

DETECTION APPARATUS AND METHOD FOR SPECIFIC DIAGNOSIS OF *PYTHIUM INSIDIOSUM*

5 **Field Of Invention**

The present invention generally relates to a detection apparatus and method for specific diagnosis of the pathogen, *Pythium insidiosum*, whereby specific primers are used for the determination of *Pythium insidiosum*.

10 **Background**

Pythium insidiosum (*P. insidiosum*) is an aquatic fungal-like pathogen and is classified in the kingdom Straminipila, phylum Oomycota, class Oomycetes, order Peronosporales, family Pythiaceae and genus Pythium, which has over 120 described species. However, *P. insidiosum* is the only species in the genus that is capable of
15 infecting mammals.

P. insidiosum is the etiological agent of pythiosis, a life-threatening, granulomatous disease of human and animals, which is characterised by cutaneous and subcutaneous lesions and which occurs primarily in the tropical and subtropical regions of the
20 world. Pythiosis can also manifest as a systemic type that involves the vascular system and usually results in arterial occlusion. The systemic type has been documented in many patients, especially those having underlying hemoglobinopathy syndromes, such as thalassemia, an inherited disease of red blood cells whereby the blood cells are vulnerable to mechanical damage and therefore die easily.

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Thalassemic patients have been found to be patients most vulnerable to pythiosis and the disease is one of the major reasons that cause clogging of red blood veins in these patients. When pythiosis occurs in patients other than those suffering from thalassemia, most of them, particularly those with debilitating illnesses such as AIDS
30 and leukaemia, exhibit a severe form of the disease and most of these patients die as a result of late diagnosis of the disease. The later the diagnosis of the disease, the longer the progression of the infection, the less effective treatment becomes. The

problem is in the inability to identify the disease at an early enough stage so that treatment can be effective.

It is difficult to identify *P. insidiosum* morphologically via known staining methods, such as Gomori's methenamine-silver nitrate stain, as there are many other fungal organisms which have appearances similar to *P. insidiosum* after staining. Although *P. insidiosum* has a distinct cultural identification feature, which is its motile biflagellate zoospores, the production of these zoospores in culture may take weeks. There are various other methods, such as immunodiffusion (ID) test and enzyme-linked immunosorbent assay (ELISA), which have been developed and can be used to diagnose pythiosis.

Both ID test and ELISA are techniques used to detect the presence of an antibody or antigen in a sample. The ID test detects the antibody or antigen by allowing reactions between antibodies and antigens. The combination of specific antibodies and antigens gives rise to precipitates that can be observed. ELISA, on the other hand, makes use of two antibodies, one of which is specific to the antigen and the other of which is coupled to an enzyme. The second antibody, coupled to the enzyme, causes a chromogenic substrate, a strongly pigmented or pigment-generating substrate, or a fluorogenic substrate to produce a signal. The ID test and ELISA are used to diagnose pythiosis in humans by the detection of immunoglobulin G (IgG) antibodies in the sera of patients. However, these methods are more expensive and less reliable due to the possibility of cross-reactivity between the antibodies.

As the disease requires as early a diagnosis as possible so as for treatment to be effective, there is a clear need for a method of detection that is rapid, effective and accurate, to address the pressing problem of late diagnosis of the disease and the deficiencies of the above-mentioned methods of diagnosis.

Summary

The present embodiment of the invention disclosed herein provides a method and an apparatus for detecting *Pythium insidiosum* in a sample. The use of specific primers

for detecting *P. insidiosum* in the sample allows the disclosed method and apparatus of detection to be effective and accurate, thus, addressing the foregoing problems.

5 In accordance with a first aspect of the invention, there is disclosed a method for detecting *P. insidiosum* in a sample containing at least one deoxyribonucleic acid (DNA) strand. The method comprises the step of obtaining at least one denatured DNA strand from the at least one DNA strand whereby each of the at least one denatured DNA strand has a sequence. Following that, the method also comprises the
10 step of providing at least two primers. The at least two primers comprise at least two of a first primer of sequence 5'GAC A CAGGG AGGTAGT GACAATAAATA 3', a second primer of sequence 5'CTTGGTAAATGCTTTCGCCT 3' and a third primer of sequence 5'GAATAATAAGATACGACCTTGG 3'. Further, the method comprises the step of contacting the at least two primers with the at least one
15 denatured DNA strand to obtain a plurality of hybridised portions. Each of the plurality of hybridised portions comprises one of the at least two primers and a portion of the at least one denatured DNA strand that has an internal sequence corresponding with a sequence of the one of the at least two primers. Furthermore, the method involves the step of extending the plurality of hybridised portions using a
20 thermo-stable enzyme to obtain a plurality of extended hybridised portions and the step of amplifying the plurality of extended hybridised portions to obtain a plurality of amplified extended hybridised portions. Lastly, the method comprises the step of comparing molecular properties of the plurality of amplified extended hybridised portions with molecular properties of a reference DNA, specific to *P. insidiosum*,
25 wherein at least one sequence of the plurality of amplified extended hybridised portions corresponds with an internal sequence of at least one portion of a sequence of the reference DNA.

