Development of a Powder Formulation of Rhizobacteria for Inoculation of Potato Seed Pieces

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This study was supported in part by funds from the California Research Advisory Board.
Accepted for publication 4 November 1980.

ABSTRACT

Kloepper, J. W., and Schroth, M. N. 1981. Development of a powder formulation of rhizobacteria for inoculation of potato seed pieces. Phytopathology 71:590-592.

A dried formulation of plant growth-promoting rhizobacteria (PGPR) that could be applied to potato seed pieces was developed. Genetically marked bacterial suspensions were mixed with various concentrations of commercially available plant gums, xanthan gum (XG), or methylcellulose and then mixed with talc. Populations of PGPR per gram of dried talc mixtures were 1×10^3 with 10% gum guar, 1×10^3 with gum locust bean, 1.4×10^3 with 1% methylcellulose, 6.9×10^5 with 50% gum arabic, and 8.2×10^7 with 20% XG. PGPR did not survive in mixtures containing gum karaya or gum tragacanth. Population of PGPR did not decline in the talc mixture with 20% XG after storage for 2 mo at 4 C, while populations decreased in mixtures containing 5 and 10% XG and 50% gum arabic. In field experiments PGPR placed on potato seed pieces in a powdered XG preparation developed root populations greater than on roots of plants inoculated with aqueous preparations. The powdered inoculum resulted in significant increases in early plant development in field tests as did the aqueous inoculum. These results suggest that the powdered formulation of PGPR in XG could be used commercially to promote plant growth.

The commercial use of plant growth-promoting rhizobacteria (PGPR) requires inoculum that retains a high cell viability and can easily be transported and applied to seed. In most studies requiring bacterial inocula, either liquid suspensions (1,2,4,7) or bacteria incorporated into peat (3,8,9) have been used. Suslow's (11,12) recent effective use of methylcellulose in a powder formulation to coat PGPR onto sugar beet seed showed that rhizobacteria survive well in a dried form under certain conditions. A dried powder formulation of PGPR is especially important for potato since the seed pieces cannot be economically pelleted as can seeds of other plants.

This paper reports the development of a powdered PGPR inoculum based upon bacterial survival in various gums and the effectiveness of that inoculum in the field.

MATERIALS AND METHODS

Dry formulations of bacterial inoculum. The ability of fluorescent PGPR in the genus *Pseudomonas* to survive drying in powdered formulations of several commercially available gums was compared with survival in methylcellulose (MC). Substrates tested included gum guar, gum locust bean, gum arabic, gum karaya, gum tragacanth, and xanthan gum (XG). A series of concentrations of each gum ranging from 1% to the maximum soluble concentration was prepared and autoclaved. Five milliliters of gum were mixed with 5 ml of a 10^9 colony forming units (cfu) per milliliter suspension of 24-hr-old potato PGPR strain A1 (4) genetically marked for resistance to rifampicin and nalidixic acid. After allowing the mixture to set 20 min, talc (approximately five times the volume of the bacteria-gum mixture) was added and mixed thoroughly. The resulting mixture was thinly spread over a metal sheet and placed at 12 C for 3–4 days until dry.

Viable populations of A1 in the dried mixtures were determined by grinding the talc mixture in a mortar and pestle, removing 1.0 g and mixing it with 10 ml of sterile water for 20 min. Serial 10-fold dilutions were prepared, and 0.1-ml aliquots of each were spread on King's medium B containing 100 ppm rifampicin and nalidixic acid.

Gums yielding populations of A1 greater than those in methylcellulose were selected for further study. These mixtures were prepared again, the initial population was sampled, and they were stored at 4 C for periodic population sampling.

Effectiveness of the dry powder inoculum. The ability of PGPR to colonize roots of potato plants was compared in four field tests of dried and liquid bacterial preparations. Plantings were made in a fine sandy loam near Minidoka, ID, and in peat soils near Tulelake, CA. Dried preparations were prepared as described above using 1% MC in 1978 and 20% XG in 1979. Potato seed pieces were dusted with the dried formulations prior to planting at a rate of 0.5 kg dust per 46 kg potato seed pieces (approximately 1 lb per 100 lb). Inoculation by liquid suspensions involved a 1–2 min dip of seed pieces into PGPR suspended in 0.1 M $MgSO_4$ prior to planting. Controls for dried inocula consisted of talc mixed with either MC or XG and water; liquid controls consisted of 0.1 M $MgSO_4$. A mechanical planter was used in all fields.

Root colonization by PGPR was measured 2 wk after plant emergence as previously described (4). Samples involved five replications per treatment, with three plants per replication selected at random; 50 linear centimeters of root was sampled from each plant.

