132	■	■	■	■		■	■	■	■	■	87
Ab_CR1143	■	■	■			■	■	■	■	■	75
Ab_CR424	■	■	■			■	■	■	■	■	96
Ab_CR461	■	■	■			■	■	■	■	■	77
Ab_CR502	■	■	■			■	■	■	■	■	82
Ab_CR604	■	■	■			■	■	■	■	■	75
Ab_CR641	■	■	■			■	■	■	■	■	81
Ab_CR891	■	■	■			■	■	■	■	■	83
Ab_CR892	■	■	■			■	■	■	■	■	91
Ab_DQ20dA1	■	■	■			■	■	■	■	■	97
Ab_DQ31A1	■	■	■			■	■	■	■	■	98
Ab_DQ40A1	■	■	■	■		■	■	■	■	■	79
Ab_DQ64A1	■	■	■			■	■	■	■	■	80

black box = present; white box = absent

Table S6 - Results of the plasmid prediction in the *A. butzleri* strains. Only plasmids with a detection value of at least 55% coverage and 85% identity were reported.

Strain	Contig	Identity (%)	Query length	Coverage (%)	Plasmid	Plasmid Accession
17CS1198-1	26	91.18	1,588	70.0	*A. butzleri* strain AC1166 plasmid AB-1166-LD	NC_025124.1
17CS1198-2	26	91.18	1,588	66.0	*A. butzleri* strain AC1166 plasmid AB-1166-LD	NC_025124.1
17CS1198-3	25	87.42	2,736	63.0	*A. butzleri* strain AC1166 plasmid AB-1166-LD	NC_025124.1
17CS0916-1	16	99.84	1,906	68.0	*A. butzleri* strain AC1167 plasmid AB-1167-LD	NC_025123.1
17CS0916-2	17	99.74	2,716	57.0	*A. butzleri* strain AC1167 plasmid AB-1167-LD	NC_025123.1
Ab_3711	44	99.89	2,932	100	*A. butzleri* strain AC1167 plasmid AB-1167-LD	NC_025123.1
Ab_4211	64	99.87	3,088	100	*A. butzleri* strain AC1166 plasmid AB-1166-LD	NC_025124.1

Table S7 - Presence and absence of virulence genes in the *A. butzleri* strains

	Flagellar genes																												
Strain	flaA_gene	flaB_gene	flgB_gene	flgC_gene	flgD_gene	flgE1_gene	flgE2_gene	flgG1_gene	flgG2_gene	flgH_gene	flgI_gene	flgK_gene	flgL_gene	fliA_gene	fliB1_gene	fliB2_gene	fliF_gene	fliD_gene	fliE_gene	fliF_gene	fliG_gene	fliH_gene	fliI_gene	fliK_gene	fliL_gene	fliM_gene	fliN1_gene	fliN2_gene
16CS0847-3																													
17CS0824																													
17CS1193-1																													
17CS1193-2																													
17CS1193-3																													
17CS1197-2																													
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17CS1198-3																													
16CS0367-1-AR																													
16CS0367-AR																													
16CS0370-1																													
16CS0375-1																													
16CS0375-2																													
16CS0831-1-1																													
16CS0831-4-1																													
17CS1200-1																													
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16CS0861-1																													
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17CS1095																													
16CS0815-2																													
16CS0817-2																													
16CS0820-1																													
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16CS0821-2																													
16CS0823-1																													
16CS0823-2																													
17CS0965-1																													
17CS0965-B																													
RMI																													
RMIII																													
NCTC_12481																													

Fortsetzung **Table S7**

	Flagellar genes																												
Strain	flaA_gene	flaB_gene	flgB_gene	flgC_gene	flgD_gene	flgE1_gene	flgE2_gene	flgG1_gene	flgG2_gene	flgH_gene	flgI_gene	flgK_gene	flgL_gene	flhA_gene	flhB1_gene	flhB2_gene	flhF_gene	fliD_gene	fliE_gene	fliF_gene	fliG_gene	fliH_gene	fliI_gene	fliK_gene	fliL_gene	fliM_gene	fliN1_gene	fliN2_gene
55	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
6V	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
7h1h	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_1426_2003	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_1711	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_2211	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_2811	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_3711	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_4211		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_4511		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_A103	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_A111	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR1132	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR1143	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR424	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR461	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR502	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR604	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR641	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR891	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_CR892	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_DQ20dA1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_DQ31A1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_DQ40A1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Ab_DQ64A1			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