In accordance with a second aspect of the invention, there is disclosed an apparatus
30 for detecting *P. insidiosum* in a sample containing at least one DNA strand. The apparatus comprises an extraction module, a hybridisation module, an amplification module and a comparison module. The extraction module is for providing at least one denatured DNA strand from the at least one DNA strand whereby each of the at least

one denatured DNA strand has a sequence. The hybridisation module is for contacting at least two primers with the at least one denatured DNA strand to obtain a plurality of hybridised portions. Each of the plurality of hybridised portions comprises one of the at least two primers and a portion of the at least one denatured DNA strand which has an internal sequence corresponding with a sequence of the one of the at least two primers. The at least two primers comprise at least two of a first primer of sequence 5'GAC A CAGGG AGGTAGT GACAATAAATA 3', a second primer of sequence 5'CTTGGTAAATGCTTTCGCCT 3' and a third primer of sequence 5'GAATAATAAGATACGACCTTGG 3'. The amplification module is for extending the plurality of hybridised portions using a thermo-stable enzyme to obtain a plurality of extended hybridised portions and for amplifying the plurality of extended hybridised portions to obtain a plurality of amplified extended hybridised portions. Lastly, the comparison module is for comparing molecular properties of the plurality of amplified extended hybridised portions with molecular properties of a reference DNA, specific to *P. insidiosum*, wherein at least one sequence of the plurality of amplified extended hybridised portions corresponds with an internal sequence of at least one portion of a sequence of the reference DNA.

In accordance with a third aspect of the invention, there is disclosed a primer set comprising at least two of: a first primer of sequence 5'GAC A CAGGG AGGTAGT GACAATAAATA 3'; a second primer of sequence 5'CTTGGTAAATGCTTTCGCCT 3'; and a third primer of sequence 5'GAATAATAAGATACGACCTTGG 3', wherein said primer set or its complement specifically hybridises to a portion of at least one denatured deoxyribonucleic acid (DNA) strand obtained from at least one DNA strand contained in a sample and wherein the primer set is for detecting *Pythium insidiosum* in the sample.

Brief Description Of The Drawings

An embodiment of the invention are described hereinafter with reference to the following drawings, in which:

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FIG. 1 shows a process flow diagram of a detection method for specific diagnosis of *Pythium insidiosum* according to an embodiment of the invention;

FIG. 2 shows a process flow diagram of a deoxyribonucleic acid (DNA) extraction
5 method of the detection method of FIG. 1;

FIG. 3 shows a process flow diagram of a hybridisation method of the detection method of FIG. 1;

10 FIG. 4 shows a process flow diagram of an amplification and comparison method of the detection method of FIG. 1;

FIG. 5 shows a process flow diagram of obtaining amplified extended hybridised portions of the amplification and comparison method of FIG. 4;

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FIG. 6 shows a process flow diagram of a first amplification process of obtaining amplified extended hybridised portions of FIG. 5;

FIG. 7 shows a process flow diagram of a second amplification process of obtaining
20 amplified extended hybridised portions of FIG. 5;

FIG. 8 shows a system flow diagram for the detection method of FIG. 1;

FIG. 9 shows a system flow diagram for the first amplification process of FIG. 6; and

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FIG. 10 shows a system flow diagram for the second amplification process of FIG. 7.

Detailed Description

A detection apparatus and a method for specific diagnosis of *Pythium insidiosum* are
30 described hereinafter for addressing the foregoing problems.

P. insidiosum is an aquatic-like pathogen and is the only species in the genus *Pythium* that is capable of infecting mammals. *P. insidiosum* is the etiological agent of

pythiosis, a life-threatening disease of human and animals, and can manifest in several forms, including a subcutaneous lesion form and a systemic form. There exist treatments, such as antigen vaccines, for pythiosis. However, the disease needs to be identified at an early enough stage for the treatments to be effective. Thus, there is a clear need for a method of detection that is not only effective and accurate, but rapid as well.

