Development of a PCR-Based Assay to Detect *Penicillium marneffei* in Insects

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Abstract

Penicillium marneffei is a pathogenic fungus endemic solely to Southeast Asia. The fungus infects humans, mainly those who have developed AIDS. The fungus is also found in bamboo rats, but there is no evidence to suggest that the disease is transmitted from rats to animals. In fact, the exact reservoir of *P. marneffei* is unknown despite decades of investigations. We hypothesize that insects may be the carriers of this infectious disease agent. To assess this hypothesis, we are attempting to develop molecular methods to detect *P. marneffei* in insects based upon previously published procedures that employed the polymerase chain reaction (PCR). Such procedures should be more efficacious than traditional culture methods. To this end, we have demonstrated that the PCR assay works in vitro to detect the fungus. However, while culture results of haemocoel ("blood") have been positive for *P. marneffei* infected wax moth larvae, PCR assays of raw whole organism extracts or unpurified haemocoel have not yielded amplification products. Future developmental efforts will focus upon using purified haemocoel extracts in the PCR assay. Nonetheless, we have demonstrated by culture techniques that wax moth larvae are capable of carrying *P. marneffei*, thus supporting the basis of our hypothesis.

Development of the PCR Assay

The method we are developing is based upon the prior work of Vanittanakom *et al.* (J. Clin. Microbiol. **40:** 1739-1742, 2002). The PCR procedure was followed as detailed in this publication. Wax moth larvae were infected with *P. marneffei* conidia. Specifically, we sought to achieve the following objectives:

1) Determine if nested primer pair successfully amplifies products from DNA and whole conidia
   RESULTS: We were successful – see Experiment #1
2) Assess if *P. marneffei* can be detected in infected wax moth larvae using culture techniques
   RESULTS: We were successful – see Experiment #2
3) Determine if primer pair RRH1/RRF1 can detect *P. marneffei* DNA from raw wax moth larval extracts or unpurified haemoceol
   RESULTS: DNA was not amplified, likely due to PCR inhibitors in the extracts

Future Plans: Repeat last objective using purified haemocoel extracts. The latter have been shown to contain viable *P. marneffei* conidia (Experiment #2).

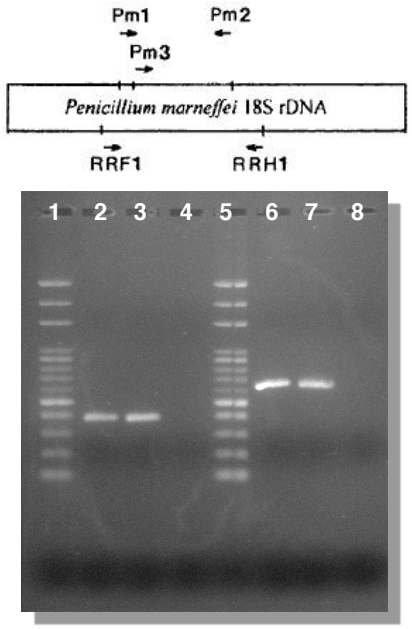
Experiment #1: Confirmation of Primer Function

Primers Pm1 and Pm2
Lane 1 – DNA Ladder
Lane 2 – Purified DNA (400 bp)
Lane 3 – Conidia (400 bp)
Lane 4 – Water (neg. control)

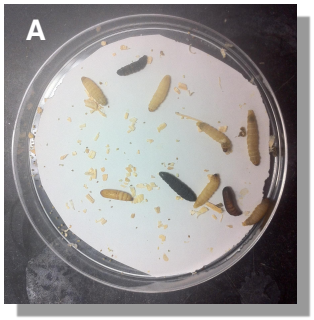
Primers RRH1and RRF1
Lane 5 – DNA Ladder
Lane 6 – Purified DNA (700 bp)
Lane 7 – Conidia (700 bp)
Lane 8 – Water (neg. control)

Conclusion: Primers work as predicted

Nested Primer Binding Sites



Experiment #2: Culture-Based Detection of *P. marneffei* in Infected Wax Moth Larvae



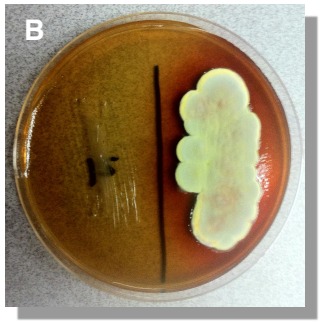


Figure A – Wax moth larvae infected with *P. marneffei*. Beige color larvae are living, dark colored larvae are dead or dying.

Figure B – Culture results from uninfected larvae (left side of plate) and infected larvae (right side).

Viability Results

19 of 20 uninfected (PBS buffer) larvae survived for at least 8 days
7 of 10 larvae infected with 2 x 10^7 *P. marneffei* conidia survived for at least 8 days
6 of 10 larvae infected with 2 x 10^8 *P. marneffei* conidia survived for at least 8 days

Conclusion: *P. marneffei* is capable of survival for at least eight days in wax moth larvae