Detection of Trichophyton benhamiae with Fluorescence In Situ Hybridization

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Introduction

The objective of this work was to detect **T. benhamiae** in **FFPE sections** of skin explants from experimentally infected guinea pigs using Fluorescence In Situ Hybridization.

T. benhamiae is a **zoonotic reemerging pathogen** causing dermatophytosis in humans.

Particularly affected are children due to close contact to pets. The condition is characterized by **dermatitis** especially in the face with severe inflammatory changes and pruritus known as Tinea faciei[1,2].

The In situ Hybridization is a common detection method already used for other pathogenetic fungi[3,4] including dermatophytes[5,6] as well for FFPE sections [4,7,8,9].

Therefore, it was necessary to modify and establish a specific protocol for this dermatophyte.

Material and methods

Fluorescence In Situ Hybridization (abbr.: FISH) is a qualitative detection method using fluorescence-labeled DNA probes.

These **DNA probes** [Tab. 1] hybridize with the **complementary target DNA** in the tissue. The labeled signal is detected by a fluorescence microscope transforming the incoming signal into a visible picture. Due to the separately detected fluorescence channels, the results are **summation images** [Fig. 1].

Probe type	Name	Fluorescence Signal
Specific for T. benhamiae	Oligo 1 Oligo 2	green
Universal for fungi	Pan-fungal Z Pan-fungal Aman	green
Enzyme probes*	Subtilisin (3,6) Mcpa	green
Positive Control	Oligo 3 Oligo 4	red
Negative Control	Green Fluorescent Protein	green

Tab.1 : The different DNA probes
* *By means of a PCR amplification it was shown that T. benhamiae is expressing those enzymes.*

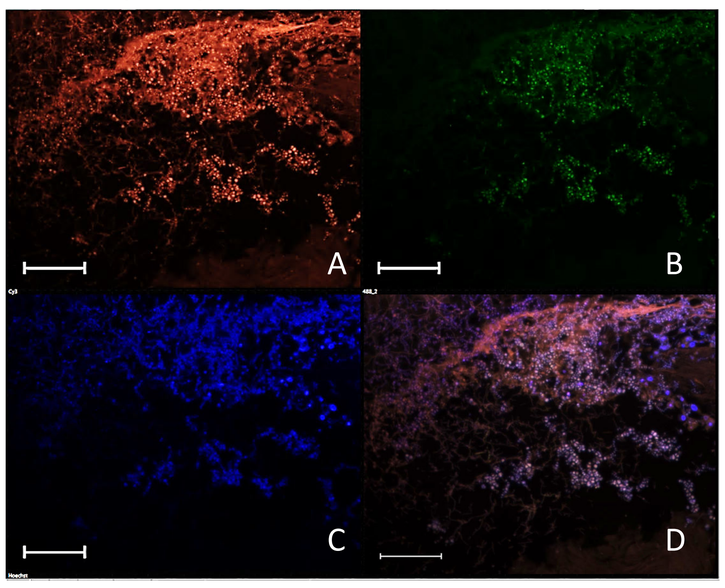
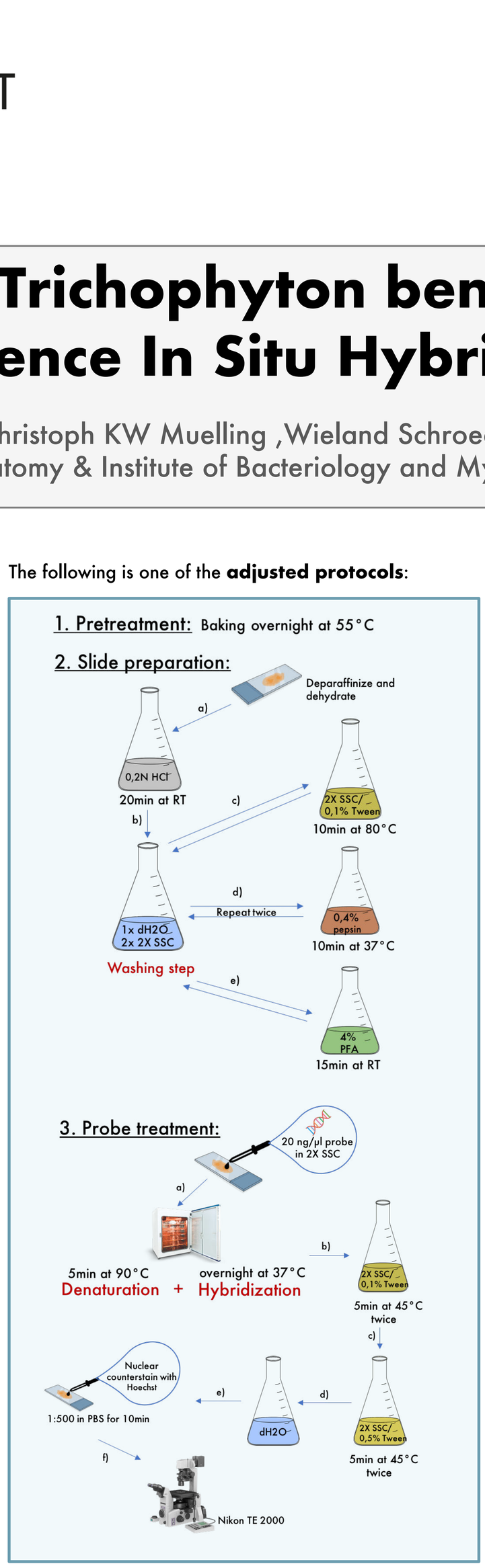


Fig. 1 : Separate images (A-C) of the fluorescence channels; summation image in D (probe: Oligo 3)

The following is one of the **adjusted protocols**:



Results and discussion

T. benhamiae infiltrates the **cornified layers** from the outside to the inside and also the **hair follicles**. The amount of distribution increases with the duration of infection.