Various methods of diagnosis, such as making use of a distinct identification feature of *P. insidiosum* in culture, have been employed, but these are considered undesirably time consuming as production of the distinct identification feature in culture may take weeks. Other attempts of diagnosis, such as immunodiffusion (ID) test and enzyme-linked immunosorbent assay (ELISA), utilise antibodies or antigens. However, these methods of diagnosis are less reliable due to the possibility of cross-reactivity between the antibodies.

Unlike the methods of diagnosis mentioned above, the method of detecting *P. insidiosum* in the present invention does not utilise antibodies or antigens. This eliminates the possibility of cross-reactivity between the antibodies, hence making detection more reliable. Instead, the detection method of the invention includes the use of primers specific to *P. insidiosum* for detecting *P. insidiosum*, which makes this detection method accurate.

A detection method 90 for specific diagnosis of *P. insidiosum* is described with reference to Fig. 1 and Fig. 8. The detection method 90 comprises a deoxyribonucleic acid (DNA) extraction method 100, a hybridisation method 200 and an amplification and comparison method 300 and is further described with reference to Fig. 2, Fig. 3 and Fig. 4.

In a step 110 of the DNA extraction method 100 as shown in Fig. 2, a sample 22, such as a tissue or a blood sample obtained from a patient infected with *P. insidiosum*, is provided for extracting DNA from cells in the sample 22 for subsequent molecular analysis.

Next in a step 112, DNA extraction solution 24 is added to the sample 22 to obtain DNA strands 26 from the sample 22. The DNA extraction solution 24 is added to lyse cells in the sample 22 and to remove membrane lipids and cellular and histone proteins bound to the DNA strands 26. The DNA strands 26 are then precipitated in alcohol, which is a component of the DNA extraction solution 24.

Following the step 112, the DNA strands 26 obtained from the step 112 are initialised in a step 114 to obtain initialised DNA strands 28. In the step 114, the DNA strands 26 are heated at a temperature of preferably 95°C for five minutes to melt the DNA strands 26.

Next in a step 116, the initialised DNA strands 28 are denatured to obtain denatured DNA strands 30. During the step 116, the initialised DNA strands 28 are heated at a temperature of preferably 95°C for one minute to break the hydrogen bonds maintaining the double-helical structure of the initialised DNA strands 28.

Following the step 116 of the DNA extraction method 100, sequences of primers 32 are obtained from a database 34 in a step 210 of the hybridisation method 200 as shown in Fig. 3. The database 34 is preferably GenBank, a collection of all publicly available nucleotide sequences. A search for 18S ribosomal deoxyribonucleic acid (rDNA) sequences from a plurality of organisms predefined in the database 34 is carried out. The 18S rDNA sequences obtained from the search are then compared with *P. insidiosum* sequence to thereby identify the primers 32 having sequences which specifically match at least a portion of the *P. insidiosum* sequence. In addition, the primers 32 preferably have a guanine-cytosine content (GC-content) of not more than 50%.

The primers 32 comprise of the following sequences:

CPL6 5'GAC A CAGGG AGGTAGT GACAATAAATA 3';

CPR8 5'CTTGGTAAATGCTTTCGCCT 3'; and

PSL1 5'GAATAATAAGATACGACCTTGG 3'.

Next in a step 212, the primers 32 are added to the denatured DNA strands 30. The primers 32 are then initialised in a step 214. During the step 214, the primers 32 are heated at a temperature of preferably 95⁰C for one minute to melt the primers 32.

- 5 Following the step 214, the primers 32 are hybridised to the denatured DNA strands 30 to obtain hybridised portions 36 in a step 216. During the hybridisation step 216, the primers 32 and the denatured DNA strands 30 are heated at a temperature of preferably 68⁰C for one minute to join the primers 32 with the denatured DNA strands 30 through base pairing. Hybridisation occurs when sequences of the primers 32
10 correspond with a portion of sequence of the denatured DNA strands 30.

- Following the step 216 of the hybridisation method 200, a thermo-stable polymerase 38, for example a thermo-stable enzyme such as DNA polymerase, is added to the hybridised portions 36 in a step 310 of the amplification and comparison method 300
15 as shown in Fig. 4. The thermo-stable polymerase is added during or after initialisation of the DNA strands 26 in the step 114.

