Laboratory-Scale Preparation of Sodium Polypectate for Use in Selective Media for Pectolytic *Erwinia* spp.

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ABSTRACT

COTHER, E. J., A. B. BLAKENEY, and S. J. LAMB. Laboratory-scale preparation of sodium polypectate for use in selective media for pectolytic *Erwinia* spp. Plant Disease 64:1086-1087.

In a laboratory method for preparing sodium polypectate from orange skins or green apples, gel consistency was substantially increased by addition of morpholinopropane sulfonic acid at 4 gm/L. Polypectates prepared in this manner produced gels with strength equal to or greater than that of Raltech pectates. Polygalacturonic acid, Na salt, from Sigma Chemical Co. failed to produce acceptable gels.

Additional key words: 3-(*N*-morpholino) propane sulfonic acid

Semiselective or enrichment media based on pectate gels are commonly used for the initial isolation and identification of soft-rot *Erwinia* spp. (1,2,4,6,9,11). Media have been formulated with sodium polypectate (3,5,8,10,12,13,15,16,18) as the gelling agent to give a solid plating medium in which pectolytic *Erwinia* will form characteristic depressions.

Sodium polypectate became scarce during 1977 and was unavailable in early 1978 when we required a suitable polypectate medium for etiologic studies on potato seed tuber breakdown. The scarcity of sodium polypectate led us to investigate its small-scale laboratory preparation and to compare the product with polypectate that became available in mid-1978.

Before we attempted preparing sodium polypectate directly from orange skins, we tried to convert various samples of commercial food grade pectins into sodium polypectates. All these attempts were disappointing, and no preparations produced gels of a consistency adequate for dilution platings or for streaking.

MATERIALS AND METHODS

Our technique was based in part on a general description published by C. W. Wilson as a patent (19).

Fresh orange peel (*Citrus sinensis* Osbeck 'Valencia,' 2 kg from about 25 fruits) was ground in a domestic meat mincer fitted with a 5-mm screen, washed well with tap water, and drained. The peel was suspended in enough water (4 L) to be stirred easily, and 33.5 g of sodium carbonate was added to maintain the pH at 8.5. The suspension was held for 14 hr at 25 C with occasional checks on pH. The pectated pulp was then washed well with water. Drying at ambient temperature (about 24 C) in a forced air dehydrator for 3 days produced dry pectated pulp that could be stored indefinitely. Eight kilograms of fresh orange peel yielded about 500 g of dry pulp.

Dried (100 g) or wet fresh (1,500 g) pulp (93% moisture) was added and stirred constantly in a stainless steel bucket containing 12 g of $Na_3PO_4 \cdot 12H_2O$ and 1.2 g NaOH dissolved in 2 L of boiling water. The mixture was heated in an autoclave at 110 C for 5 min, and the suspension was filtered through 2-mm fiberglass mesh. Sodium polypectate was precipitated from the filtrate by addition, with stirring, of two volumes of acetone. The fibrous precipitate was collected after 1 hr by filtering through several layers of cheesecloth, resuspended in a half volume of acetone, and refiltered. The polymer was dried at 30 C in a vacuum oven and ground to a powder in a small laboratory mill.

Two modifications were made to this procedure. The pulp was blended in the phosphate/caustic solution for 30 sec in a Waring Blendor, or after being filtered the precipitate was redispersed in 2 L of boiling water, held at 100 C for 5 min, filtered through Miracloth (Calbiochem, La Jolla, CA), and precipitated as before.

Apples were investigated as an alternative source for polypectate. Five kilograms of green apples (*Malus domestica* Borkh 'Granny Smith,' about 32 apples) were cut and pulped in a domestic rasptype juicing machine; the juice-free pulp was collected and washed in two changes of distilled water. The rehydrated pulp (about 1.13 kg) was mixed with water to form a slurry, and $NaCO_3$ (12.5 g) was added to maintain the pH at 8.5. After 12 hr the pulp was again washed twice with water, drained, and treated in a similar manner to the orange skin pulp. Because of the finer particle size of the apple pulp, the phosphate/caustic pulp suspension was filtered through Miracloth before acetone was added.

In mid-1978, sodium polypectate became available again from Sigma Chemicals (St. Louis, MO) and from Raltech Scientific Services Inc. (Madison, WI). Pectates prepared in the laboratory were compared with pectates from these sources in crystal violet pectate (CVP) medium (5) at the following rates: Raltech, 20 g/L; laboratory pectates, 20 g/L; Sigma polygalacturonic acid (sodium salt, P-1879) 30–40 g/L.

The acceptability of each pectate as a selective growth medium was assessed by inoculating petri dishes with isolates of *Erwinia chrysanthemi* Burkholder, McFadden and Dimock, *E. carotovora* var. *carotovora* Dye, *Xanthomonas campestris* (Pammel) Dowson, and *Pseudomonas solanacearum* (Smith) Smith. Decaying tuber tissue from different potato fields was also streaked on CVP. Plates were incubated at 25 C for 3 days and compared for type and clarity of cavities formed.