Fortsetzung **Table S7**

	Flagellar genes								Chemotaxis cluster								Urease cluster						Capsule cluster						
Strain	fliP_gene	fliQ_gene	fliR_gene	flhS_gene	flhV2_gene	hag_gene	pomA_gene	motB_gene	cheW_gene	cheV_gene	cheY1_gene	cheA_gene	cheR_gene	cheB_gene	cheY2_gene	cheY3_gene	ureD_gene	ureA_gene	ureC_gene	ureE_gene	ureF_gene	ureG_gene	fcl_gene	glmM2_gene	gmhA_gene	gmhB2_gene	hddA_gene	hddC_gene	rmd_gene
16CS0847-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS0824	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1193-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1193-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1193-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1197-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1197-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1197	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1198-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1198-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1198-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0367-1-AR	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0367-AR	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0370-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0375-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0375-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0831-1-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0831-4-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
17CS1200-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1201	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1205-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1205-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1206-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1208-3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1167	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1168	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1056	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1066	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1067	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1068-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1068-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS0916-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS0916-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0857-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0861-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1092-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1092-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS1095	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0815-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0817-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0820-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0820-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0821-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0823-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
16CS0823-2	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS0965-1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
17CS0965-B	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
RMI	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
RMIII	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
NCTC_12481	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							

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	Flagellar genes								Chemotaxis cluster								Urease cluster						Capsule cluster						
Strain	fliP_gene	fliQ_gene	fliR_gene	fliS_gene	flhV2_gene	hag_gene	pomA_gene	motB_gene	cheW_gene	cheV_gene	cheY1_gene	cheA_gene	cheR_gene	cheB_gene	cheY2_gene	cheY3_gene	ureD_gene	ureA_gene	ureC_gene	ureE_gene	ureF_gene	ureG_gene	fcl_gene	glmM2_gene	gmhA_gene	gmhB2_gene	hddA_gene	hddC_gene	rmd_gene
55	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
6V	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
7h1h	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_1426_2003	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_1711	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_2211	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_2811	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_3711	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_4211	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_4511	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							■	■	■	■	■	■	■
Ab_A103	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_A111	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_CR1132	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_CR1143	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_CR424	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_CR461	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_CR502	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_CR604	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_CR641	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_CR891	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_CR892	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_DQ20dA1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_DQ31A1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													
Ab_DQ40A1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							
Ab_DQ64A1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							

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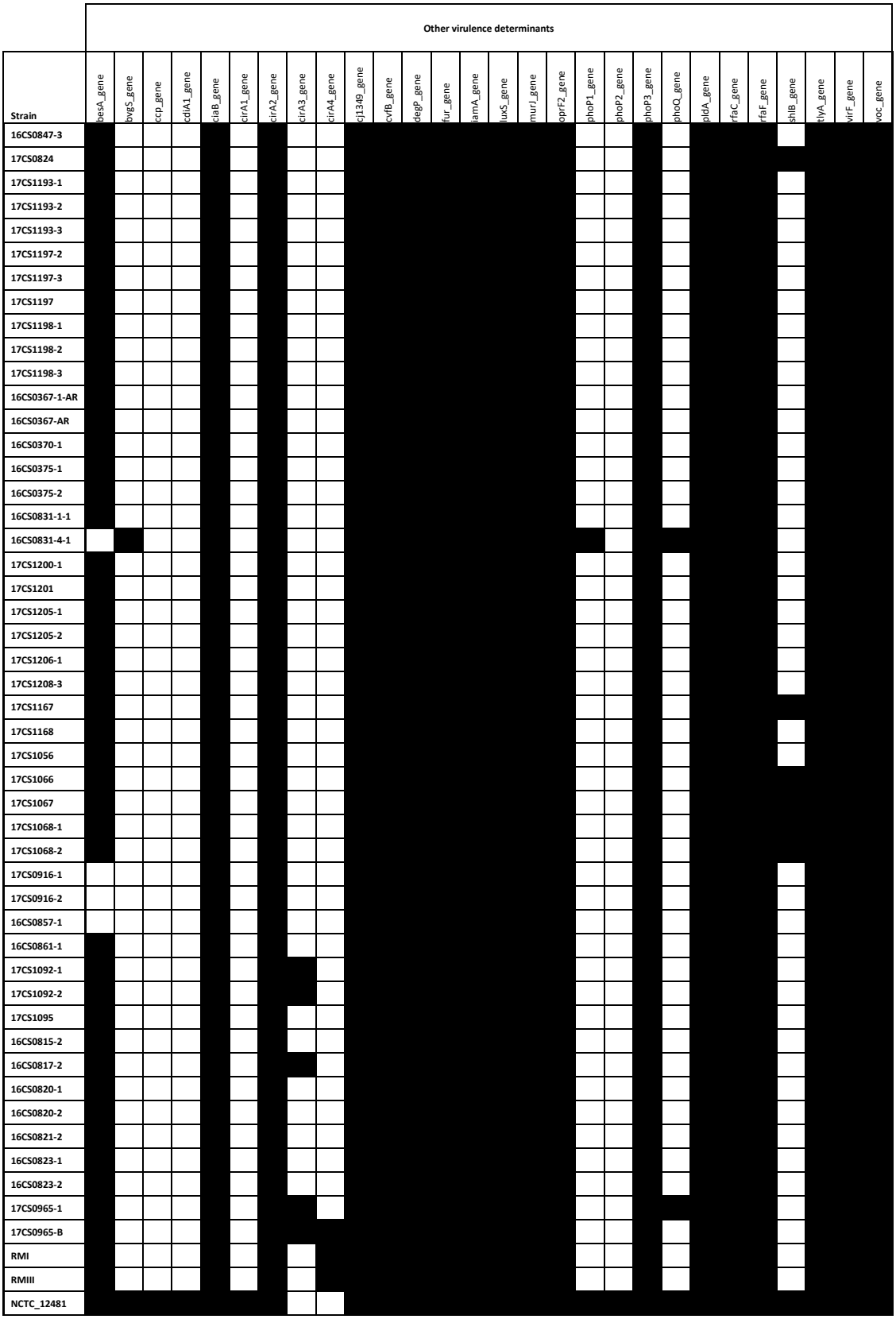

