Long-Term Storage of *Phytophthora* Cultures in Water[1]

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Abstract

Long-term storage of cultures of *Phytophthora* species is a challenge for any lab managing a working collection of isolates. Storage in liquid nitrogen is generally considered to be optimal for archival storage, and successful recovery of most species is regularly achieved after many years. Nitrogen storage has its drawbacks, however, especially for a working collection. It requires species specific freezing conditions, and thawing must be done carefully. Equipment is bulky, and regular addition of liquid nitrogen is necessary. Storage in vials in water at room temperature is an efficient, effective alternative for many collections. Isolates are grown on agar, and plugs are removed from the colony margin and placed in sterile water in 1.5 ml plastic tubes, with or without pieces of sterilized hemp seed. Tubes are kept in coded racks at room temperature, in the dark. Agar plugs can be removed one at a time as needed and plated on selective agar to resume growth. In a recent test, we had nearly 100 percent recovery of several *Phytophthora* species, including *P. ramorum*, after 7 years in water storage.

Introduction

Long-term storage of cultures of *Phytophthora* species is a challenge for any lab managing a working collection of isolates. Storage in liquid nitrogen is generally considered to be optimal for archival storage, and successful recovery of most species is regularly achieved after many years. Nitrogen storage has its drawbacks, however, especially for a working collection. It requires species-specific freezing conditions, and thawing must be done carefully. Equipment is bulky, and regular addition of liquid nitrogen is necessary.

In 1976 Boesewinkel described a new storage method for storing *Phytophthora* using colonized agar blocks in bottles (90 x 26 mm) of sterilized distilled water. This method eliminated the space and cost issues associated with nitrogen storage. We have adapted the method, using smaller vessels and adding hemp seeds.

Methods

Culture Storage

Water vials are 1.5 ml of de-ionized water placed in 1.5 ml cryogenic vials. Hemp seed/water vials are one and a half ml of de-ionized water and one hemp seed placed in 1.5 ml cryogenic vials. The vials are sealed, sterilized by autoclaving and cooled to room temperature.

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Isolates are grown on agar (usually corn meal agar amended with 20 ppm β sitosterol) for five to ten days. Each isolate is stored in a pair of vials, one 5mm plug placed in a hemp seed/water vial and three to five plugs in a water vial. The vials are placed in CryoBoxes and stored in the dark at room temperature.

Culture selection

Species selection for this test was based on availability in long term storage and interest to the larger community. Cultures that have not been identified to species were not included in this test. When possible, five isolates per species were included.

Water Tube Culture Retrieval

A single plug was retrieved from a water vial and plated on the selective medium CARP (17 g BBL Corn Meal Agar, 1 L de-ionized water, 20ppm Delvocid (50 percent natamycin salt), 200ppm Ampicillin, sodium salt and 10ppm Rifamycin SV sodium salt). At 14 days plates were examined for growth. If new mycelium had grown into the agar it was scored a success. If no new mycelium was present the water storage vial was re-sampled, this time plating all remaining plugs. Again if there was new growth in 14 days it was scored as a success and if there was no growth it was considered a failure.

Hemp/water tube culture retrieval

Both the hemp seed and plug were plated on CARP. Frequently in the hemp tubes a mass of mycelium forms and this was plated as well. At 14 days the plates were examined for new growth. New mycelium emerging from the plug, hemp seed or mycelial mass was scored a success.

Results

Cultures were successfully retrieved from 72 of the 78 water vials after three to eight years in storage (Table 1). Of the six failures, the four 7-year-old *P. lateralis* and one 5-year-old *P. ilicis* vials had only one remaining plug and so couldn't be re-sampled. The 8-year-old *P. megasperma* had five non-viable plugs.

Table 1ð Water vial culture success ratios (number of successful retrievals/number of vials sampled) by years stored.

	YEARS IN STORAGE						
SPECIES	8	7	6	5	4	3	Total
P. cactorum	1/1	5/5					6/6
P. cambivora			2/2	2/2			4/4
P. cinnamomi				3/3			3/3
P. citricola	2/2		1/1	1/1			4/4
P. cryptogea				2/2		2/2	4/4
P. sp. *"*DF1*"*	5/5						5/5
P. europaea			1/1				1/1
P. gonapodyides				2/2	5/5		7/7
P. ilicis				0/1	1/1		1/2
P. lateralis		0/4	3/3				3/7
P. megasperma BHR	7/8						7/8
P. nemorosa			5/5				5/5
P. sp. *"Pg chlamydo"*	5/5						5/5
P. pseudosyringae				4/4			4/4
P. pseudotsugae	1/1						1/1
P. ramorum			7/7				7/7
P. siskiyouensis					5/5		5/5
Totals	21/22	5/9	19/19	14/15	11/11	2/2	72/78

Cultures were successfully retrieved from 34 of the 38 hemp/water vials after three to six years in storage (Table 2). Of the four failures, two had bacterial contamination. Of the successes, in all but one case, both the hemp/water plug and the hemp seed/mycelium mass were viable.

Table 2ð Hemp seed/water vial culture success ratios (number of successful retrievals/number of vials sampled) by years stored.

	YEARS IN STORAGE				
SPECIES	6	5	4	3	Total
P. cactorum					
P. cambivora	5/5	2/2			7/7
P. cinnamomi		3/3			3/3
P. citricola	1/1	1/1			2/2
P. cryptogea		2/2		2/2	4/4
P. sp. *"DF1"*					
P. europaea					
P. gonapodyides		2/2	4/5		6/7
P. ilicis		0/1			0/1
P. lateralis					
P. megasperma BHR					
P. nemorosa					
P. sp. *"Pg chlamydo"*					
P. pseudosyringae		3/4			3/4
P. pseudotsugae					
P. ramorum	2/2	3/3			5/5
P. siskiyouensis			4/5		4/5
Totals	8/8	16/18	8/10	2/2	34/38

Comparing the success of the two methods was possible in 31 cases, where isolates had been stored in both water and hemp/water vials at the same time (Table 3).

Table 3—Culture success comparing water vial to hemp/water vial.

	Water Vial	
Hemp/Water Vial	Success	Failure
Success	27	0
Failure	3	1

Discussion

There are many advantages to this type of culture storage, cost, ease and high success rate to name a few. The ability to remove one plug at a time is more efficient than thawing and plating an entire vial. However, there are a few cautions. Repeated visits to the same tube increase the odds of contamination. Our "room temperature" ranges from about 18-24^{o}C, but some species may need special conditions, especially cooler or more controlled temperatures. After retrieval of a culture it's a good idea to restore anything that's been in storage more than two years.

We have been using water storage exclusively for at least 12 years. Recently, *P. lateralis* was successfully cultured from 4 hemp vials stored in 1995. While there is no way of knowing which isolates are still viable until they are retrieved, over the course of the 12 years our success rate has been high.

Literature Cited

Boesewinkel, H.J. 1976. Storage of Fungal Cultures in Water. Trans. Br. Mycol. Soc. 66:183-185.