- Next in a step 312, extended hybridised portions 40 are obtained by extending the hybridised portions 36, specifically the primers 32 which are hybridised to the
20 denatured DNA strands 30, using the thermo-stable polymerase 38. The thermo-stable polymerase 38 extends the hybridised portions 36 by catalysing formations of polynucleotides of DNA using the denatured DNA strands 30 as templates. The step 312 is carried out at a temperature of preferably 72⁰C for one minute.

- 25 The extended hybridised portions 40 are then amplified in a step 314 to obtain amplified extended hybridised portions 42. The amplification step 314 comprises a first amplification process 320 and a second amplification process 330 as shown in Fig. 5. The first amplification process 320 is described with reference to Fig. 6 and Fig. 9 respectively while the second amplification process 330 is described with
30 reference to Fig. 7 and Fig. 10 respectively.

The first amplification process 320, as shown in Fig. 6, comprises a step 322 to obtain denatured DNA strands 64. The extended hybridised portions 40, which are also

initialised DNA strands 62, are denatured in the step 322 to obtain the denatured DNA strands 64. The initialised DNA strands 62 are heated at a temperature of preferably 95⁰C for one minute. The first amplification process 320 also comprises hybridising the primers 32, which are initialised, with the denatured DNA strands 64 in a step 324 to obtain hybridised portions 66. The primers 32 and the denatured DNA strands 64 are heated at a temperature of preferably 68⁰C for one minute. Further, the first amplification process 320 comprises extending the hybridised portions 66, specifically the primers 32 which are hybridised to the denatured DNA strands 64, in a step 326 to obtain extended hybridised portions 68. The step 326 is carried out at a temperature of preferably 72⁰C for one minute. The first process 320 is performed for preferably thirty times.

The second amplification process 330, as shown in Fig. 7, comprises a step 332 to obtain denatured DNA strands 72 by denaturing the extended hybridised portions 68, which are also initialised DNA strands 70. In the step 332, the initialised DNA strands 70 are heated at a temperature of preferably 95⁰C for one minute. The second amplification process 330 also comprises hybridising the primers 32 with the denatured DNA strands 72 in a step 334 to obtain hybridised portions 74. The primers 32 and the denatured DNA strands 72 are heated at a temperature of preferably 58⁰C for one minute. The second amplification process 330 further comprises extension of the hybridised portions 74, specifically the primers 32 which are hybridised to the denatured DNA strands 72, in a step 336 by heating at a temperature of preferably 72⁰C for one minute to obtain extended hybridised portions 76. The amplified extended hybridised portions 42 are then obtained by performing the second amplification process 330 for preferably twenty times.

Following the step 314, molecular properties of the amplified extended hybridised portions 42 are compared with molecular properties of a reference DNA 44, specific to *P. insidiosum*, using gel electrophoresis in a step 316. Gel electrophoresis is a technique for separating and identifying molecules based on physical characteristics, such as size, shape or isoelectric point.

The following example demonstrates certain aspects of the detection method 90 for specific diagnosis of *P. insidiosum*, when applied using a detection apparatus for specific diagnosis of *P. insidiosum*, and should not be taken as limiting the scope thereof.

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In an example according to the detection method 90 for specific diagnosis of *P. insidiosum*, a detection apparatus for specific diagnosis of *P. insidiosum* comprises a DNA extraction apparatus, a polymerase chain reaction (PCR) apparatus and a gel electrophoresis apparatus.

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The DNA extraction apparatus comprises a 1.5ml white plastic tube, preferably a microcentrifuge tube, for containing the sample 22, such as a blood sample obtained from a patient infected with *P. insidiosum*, and the DNA extraction solution 24 for extracting DNA. The DNA extraction solution 24 preferably comprises 10ml lysis buffer, 5ml TE 10-1 buffer, 15ml 70% alcohol, 15ml isopropanol, 100ml TE-saturated
15 buffer, 20ml phenol-chloroform-isoamyl alcohol, 20ml chloroform-isoamyl alcohol, 100µl mercaptoethanol, 400µl RNase A, 1500µl proteinase K and 500µl 3M sodium acetate. The DNA extraction solution 24 is added to the blood sample and is responsible for lysing blood cells in the blood sample, for removing membrane lipids and cellular and histone proteins bound to DNA strands in the blood cells and for
20 precipitating the DNA strands.