Strain	Other virulence determinants																														
	besA_gene	bvgS_gene	ccp_gene	cdiA1_gene	ciaB_gene	cirA1_gene	cirA2_gene	cirA3_gene	cirA4_gene	cj1349_gene	cvfB_gene	degP_gene	fur_gene	iamA_gene	luxS_gene	murJ_gene	oprF2_gene	phoP1_gene	phoP2_gene	phoP3_gene	phoQ_gene	pldA_gene	rfaC_gene	rfaF_gene	shlB_gene	tlyA_gene	virF_gene	voc_gene
16CS0847-3	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS0824	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1193-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1193-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1193-3	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1197-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1197-3	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1197	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1198-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1198-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1198-3	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0367-1-AR	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0367-AR	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0370-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0375-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0375-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0831-1-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0831-4-1		■			■		■			■	■	■	■	■	■	■	■	■		■	■	■	■	■		■	■	■
17CS1200-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1201	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1205-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1205-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1206-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1208-3	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1167	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1168	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1056	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1066	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1067	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1068-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1068-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS0916-1					■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS0916-2					■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0857-1					■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0861-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1092-1	■				■		■	■		■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1092-2	■				■		■	■		■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS1095	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0815-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0817-2	■				■		■	■		■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0820-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0820-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0821-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0823-1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
16CS0823-2	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS0965-1	■				■		■	■		■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
17CS0965-B	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
RMI	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
RMIII	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
NCTC_12481	■	■	■	■	■	■	■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

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	Other virulence determinants																												
Strain	besA_gene	bvgS_gene	ccp_gene	cdiA1_gene	ciaB_gene	cirA1_gene	cirA2_gene	cirA3_gene	cirA4_gene	cj1349_gene	cvfB_gene	degP_gene	fur_gene	iamA_gene	luxS_gene	murJ_gene	oprF2_gene	phoP1_gene	phoP2_gene	phoP3_gene	phoQ_gene	pldA_gene	rfaC_gene	rfaF_gene	shlB_gene	tlyA_gene	virF_gene	voc_gene
55	■				■	■	■			■	■	■	■	■	■	■	■	■	■	■		■	■	■		■	■	■
6V	■	■	■		■	■	■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■
7h1h	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_1426_2003	■		■		■	■	■			■	■	■	■	■	■	■	■	■	■	■		■	■	■		■	■	■
Ab_1711					■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_2211	■	■	■		■	■	■			■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■
Ab_2811					■	■	■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_3711					■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_4211	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_4511					■	■	■			■	■	■	■	■	■	■	■	■		■		■	■	■		■	■	■
Ab_A103	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_A111	■				■		■	■	■	■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_CR1132	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_CR1143					■	■	■			■	■	■	■	■	■	■	■	■		■		■	■	■		■	■	■
Ab_CR424					■		■			■	■	■	■	■	■	■	■	■		■		■	■	■		■	■	■
Ab_CR461					■	■	■		■	■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_CR502					■		■	■		■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_CR604					■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_CR641					■	■	■			■	■	■	■	■	■	■	■	■		■		■	■	■		■	■	■
Ab_CR891					■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_CR892					■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_DQ20dA1		■		■	■		■			■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■
Ab_DQ31A1	■	■			■	■	■			■	■	■	■	■	■	■	■	■		■		■	■	■	■	■	■	■
Ab_DQ40A1	■				■		■			■	■	■	■	■	■	■	■			■		■	■	■		■	■	■
Ab_DQ64A1					■		■	■	■	■	■	■	■	■	■	■	■			■		■	■	■	■	■	■	■

