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Photobacterium damselae PCR Detection Kit

MBK0009
50 Reactions

Intended use

Identification of *Photobacterium damselae* DNA and discrimination between subspecies damselae and piscicida by polymerase chain reaction (PCR).

Introduction

Photobacterium damselae subsp. piscicida is the causative agent of fish pasteurellosis (or pseudotuberculosis), a bacterial septicemia described in a wide variety of marine fish. The disease has great economic impact in aquaculture of the Mediterranean area and Japan (1). Current identification methods rely on culture isolation followed by serological and biochemical tests. However, microbiological diagnosis can be hampered by the slow growth of this species, which can be easily obscured by other fast growing bacteria (2), or by the presence of viable but nonculturable cells (3). Moreover, phenotypic characterization revealed a considerable degree of variability within this subspecies and *P. damselae subsp. damselae* (4), (5). PCR-based detection systems are a good alternative to microbiological standard methods, offering sensitivity and shortened analysis time, but their application to *P. damselae subsp. piscicida* identification has been limited by the high degree of DNA base sequence similarity between the two subspecies *piscicida* and *damselae* (6).

Product description

The "*PHOTOBACTERIUM DAMSELAE* PCR detection Kit" allows the detection of DNA from *P. damselae* using Polymerase Chain Reaction (PCR). **PCR primer combination specifically discriminates both subspecies *piscicida* and *damselae***. The kit contains reagents and enzymes for the specific amplification of two target sequences: a 448 bp region specific for the subsp. *damselae* and a 297 bp region present in the genome of all *Photobacterium damselae* subsp. *piscicida.* Few *P. damselae* subsp. *damselae* isolates, showing the 297 bp amplicon, can be identified by the presence of the 448 bp product. To reveal possible PCR inhibition, an internal control, giving an amplicon of 112 bp, is also supplied in the PCR mix.

Kit contents

2 x 1250µl	**Photobacterium damselae Mix**
1 x 15µl	**Hot-Rescue DNA Polymerase (5U/µl)**
1 x 15µl	***Photobacterium damselae* Positive Control**
1 x 1000µl	**PCR-Grade Water**

Storage

Store the kit at -20°C.
Repeated thawing and freezing cycles may reduce the sensitivity and should be avoided. It is suggested freezing the reagents in aliquots for intermittently use.

Precautions

The operator should always pay attention to:

- use pipette tips with filter;
- store positive material (specimens, controls and amplicons)
- separately from all other reagents and, if possible, add it to the
- reaction mix in a facility separated space;
- do not use the same precision pipettes for reaction mix and
- DNA-samples;
- thaw all components samples at room temperature before
- starting an assay;
- when thawed, mix the components and centrifuge briefly;
- work on ice or in a cooling block.

Procedure

DNA ISOLATION

Sample preparation
Various protocols or commercial kits can be used to extract bacterial DNA from clinical specimens or bacterial cultures.
Carry out the DNA isolation according to the procedure making sure that the extracted DNA is free from PCR inhibitors.

PCR SETUP
The use of thin-walled PCR tubes for all amplification steps is recommended. Include a positive control and at least one negative control (water) in each PCR run. Before each use thaw all reagents completely, mix and centrifuge. The PCR assay must be prepared

following the pipetting scheme below:

Component	For 1 reaction
Photobacterium damselae mix	48.8µl
Hot-Rescue DNA Polymerase	0.2µl
DNA sample	1.0µl
Total volume	**50.0µl**

Make a master mix combining the reagents together in a 1.5 ml tube.
Aliquot 49µl of master mix into each PCR reaction tube before adding 1µl* sample, negative and positive control.
*It is preferable to employ 5ng DNA/reaction.

In order to avoid cross contamination, first pipet the negative control, then the samples and finally the positive control.
Centrifuge briefly.
For PCR thermal cycler without heated lid, each reaction mixture will have to be covered with a drop of mineral oil.

THERMAL PROFILE
Program the PCR thermal cycler with the following parameters:

1 cycle	95°C for 1 min
40 cycles	95°C for 30 sec 65°C for 30 sec 72°C for 1 min
1 cycle	72°C for 10 min
	cool down to 4°C

AGAROSE GEL ELECTROPHORESIS
Mix 20 µl of the PCR reaction with 5 µl of DNA loading buffer. Separate the DNA in the presence of a DNA standard, on a 3% agarose gel containing ethidium bromide, for about 45-60 min. The DNA standard should be specific for the low range (100-1000 bp).

The following results are possible:

Band pattern	Interpretation
Band at 112 bp	NEGATIVE SAMPLE
Band at 297 bp and a possible additional band at 112 bp	POSITIVE SAMPLE for *P. damselae* subsp. *piscicida*
Band at 448 bp and possible additional bands at 297 bp and 112 bp	POSITIVE SAMPLE for *P. damselae* subsp. *damselae*
No band	NO DIAGNOSIS (see troubleshooting section)

Troubleshooting

No bands of 279 and 448 bp from the positive control	Incorrect programming of the thermal cycler: repeat the PCR with the correct settings. Degraded reagent: store PCR reagents at -20°C and keep on ice once thawed. Avoid multiple freeze/thaw cycles. Incorrect tubes: use thin-walled PCR reaction tubes.
No bands of 279 and/or 448 bp (*P. damselae*) and 112 bp (Internal Control) from the sample PCR	PCR reaction inhibition: repurify the DNA sample to remove inhibitors.
Bands of 279 and/or 448 bp from the negative control.	Contamination of PCR reaction: vigorous cleaning is recommended before repeat the amplification.

References

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Gauthier G., Lafay B., Ruimy R., Breittmayer V., Nicolas J. L., Gauthier M. and Christen R. (1995) Small-subunit rRNA sequences and whole DNA relatedness concur for the reassignment of *Pasteurella piscicida* (Snieszko et al.) Janssen and Surgalla to the genus *Photobacterium* as *Photobacterium damsela* subsp*. piscicida* comb. nov. *Int J Syst Bacteriol.* 45:139-44.

Images

Fig. 1
Lane 1: ~ bp DNA ladder (Invitrogen).
Lanes 2-5: Amplification of **10^4, 10^3, 10^2** and **10** *P. damselae* DNA molecules.
Lane 6: Negative control.

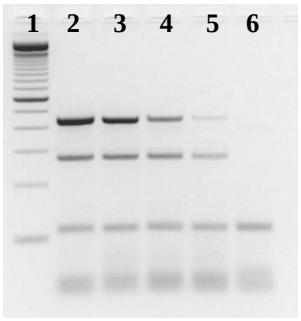

Fig. 2
Lane 1: ~ bp DNA ladder (MBI Fermentas).
Lanes 2-9: Amplification of some *P. damselae* subsp. *piscicida* strains.
Lanes 10-18: Amplification of some *P. damselae* subsp. *damselae* strains.
Lane 19: Negative control.