The ability of PGPR applied as dry seed piece treatments to increase potato stolon growth (4) was compared with liquid suspension treatments. Plants were selected at random 2 wk after emergence and length of stolons was measured. Five replications, each with three plants, were used per treatment. Data were

TABLE 1. Effect of different gums on the viability of plant growth-promoting rhizobacteria in dried powders[a]

Gum tested	Percent gum[b]	Rhizobacterial population[c] (cfu per gram dried powder)
Guar	10	1×10^3
Locust bean	15	1×10^3
Arabic	50	6.9×10^5
Arabic	60	5.0×10^3
Karaya	1–50	0
Tragacanth	1–50	0
Xanthan	5	1.3×10^5
Xanthan	10	6.6×10^6
Xanthan	20	8.2×10^7
Methylcellulose	1	1.4×10^3

[a] Experiments were repeated twice with similar results.
[b] Various concentrations were used for each gum; concentrations reported here are those yielding the highest bacterial populations.
[c] A 10^9 cfu/ml suspension of potato PGPR A1 was mixed with each gum and talc prior to drying. Populations were measured 1 wk after preparation.

0031-949X/81/06059203/$3.00/0

analyzed by using a two-way analysis of variance. If a significant F-test resulted, significant differences in treatment means were determined by using the LSD test.

RESULTS

Dry formulations of bacterial inoculum. Dried-dust formulations of XG and gum arabic yielded higher A1 populations than methylcellulose (MC) (Table 1). The maximum population with XG was 8.2×10^7 per gram, which was more than four log units greater than MC. The concentration of gum had a marked effect on bacterial viability. Gum arabic supported higher viable populations at a concentration of 50 than at 60%, and XG supported the highest populations at 20%.

The initial high populations of rhizobacteria in the gum formulations were not necessarily sustained during storage. After storage for 2 mo at 4 C, PGPR populations in the 50% gum arabic mixture decreased 74%. In the 20% XG mixture, however, for unknown reasons a 52% greater number was detected after 2 mo of storage than initially. In experiments measuring long-term survival of the rhizobacteria, formulations of 20% XG were best, yielding populations of 10^7 per gram after 3 mo of storage. However, populations in mixtures of 10 and 5% XG dropped sharply (Fig. 1).

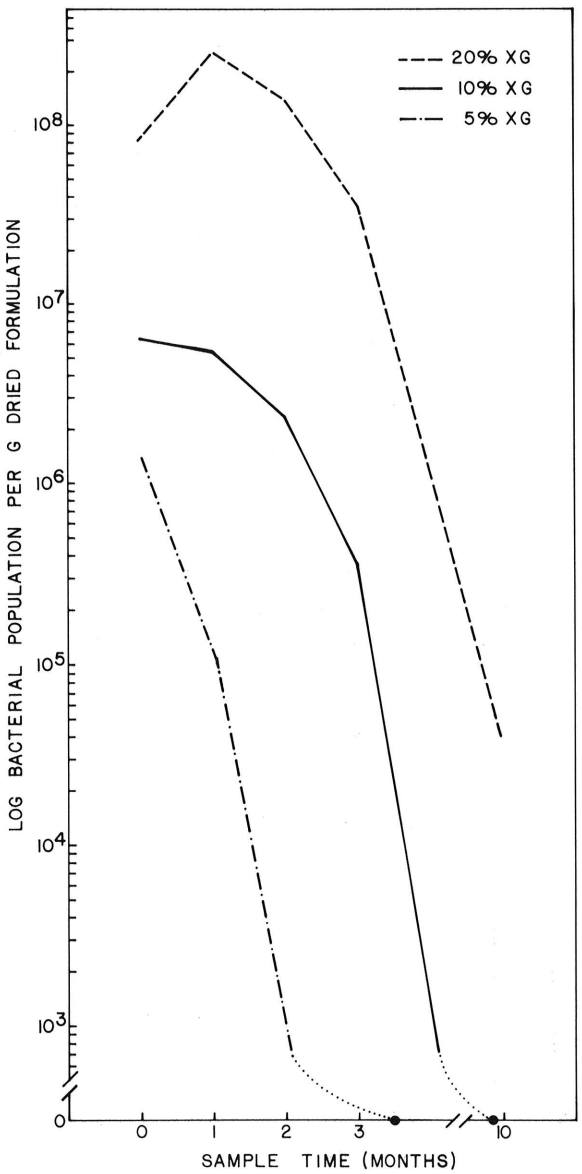

Fig. 1. Survival of plant growth-promoting bacteria in dried formulations of xanthan gum.