Fortsetzung **Table S7**

	Type IV secretion system genes (T4SS)																														
Strain	cssS_gene	cusR_gene	D4963p_10560_gene	tap4_gene	D4963p_10580_gene	hipA2_gene	D4963p_10600_gene	hipA3_gene	hipA4_gene	D4963p_10630_gene	D4963p_10640_gene	D4963p_10650_gene	D4963p_10660_gene	D4963p_10670_gene	D4963p_10680_gene	D4963p_10690_gene	D4963p_10700_gene	D4963p_10710_gene	D4963p_10720_gene	D4963p_10730_gene	D4963p_10740_gene	topB_gene	D4963p_10760_gene	virB1_gene	D4963p_10780_gene	D4963p_10790_gene	D4963p_10800_gene	D4963p_10810_gene
16CS0847-3				■																								
17CS0824				■																								
17CS1193-1				■																								
17CS1193-2				■																								
17CS1193-3				■											■													
17CS1197-2				■																								
17CS1197-3				■																								
17CS1197				■																								
17CS1198-1				■																								
17CS1198-2				■																								
17CS1198-3				■																								
16CS0367-1-AR				■																								
16CS0367-AR				■																								
16CS0370-1				■																								
16CS0375-1				■																								
16CS0375-2				■																								
16CS0831-1-1				■																								
16CS0831-4-1				■																								
17CS1200-1				■																								
17CS1201				■																								
17CS1205-1				■																								
17CS1205-2				■																								
17CS1206-1				■																								
17CS1208-3				■																								
17CS1167				■												■												
17CS1168				■																								
17CS1056				■												■												
17CS1066				■												■												
17CS1067				■												■												
17CS1068-1				■												■												
17CS1068-2				■												■												
17CS0916-1				■																								
17CS0916-2				■																								
16CS0857-1				■																								
16CS0861-1				■	■	■	■	■	■																			
17CS1092-1				■	■	■	■	■	■																			
17CS1092-2				■	■	■	■	■	■																			
17CS1095				■																								
16CS0815-2				■																								
16CS0817-2				■	■	■	■	■	■						■													
16CS0820-1				■	■	■																						
16CS0820-2				■																								
16CS0821-2				■	■	■																						
16CS0823-1				■																								
16CS0823-2				■																								
17CS0965-1				■	■	■	■	■	■	■					■													
17CS0965-B				■																								
RMI				■																								
RMIII				■																								
NCTC_12481			■	■																								

Fortsetzung **Table S7**

	Type IV secretion system genes (T4SS)																												
Strain	cssS_gene	cusR_gene	D4963p_10560_gene	tap4_gene	D4963p_10580_gene	hipA2_gene	D4963p_10600_gene	hipA3_gene	hipA4_gene	D4963p_10630_gene	D4963p_10640_gene	D4963p_10650_gene	D4963p_10660_gene	D4963p_10670_gene	D4963p_10680_gene	D4963p_10690_gene	D4963p_10700_gene	D4963p_10710_gene	D4963p_10720_gene	D4963p_10730_gene	D4963p_10740_gene	topB_gene	D4963p_10760_gene	virB1_gene	D4963p_10780_gene	D4963p_10790_gene	D4963p_10800_gene	D4963p_10810_gene
55			■	■																								
6V			■	■											■													
7h1h				■											■													
Ab_1426_2003				■																								
Ab_1711				■	■	■	■	■	■																			
Ab_2211			■	■																								
Ab_2811				■																								
Ab_3711				■	■	■																						
Ab_4211				■	■	■	■	■	■																			
Ab_4511				■																								
Ab_A103				■																								
Ab_A111				■																								
Ab_CR1132				■	■	■	■	■	■																			
Ab_CR1143				■																								
Ab_CR424				■	■	■	■	■	■																			
Ab_CR461				■																								
Ab_CR502				■																								
Ab_CR604				■																								
Ab_CR641				■																								
Ab_CR891				■																								
Ab_CR892				■																								
Ab_DQ20dA1				■																								
Ab_DQ31A1				■																								
Ab_DQ40A1				■																								
Ab_DQ64A1				■												■												