After the DNA strands from the blood cells are extracted, the DNA strands are initialised and denatured as in the step 112 and the step 116 respectively to obtain
25 denatured DNA strands. Following this, the PCR apparatus is used to amplify the denatured DNA strands to obtain amplified extended hybridised portions. The PCR apparatus comprises three 200µl white plastic tubes which are preferably microcentrifuge tubes. The first tube contains MgCl₂, dNTP, Taq DNA polymerase and the primers 32 having the sequences CPL6, CPR8 and PSL1, which specifically
30 match at least a portion of the *P. insidiosum* sequence. The second tube contains human DNA as a negative control while the third tube contains *P. insidiosum* DNA as a positive control.

Next, gel electrophoresis is employed to identify the amplified extended hybridised portions obtained. The gel electrophoresis apparatus comprises 3g of agarose, 150ml 10X electrophoresis buffer and three 1.5ml plastic tubes which are preferably microcentrifuge tubes. The first tube contains *P. insidiosum* DNA marker, preferably one that is $\varnothing \times 174$ Hae III digested. The second tube contains gel-loading buffer while the third tube contains gel-staining solution.

Since the blood sample is taken from a patient infected with *P. insidiosum*, the amplified extended hybridised portions are representative of one of *P. insidiosum*. Thus, after gel electrophoresis is carried out as in the step 316, the molecular properties of the amplified extended hybridised portions reflect the molecular properties of *P. insidiosum* DNA.

In the foregoing manner, a detection apparatus and a detection method for specific diagnosis of *Pythium insidiosum* are described according to one embodiment of the invention for addressing the foregoing disadvantages of conventional methods. Although only one embodiment of the invention is disclosed, the invention is not to be limited to the specific forms or arrangements of parts so described and it will be apparent to one skilled in the art in view of this disclosure that numerous changes and/or modification can be made without departing from the scope and spirit of the invention.

Claims

1. A method for detecting *Pythium insidiosum* in a sample containing at least one deoxyribonucleic acid (DNA) strand, the method comprising the steps of:

obtaining at least one denatured DNA strand from the at least one DNA strand, each of the at least one denatured DNA strand having a sequence;

providing at least two primers comprising at least two of:

a first primer of sequence

5'GAC A CAGGG AGGTAGT GACAATAAATA 3';

a second primer of sequence

5'CTTGGTAAATGCTTTCGCCT 3'; and

a third primer of sequence

5'GAATAATAAGATACGACCTTGG 3';

contacting the at least two primers with the at least one denatured DNA strand to obtain a plurality of hybridised portions, each of the plurality of hybridised portions comprising one of the at least two primers and a portion of the at least one denatured DNA strand having an internal sequence corresponding with a sequence of the one of the at least two primers;

extending the plurality of hybridised portions using a thermo-stable enzyme to obtain a plurality of extended hybridised portions;

amplifying the plurality of extended hybridised portions to obtain a plurality of amplified extended hybridised portions; and

comparing molecular properties of the plurality of amplified extended hybridised portions with molecular properties of a reference DNA, specific to *P. insidiosum*,

wherein at least one sequence of the plurality of amplified extended hybridised portions corresponds with an internal sequence of at least one portion of a sequence of the reference DNA.

2. The method as in claim 1, the step of obtaining at least one denatured DNA strand comprising the step of:

adding DNA extraction solution to the sample for extracting the at least one DNA strand.

3. The method as in any of the preceding claims, further comprising the steps of:
initialising the at least one DNA strand; and
denaturing the at least one DNA strand.
- 5 4. The method as in claim 1, the step of providing at least two primers comprising the steps of:
searching for 18S ribosomal deoxyribonucleic acid (rDNA) sequences
from a plurality of organisms predefined in a database; and
10 comparing the 18S rDNA sequences with *P. insidiosum* sequence to
thereby obtain at least two primer sequences which specifically match at least
one portion of the *P. insidiosum* sequence,
wherein the at least two primer sequences which specifically match the
at least one portion of the *P. insidiosum* sequence have a guanine-cytosine
15 content (GC-content) of not more than 50%.
5. The method as in claim 1, the step of contacting the at least two primers with
the at least one denatured DNA strand comprising the step of:
adding the at least two primers to the at least one denatured DNA
20 strand.
6. The method as in claim 5, further comprising the steps of:
initialising the at least two primers; and
hybridising the at least two primers with the at least one denatured
25 DNA strand.
7. The method as in claim 1, the step of extending the plurality of hybridised
portions using a thermo-stable enzyme comprising the step of:
extending the plurality of hybridised portions using a thermo-stable
30 polymerase to obtain the plurality of extended hybridised portions.
8. The method as in any of the preceding claims, further comprising the steps of:

