

COMPARISON OF STORAGE METHODS TO PRESERVE THE PATHOGENIC OOMYCETE *PYTHIUM INSIDIOSUM*

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Abstract. *Pythium insidiosum* causes life-threatening pythiosis in humans and other animals. Study of this organism requires maintaining it in culture which can be difficult. The aim of this study was to compare several methods of maintaining *P. insidiosum* in cultures in order to determine the best method to accomplish this. We compared the following methods for maintaining *P. insidiosum*: routine repeated subculture, distilled water immersion, liquid paraffin overlay and storage at an ultra-low temperature with liquid nitrogen. Ribosomal deoxyribonucleic acid (rDNA) sequences of *P. insidiosum* were analyzed to confirm the identity of the organism after preservation. We conducted an initial 2-week assessment but due to relatively low viability with storage at an ultra-low temperature with liquid nitrogen, this method was not used for the long term study. The remaining methods were reassessed every 3 months for 15 months. The routine repeated subculture, distilled water immersion and liquid paraffin overlay methods showed the viability rates of 100%, 100% and 90% at 9 months, 100%, 90% and 80% at 12 months and 100%, 60% and 40% at 15 months, respectively. The rDNA sequences of the preserved strains showed no differences compared to the original strains. We conclude the distilled water immersion method is optimal due to being simple to perform, inexpensive and relatively efficient for maintaining *P. insidiosum* for at least 9 months. This method reduces the frequency of routine repeated subculture passages by 9 fold.

Keywords: *Pythium insidiosum*, pythiosis, preservation, storage, culture collection

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