Fortsetzung **Table S7**

	Type IV secretion system genes (T4SS)																													
Strain	dnaG1_gene	D4963p_10830_gene	D4963p_10840_gene	D4963p_10850_gene	traG_gene	virB11_gene	virB10_gene	virB9_gene	virB8_gene	D4963p_10910_gene	D4963p_10920_gene	D4963p_10930_gene	D4963p_10940_gene	D4963p_10950_gene	D4963p_10960_gene	D4963p_10970_gene	virB4_gene	D4963p_10990_gene	D4963p_11000_gene	D4963p_11010_gene	D4963p_11020_gene	D4963p_11030_gene	D4963p_11040_gene	D4963p_11060_gene	D4963p_11050_gene	D4963p_11070_gene	intA1_gene	total no. of detected genes
16CS0847-3																													75
17CS0824														■												■		80
17CS1193-1																													77
17CS1193-2																													77
17CS1193-3														■															79
17CS1197-2																													75
17CS1197-3																													75
17CS1197																													75
17CS1198-1																													77
17CS1198-2																													77
17CS1198-3																													77
16CS0367-1-AR														■										■					79
16CS0367-AR														■										■					79
16CS0370-1														■										■					79
16CS0375-1														■										■					79
16CS0375-2														■										■					79
16CS0831-1-1														■										■					79
16CS0831-4-1																								■					74
17CS1200-1														■															78
17CS1201														■															78
17CS1205-1														■															78
17CS1205-2														■															78
17CS1206-1														■															78
17CS1208-3														■															78
17CS1167														■															80
17CS1168																													75
17CS1056														■															79
17CS1066														■															80
17CS1067														■															80
17CS1068-1														■															80
17CS1068-2														■															80
17CS0916-1																													76
17CS0916-2																													76
16CS0857-1																													76
16CS0861-1																													82
17CS1092-1																										■			84
17CS1092-2																										■			84
17CS1095																													77
16CS0815-2																													75
16CS0817-2														■															85
16CS0820-1																													78
16CS0820-2																													76
16CS0821-2																													78
16CS0823-1																													76
16CS0823-2													■	■															79
17CS0965-1														■															87
17CS0965-B																													73
RMI																													78
RMIII																													78
NCTC_12481																													86

Fortsetzung **Table S7**

	Type IV secretion system genes (T4SS)																												total no. of detected genes
Strain	dnaG1_gene	D4963p_10830_gene	D4963p_10840_gene	D4963p_10850_gene	traG_gene	virB11_gene	virB10_gene	virB9_gene	virB8_gene	D4963p_10910_gene	D4963p_10920_gene	D4963p_10930_gene	D4963p_10940_gene	D4963p_10950_gene	D4963p_10960_gene	D4963p_10970_gene	virB4_gene	D4963p_10990_gene	D4963p_11000_gene	D4963p_11010_gene	D4963p_11020_gene	D4963p_11030_gene	D4963p_11040_gene	D4963p_11060_gene	D4963p_11050_gene	D4963p_11070_gene	intA1_gene	
55																													76
6V																													78
7h1h														■															79
Ab_1426_2003														■															76
Ab_1711																													81
Ab_2211																													78
Ab_2811																													71
Ab_3711																													78
Ab_4211																													75
Ab_4511														■															79
Ab_A103																													71
Ab_A111																													79
Ab_CR1132																													82
Ab_CR1143																													72
Ab_CR424																													76
Ab_CR461																										■			73
Ab_CR502																													77
Ab_CR604																													76
Ab_CR641																													72
Ab_CR891																													76
Ab_CR892																													76
Ab_DQ20dA1																													75
Ab_DQ31A1																								■		■			78
Ab_DQ40A1																													77
Ab_DQ64A1														■															79

black box = present; white box = absent

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