The breaking strength of each pectate gel was measured on 3.5 cm thick, 2-day-old gels in stainless steel beakers, using an Instron Model 1140 Universal Texture Testing Instrument (Instron Ltd., Bucks., U.K.) with a model A31/1001 compression load cell (full-scale reading, 500 g) and a 57-mm diameter probe traveling at 50 mm/min. This method of gel characterization was briefly reviewed by Towle and Christensen (17).

The phosphate content of each polypectate sample was determined by the AOAC method (7).

RESULTS AND DISCUSSION

All methods of preparation gave acceptable yields of sodium polypectate: the direct method, with 1.5 kg of fresh peel, gave 16.3 g of dried polypectate; and with 100 g previously dried pectinated

0191-2917/80/12108602/$03.00/0

Table 1. Properties of crystal violet pectate gels from different sodium polypectate sources

Source	Gel		Polypectate phosphate (mg/100 g)	Subjective streakability of gel (0–10 scale)[b]
	pH after autoclaving	Tensile strength (g/cm^2)[a]		
Raltech	6.1	84	56	8
Raltech + MOPS[c]	7.2	86	···	10
Sigma	4.2	0.5	0	1
Sigma + MOPS	6.4	10	···	2
Laboratory preparations				
Single precipitation	7.8	0.1	173	1
Double precipitation	7.0	2	93	4
Apple pulp	8.0	0.4	286	0
Direct from peel	7.8	0.5	213	0
Single precipitation + MOPS	7.2	90	···	8
Double precipitation + MOPS	6.8	181	···	10
Apple + MOPS	7.2	110	···	10
Direct + MOPS	7.2	184	···	10

[a] Measured with an Instron Model 1140 Universal Texture Testing Instrument.
[b] Based on increasing ease of inoculation with bacteria using 1 mm nichrome loop.
[c] MOPS = 3-(*N*-morpholino) propane sulfonic acid.

pulp, single precipitation yielded 19 g, double precipitation 18 g, and blended single precipitation 20.6 g. Yield from 1.13 kg of green apple pulp was only 10.1 g. The original CVP medium (5) had to be modified for these new polypectates. Sodium hydroxide was deleted from all laboratory polypectates and the volume reduced from 9 ml to 5 ml/L of 1M NaOH for Raltech pectate. With all pectates, gel consistency declined as pH increased, and gels eventually became sloppy and unusable. The laboratory polypectates initially formed unacceptable gels in CVP medium because pH rose during autoclaving. The addition of 3-(*N*-morpholino) propane sulfonic acid (MOPS, Calbiochem) at 4 g/L buffered the gels at pH 7.2 and prevented pH changes during autoclaving.

MOPS substantially improved the consistency of our media (Table 1) and had no noticeable effect on the selectivity of the medium. Growth of *Erwinia* spp. and cavity formation were similar on Raltech and laboratory pectate gels. *Pseudomonas* and *Xanthomonas* spp. grew very poorly on all media.

MOPS is a zwitterionic buffer with a pKa of 7.2 at 20 C. It could be acting as a cross-linking agent for the pectate polymers (14).

Two batches of sodium polypectate (97C-3898 and 117C-3878) were purchased from Sigma Chemical Co. Both batches gave completely unacceptable gels in CVP medium even when we used concentrations of 30–50 g/L. Varying amounts of NaOH and MOPS gave stable neutral pHs, but no concentration produced a satisfactorily gelled medium. Other modifications of the CVP medium with Sigma polypectate were tried with other buffer salts, microcrystalline and fibrous cellulose, varying agar levels, and trisodium phosphate. None of these approaches gave any promise of improving gel strength.

Because of the diverse nature of natural pectins and variety of chemical approaches possible for preparing a sodium polypectate, this compound cannot be considered a definite chemical species. Sodium polypectates prepared in different ways will naturally differ in their gel forming abilities. Cuppels and Kelman (5), who originally proposed CVP medium, used a sodium polypectate from Sunkist Growers Inc. (Ontario, CA), prepared following Wilson's original patent (19). Sodium polypectates prepared this way contain appreciable levels of phosphate (Table 1). Sigma preparations contain no phosphate, however, and have presumably been prepared in some other manner. Their poor gel forming characteristics may relate to absence of phosphate, as these ions may be involved in cross-linking pectin molecules.

Sodium polypectate can be produced by our method in less than 4 days or in 2 days if dry pectinated pulp is produced in advance. Much of the residual carotenoid pigment from the skins is extracted during the acetone precipitations, and this procedure is an advantage over ethanol used in the original patent. Because of their higher pectin content, grapefruit and lemons may yield considerably more sodium polypectate than orange peel yields. The double precipitation method from dry pulp is now used in our laboratory and will be useful in others if sodium polypectate becomes unprocurable.

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