

the fungus. Direct microscopic examination of skin, hair and nail material is often sufficient for the preemptive diagnosis of a fungal infection, but it does not lead to a specific species diagnosis. Although rapid and cheap, this technique has a relatively low sensitivity and shows false negative results in up to 15 % of all cases.

- 5 Application of culture enables specific species identification in 10-15 days in approximately 95 % of cases. However, for some slow growing or atypical isolates time of diagnosis is up to 3-6 weeks. Therefore, a simple rapid and specific method for the diagnosis of dermatophyte infections is required.

10 PCR-based methods have been introduced for the diagnosis of fungal infections. For example, US2010/0311041 discloses a method for extracting nucleic acids from fungi, a PCR method for detecting fungi in patient samples and a PCR kit for detecting dermatophytes and for diagnosing infections by the three genera *Trichophyton*, *Microsporum* and *Epidermophyton*.

15 However, the methods disclosed in the state of the art have shortcomings. In particular, they do not allow the rapid and reliable distinction of closely related strains from the genus *Trichophyton* and *Microsporum*, which includes zoophilic and non-zoophilic species.

Therefore, a problem underlying the present invention is to provide methods and reagents for the diagnosis of a skin, hair and nail disease, preferably a skin, hair and nail infection, more preferably a fungal skin, hair and nail infection.

- 20 Another problem underlying the present invention is to provide methods and reagents for identifying and distinguishing from closely related species a pathogen from the genus *Trichophyton*, in particular *T. benhamiae* (yellow) and *T. concentricum*.

The problem underlying the present invention is solved by the subject matter of the attached independent and dependent claims.

25 SUMMARY

In one aspect, the present disclosure refers to a primer pair comprising a forward primer and a reverse primer capable of amplifying a nucleic acid from a pathogen associated with a skin, hair and nail infection, wherein the amplified nucleic acid comprises SEQ ID NO22, wherein the forward primer hybridizes specifically to a region of the pathogen's genome upstream of
30 SEQ ID NO22 and the reverse primer hybridizes specifically to a region of the pathogen's genome downstream of SEQ ID NO22, wherein the distance between the final base pair of the forward primer, in its 5'-3' orientation, and the first base pair of SEQ ID NO22, in its 5'-3' orientation, is 200 or less base pairs, wherein the distance between the final base pair of the

reverse primer, in its 5'-3' orientation, and the final base pair of SEQ ID NO22, in its 5'-3' orientation, is 200 or less base pairs, wherein each primer has a length of 10 to 40 nucleotides and wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.

In another aspect, the present disclosure refers to a nucleic acid probe capable of hybridizing specifically to a nucleic acid sequence from a pathogen associated with a skin, hair and nail infection, wherein said nucleic acid sequence from a pathogen is SEQ ID NO22 or its complementary strand, wherein the probe has a length of not more than 200 nucleotides fused to a detectable signal molecule and wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.

In another aspect, the present disclosure refers to a nucleic acid comprising (a) a forward primer sequence and a reverse primer sequence according to the primer pair as disclosed herein and, between the forward and the reverse primer sequence, (b) the nucleic acid sequence from a pathogen associated with a skin, hair and nail infection, wherein the amplified nucleic acid comprises SEQ ID NO22, wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei* and wherein the nucleic acid is obtained by amplifying a sample comprising said pathogen and using the primer pair as disclosed herein, wherein the nucleic acid comprises a detectable label selected from the group consisting of a fluorescent, radioactive, colloidal gold and enzymatically active label.

In yet another aspect, the present disclosure refers to a carrier comprising the nucleic acid as disclosed herein.

In yet another aspect, the present disclosure refers to a method comprising the step detecting in a sample a nucleic acid sequence comprising SEQ ID NO22 from a pathogen associated with a skin, hair and nail infection and wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.

In yet another aspect, the present disclosure refers to a use of the primer pair as disclosed herein, the nucleic acid as disclosed herein or the carrier as disclosed herein in the manufacture of a diagnostic agent for diagnosis of a skin, hair and nail infection associated with a pathogen selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.

In yet another aspect, the present disclosure refers to a kit comprising the primer pair as disclosed herein, the nucleic acid as disclosed herein and/or the carrier as disclosed herein, preferably for the diagnosis of a skin, hair and nail infection associated with a pathogen and wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T.*
5 *interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.

