Preservation of Plant-Pathogenic Bacteria on Silica Gel

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ABSTRACT

Leben, C., and Sleesman, J. P. 1982. Preservation of plant-pathogenic bacteria on silica gel. Plant Disease 66:327.

Thirteen species of plant-pathogenic bacteria, members of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas*, were preserved dry on silica gel held at −20 C for 5 yr. Survivors were pathogenic. The method is simple, and the only major equipment required is a household freezer.

A simple, generally available method for preserving a range of plant-pathogenic bacteria unchanged for future study or comparisons has been needed for many years. We report survival data for 13 species stored dry on silica gel held at −20 C. Survival after 20 mo at this temperature has been described as "excellent" (2). This account, which presents data after 60 mo of storage, seems warranted because of the simplicity and success of the method.

MATERIALS AND METHODS

Materials and methods have been described in detail previously (2). Bacterial cells taken from 7-day-old cultures (an age favoring survival [1]) were suspended in an aqueous milk or milk-glycerol menstruum, and 0.5 ml of menstruum was added per 3 g of silica gel particles. Particles were dried and stored in the freezing compartment (−20 C) of a household refrigerator. We emphasize again that it is essential to precool vessels and suspensions so that the heat generated when liquid is added to silica gel is dissipated rapidly.

We reisolated bacteria by scattering about 50 particles on sucrose nutrient agar (Difco nutrient agar, 23 g; sucrose, 10 g/L) in two petri dishes and counting particles surrounded by bacterial colonies after incubating the dishes at 24 C for 2–7 days. Methods described previously (2) were used to test the pathogenicity of bacteria from representative colonies.

RESULTS AND DISCUSSION

Data on the survival of pathogens dried with the two menstrua are given in Table 1. Recovery was better with milk-glycerol than with milk alone, but even with milk, surviving bacteria were reisolated from more than half of the particles. Colonies were normal in appearance. With each menstruum, all species were pathogenic.

This method is so simple and successful that we have adopted it as our method of choice for storing pathogens. The method is especially useful because the storage vessel may be opened a number of times to establish stock cultures for day-to-day use.

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LITERATURE CITED

1. Sleesman, J. P., and Leben, C. 1976. Bacterial desiccation: Effects of temperature, relative humidity, and culture age on survival. Phytopathology 66:1334-1338.
2. Sleesman, J. P., and Leben, C. 1978. Preserving phytopathogenic bacteria at −70 C or with silica gel. Plant Dis. Rep. 62:910-913.

Table 1. Survival of plant-pathogenic bacterial cells dried on silica gel particles after 60 mo at −20 C

Bacterium[a]	Percentage of about 50 particles bearing surviving pathogens	
	Cells dried in milk	Cells dried in milk-glycerol
Agrobacterium tumefaciens	100	100
Corynebacterium michiganense	100	100
C. nebraskense	100	100
Erwinia carotovora (var. *carotovora*)	96	100
E. stewartii	100	98
Pseudomonas glycinea (*P. syringae*)	52	97
P. lachrymans (*P. syringae*)	100	97
P. phaseolicola (*P. syringae*)	100	100
P. solanacearum	73	100
P. syringae (from bean)	100	100
Xanthomonas campestris	100	100
X. nigromaculans f. sp. *zinniae*	100	100
X. phaseoli (*X. campestris*)	100	100

[a] Names in parentheses are preferred in Bergey's Manual of Determinative Bacteriology (8th ed., 1974).