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initialising the at least one DNA strand and the at least two primers, initialised by heating at 95⁰C for five minutes;

providing a first amplification process comprising the steps of:

denaturing the at least one DNA strand by heating at 95⁰C for one minute;

hybridising the at least two primers with the at least one denatured DNA strand by heating at 68⁰C for one minute; and

extending the plurality of hybridised portions by heating at 72⁰C for one minute;

providing a second amplification process comprising the steps of:

denaturing the at least one DNA strand by heating at 95⁰C for one minute;

hybridising the at least two primers with the at least one denatured DNA strand by heating at 58⁰C for one minute; and

extending the plurality of hybridised portions by heating at 72⁰C for one minute; and

performing the first amplification process for thirty times and performing the second amplification process for twenty times.

9. The method as in claim 1, the step of comparing molecular properties of the plurality of amplified hybridised portions with molecular properties of a reference DNA comprising the step of:

comparing the molecular properties of the plurality of amplified extended hybridised portions with the molecular properties of the reference DNA using gel electrophoresis.

10. An apparatus for detecting *Pythium insidiosum* in a sample containing at least one deoxyribonucleic acid (DNA) strand, the apparatus comprising:

an extraction module for providing at least one denatured DNA strand from the at least one DNA strand, each of the at least one denatured DNA strand having a sequence;

a hybridisation module for contacting at least two primers with the at least one denatured DNA strand to obtain a plurality of hybridised portions,

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each of the plurality of hybridised portions comprising one of the at least two primers and a portion of the at least one denatured DNA strand having an internal sequence corresponding with a sequence of the one of the at least two primers, the at least two primers comprising at least two of:

- 5 a first primer of sequence
5'GAC A CAGGG AGGTAGT GACAATAAATA 3';
a second primer of sequence
5'CTTGGTAAATGCTTTCGCCT 3'; and
a third primer of sequence
10 5'GAATAATAAGATACGACCTTGG 3';

an amplification module for extending the plurality of hybridised portions using a thermo-stable enzyme to obtain a plurality of hybridized portion and for amplifying the plurality of extended hybridised portions to obtain a plurality of amplified extended hybridised portions; and

- 15 a comparison module for comparing molecular properties of the plurality of amplified extended hybridised portions with molecular properties of a reference DNA, specific to *P. insidiosum*,

wherein at least one sequence of the plurality of amplified extended hybridised portions corresponds with an internal sequence of at least one portion of a sequence of the reference DNA.

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11. The apparatus as in claim 10, the extraction module comprising:
means for adding DNA extraction solution to the sample for extracting the at least one DNA strand.

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12. The apparatus as in claim 11, further comprising:
means for initialising the at least one DNA strand; and
means for denaturing the at least one DNA strand.

- 30 13. The apparatus as in claim 10, the hybridisation module comprising:
means for adding the at least two primers to the at least one DNA strand,

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wherein the at least two primers are obtained by searching for 18S ribosomal deoxyribonucleic acid (rDNA) sequences from a plurality of organisms predefined in a database and comparing the 18S rDNA sequences with *P. insidiosum* sequence to thereby obtain at least two primer sequences which specifically match at least one portion of the *P. insidiosum* sequence, the at least two primer sequences having a guanine-cytosine content (GC-content) of not more than 50%.

14. The apparatus as in claim 13, further comprising:

means for initialising the at least two primers; and

means for hybridising the at least two primers with the at least one denatured DNA strand.

15. The apparatus as in claim 10, the amplification module comprising:

a thermo-stable polymerase; and

means for extending the plurality of hybridised portions using the thermo-stable polymerase to obtain the plurality of extended hybridised portions.

16. A primer set comprising at least two of:

a first primer of sequence

5'GAC A CAGGG AGGTAGT GACAATAAATA 3';

a second primer of sequence

5'CTTGGTAAATGCTTTTCGCCT 3'; and

a third primer of sequence

5'GAATAATAAGATACGACCTTGG 3',

wherein said primer set or its complement specifically hybridises to a portion of at least one denatured deoxyribonucleic acid (DNA) strand obtained from at least one DNA strand contained in a sample and wherein the primer set is for detecting *Pythium insidiosum* in the sample.