Effectiveness of the dry powder inoculum. PGPR applied as dried XG formulations colonized roots of plants to a greater degree than when applied as aqueous suspensions (Table 2). In two fields, XG-talc mixtures of PGPR resulted in root populations that were 1.0–2.3 log units greater than populations initiated with aqueous inoculum. Plants treated with MC formulations had lower populations of PGPR on roots than those treated with aqueous suspensions, while plants treated with XG formulations had higher populations than those treated with aqueous suspensions (Table 2). Both MC and XG dried inoculum preparations enhanced stolon development (which relates to yield increases [4]) in the field (Table 3) as well or better than aqueous inocula.

TABLE 2. Populations of plant growth-promoting rhizobacteria (strain A1) on roots of potato treated with dried inocula compared with aqueous bacterial suspensions 2 wk after plant emergence

Year and field location	Seed piece treatment[a]	Rhizobacterial population on roots (cfu per cm)[b]
1978		
Idaho	A1 suspension	1.2×10^4
	A1 MC talc	7.8×10^2
Tulelake, CA	A1 suspension	6.8×10^2
	A1 MC talc	3.4×10^2
1979		
Tulelake, CA	A1 suspension	7.0×10^0
	A1 XG talc	2.3×10^2
Tulelake, CA	B10 suspension	7.0×10^0
	B10 XG talc	1.1×10^3
	E6 suspension	3.0×10^2
	E6 XG talc	2.2×10^3

[a] Seed pieces were dipped into aqueous suspensions of bacteria or dusted with powdered talc formulations. MC = methylcellulose, XG = xanthan gum.
[b] Average of five replications per treatment with three plants per replication and 50 cm of root per plant.

TABLE 3. Increased stolon length 2 wk after emergence of potatoes treated with dried inocula or aqueous suspensions of plant growth-promoting rhizobacteria

Year and location	Field	Seed piece treatment[a]	Length of stolons (cm)[b]
1978			
Idaho	1	A1 suspension	8*[c] $LSD_{0.01} = 4$
		A1 MC talc	7*
		Untreated control	2
1978			
Tulelake, CA	2	A1 suspension	27* $LSD_{0.05} = 3$
		A1 MC talc	25*
		Liquid control	17
		MC talc control	19
1979			
Tulelake, CA	3	E6 XG talc	40* $LSD_{0.05} = 8$
		A1 XG talc	25*
		B10 XG talc	24*
		E6 suspension	18*
		A1 suspension	17*
		B10 suspension	27*
		Liquid control	4
		XG talc control	7
1979			
Tulelake, CA	4	E6 XG talc	36* $LSD_{0.01} = 9$
		B10 XG talc	34*
		E6 suspension	39*
		B10 suspension	26*
		Liquid control	6
		XG talc control	14

[a] Seed pieces were dipped into aqueous suspensions of bacteria or dusted with powdered talc formulations. MC = methylcellulose, XG = xanthan gum.
[b] Average of five replications per treatment with three plants per replication.
[c] *, significant difference, $P = 0.05$.

DISCUSSION

Rhizobacteria can be formulated as a dry powder inoculum with XG as a base, enabling their commercial application to potato seed. The XG enhancement of rhizobacterial cell survival is probably related to the nature of the lipopolysaccharide and its coating properties. It is derived from *X. campestris* (10) and is reported to be important in the survival of *Xanthomonas* spp. (5,6,13).

The physiological state of PGPR cells when they first encounter the soil environment undoubtedly affects their survival of seed pieces. Unlike cells in aqueous suspensions, PGPR cells in XG formulations are physiologically inactive and protected from environmental stresses.

With potato seed pieces, the soil is frequently dry at planting time. Thus, the bacteria must survive during unfavorable conditions until moisture is present and nutrients are provided by the germinating seed pieces and growing roots. PGPR applied in the XG formulation presumably survived better on seed pieces, which led to increased root colonization.

The application of both aqueous and powdered inocula resulted in significant increases in stolon development in field tests compared to nontreated plants. Increases in stolon length early in the season have been shown (4) to relate to yield increases. The increases, however, were not significantly different between plants treated with dry or aqueous inoculum. Although populations of PGPR were greater following the use of powders, presumably the population resulting from aqueous inoculum still was sufficient to cause increases in stolon length.

The successful use of XG mixtures of PGPR reported here for potatoes suggests that the inoculum can be used on other crops, including those propogated via true seed. The dried inoculum could either be pelleted onto seeds, dusted onto them, or mixed into soil at planting time. Although the bacterial population declines with storage time, the reduction is not sufficiently great to constitute a problem with storage for 3–4 mo. The population per gram of talc also can be increased by using greater numbers of bacteria when making the preparation. It is expected that modifications and improvements of the present formulation will lead to improved viability and better storage life.

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