DETAILED DESCRIPTION OF THE INVENTION

In a first aspect, the problem underlying the present invention is solved by a primer pair comprising a forward primer and a reverse primer capable of amplifying a nucleic acid from a pathogen associated with a skin, hair and nail infection comprising SEQ ID NO22, preferably
10 SEQ ID NO1.

In a second aspect, the problem underlying the present invention is solved by a nucleic acid capable of hybridizing specifically to a nucleic acid sequence from a pathogen associated with a skin, hair and nail infection comprising SEQ ID NO22, preferably SEQ ID NO1, or its complementary strand or a vector or cell comprising said nucleic acid sequence.

Claims

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1. A primer pair comprising a forward primer and a reverse primer capable of amplifying a nucleic acid from a pathogen associated with a skin, hair and nail infection, wherein the amplified nucleic acid comprises SEQ ID NO22, wherein the forward primer hybridizes specifically to a region of the pathogen's genome upstream of SEQ ID NO22 and the reverse primer hybridizes specifically to a region of the pathogen's genome downstream of SEQ ID NO22,
 wherein the distance between the final base pair of the forward primer, in its 5'-3' orientation, and the first base pair of SEQ ID NO22, in its 5'-3' orientation, is 200 or less base pairs,
 wherein the distance between the final base pair of the reverse primer, in its 5'-3' orientation, and the final base pair of SEQ ID NO22, in its 5'-3' orientation, is 200 or less base pairs,
 wherein each primer has a length of 10 to 40 nucleotides and
 wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.
2. A nucleic acid probe capable of hybridizing specifically to a nucleic acid sequence from a pathogen associated with a skin, hair and nail infection, wherein said nucleic acid sequence from a pathogen is SEQ ID NO22 or its complementary strand,
 wherein the probe has a length of not more than 200 nucleotides fused to a detectable signal molecule and
 wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.
3. A nucleic acid comprising (a) a forward primer sequence and a reverse primer sequence according to the primer pair of claim 1 and, between the forward and the

reverse primer sequence, (b) the nucleic acid sequence from a pathogen associated with a skin, hair and nail infection, wherein the amplified nucleic acid comprises SEQ ID NO22,

wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei* and

wherein the nucleic acid is obtained by amplifying a sample comprising said pathogen and using the primer pair of claim 1,

wherein the nucleic acid comprises a detectable label selected from the group consisting of a fluorescent, radioactive, colloidal gold and enzymatically active label.

4. The primer pair according to claim 1 or the nucleic acid according to claim 2, comprising a detectable label, preferably from the group comprising a fluorescent, radioactive, colloidal gold or enzymatically active label.

5. A carrier comprising the nucleic acid according to any of claims 2 or 4.

6. The carrier according to claim 5, wherein the carrier is a silane coated glass, plastic or silicon material microarray plate.

7. A method comprising the step detecting in a sample a nucleic acid sequence comprising SEQ ID NO22 from a pathogen associated with a skin, hair and nail infection and wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.

8. The method according to claim 7, comprising the steps

a) providing a sample, preferably nail, hair or skin material, from a patient,

b) amplifying any nucleic acid comprising SEQ ID NO22 present in the sample using the primer pair according to any of claims 1 or 4, thus generating an

amplicon if a nucleic acid sequence comprising SEQ ID NO22 from the pathogen is present in the sample.

- 5 9. The method according to claim 8, further comprising the step

c) detecting the amplicon.
- 10 10. The method according to step 9, wherein the amplicon is detected by fluorescence, radioactivity, colloidal gold or chemiluminescence.
- 15 11. Use of the primer pair according to any of claims 1 or 4, the nucleic acid according to claim 2 or the carrier according to any of claims 5 to 6 in the manufacture of a diagnostic agent for diagnosis of a skin, hair and nail infection associated with a pathogen selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.
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12. A kit comprising the primer pair according to any of claims 1 or 4, the nucleic acid according to claim 2 and/or the carrier according to any of claims 5 to 6, preferably for the diagnosis of a skin, hair and nail infection associated with a pathogen and
25 wherein the pathogen is selected from the group comprising *T. tonsurans*, *T. equinum*, *T. interdigitale*, *T. benhamiae (african)*, *T. benhamiae (yellow)*, *T. concentricum* and *T. erinacei*.
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