The fluorescence signal of T. benhamiae depends on the used probes [Tab.1], the cell nuclei are shown in blue.

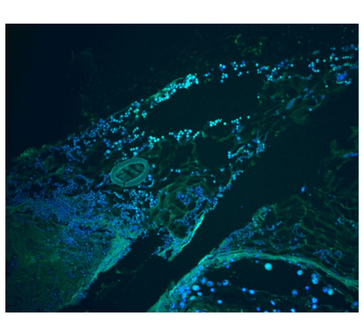


Fig. 2: probe: Subtilisin 6

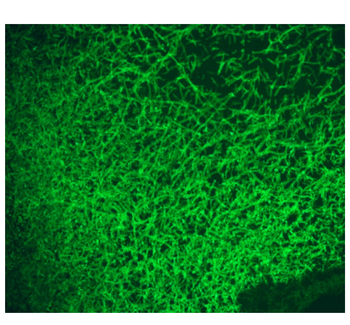


Fig. 3: probe: Oligo 1

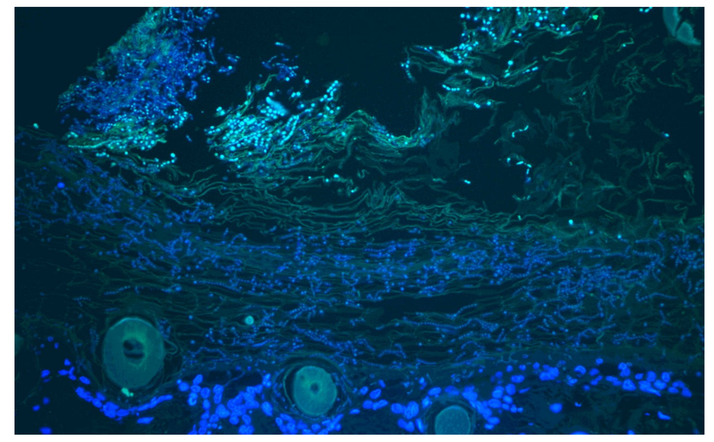


Fig. 4: T. benhamiae is widely distributed in the cornified layers of the epidermis (probe: Oligo 2)

Unfortunately, the detected results were not reproducible. Especially the **accessibility of target DNA** in the FFPE slides seems to be a barrier for the probes. Although the DNA probes produced a correct signal when applied directly on T. benhamiae samples, the results for the FFPE slides were inconsistent. Instead of pepsin digestion we tried **proteinase K and LysingEnzyme** (Sigma). Furthermore, the duration of the heat treatment was raised up to 20 minutes as well as the hydrochloric acid treatment. Especially the heat treatment is a critical step, as it has a great impact on the hybridization success[9].

Another problem that occurred were DNA probes **bleeding through** every channel of the microscope or at wrong locations even though negative controls were correct. **Non-specific fluorescences** were described in other cases[4] as well. The hybridization buffer was replaced by one consisting out of formamide, SDS, dextran sulfate and salmon DNA to avoid mismatches.

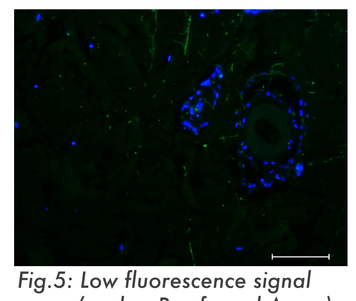


Fig.5: Low fluorescence signal (probe: Pan-fungal Aman)

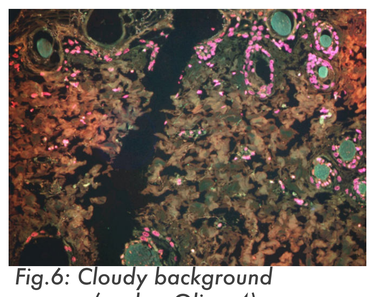


Fig.6: Cloudy background (probe: Oligo 4)

Furthermore, there were slides with a weak fluorescence signal or/and a high background signal [Fig. 5, 6] , a common problem for ISH with fungi[10]. The amount of washing steps was increased, it helped marginally.

Conclusion

FISH as a qualitative method can be transferred to detect T. benhamiae. The detection of T. benhamiae in guinea pig skin explants, e.g. skin sections thereof, resulted in non-reproducible stainings. Although several modifications to the protocol were made, the results stayed below expectations. As the current set-up seems vulnerable to minor modifications, an approach to solve the issue would be to design new probes.

Contact

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References

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