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SOLUBILIZATION OF GELLAN GELS BY CHELATION OF CATIONS

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SUMMARY

Chelation solubilization of gellan under mild conditions has been accomplished for the first time by exposure to either 10 mM sodium citrate buffer (pH 6.0) or to 1 mM sodium hexametaphosphate (pH 6.6). The citrate system was preferred for most applications since its is a ubiquitous cellular component, its solutions are autoclavable, and because viable plant tissues, fungi, and bacteria could be recovered from culture. Such recovery is not possible from more commonly used media such as agar.

INTRODUCTION

Gellan gum is an industrial polysaccharide produced extracellularly by *Pseudomonas elodea.* In the presence of divalent cations, gellan forms clear and firm gels. Gellan gels provide an excellent matrix for nutrient media for plant tissue culture research and this is the area of their greatest application. The polysaccharide has a tetrasaccharide repeating unit consisting of two β-D-glucose, one β-D-glucuronic acid and one α-L-rhamnose residues (Jansson et al., 1983). Gellan was recently approved for some food applications by the U.S. Food and Drug Administration. In connection with the approval process, a method was developed to quantify gellan in foods (Baird and Smith, 1989). The initial step was extraction of gellan with a dilute solution of the sequestrant sodium hexametaphosphate at 90°C. We required a procedure to isolate viable plant roots and fungal spores from mycorrhizal culture in gellan for further study, and to isolate root exudates for characterization. To this end, efforts were directed to developing mild conditions for chelating divalent cations from the gellan matrix, thereby reversing the gelation process and rendering gellan soluble.

MATERIALS AND METHODS

Preparation of gels: Dissolution rate comparisons were conducted on 0.40, 0.75, and 1.00% gels prepared from gellan gum (Gel-Gro, ICN Biochemicals, Cleveland, OH) with counterions Mg^{2+}, Ca^{2+} or those (Na^{+}, K^{+}, Ca^{2+}, Mg^{2+},Mn^{2+}, Zn^{2+}, Cu^{2+}) in a plant root culture nutrient medium (Bécard and

Fortin, 1988). For example, 0.40% gellan gels with Mg^{2+} were prepared by sprinkling 1.60 g gellan into 400 mL of a stirred, boiling solution of $MgSO_4$ (0.025% w/v) over one min., removing the heat source, and continuing stirring until the temperature dropped to about 60°C, when aliquots of the solution were poured into appropriate vessels for gelation. For most experiments 30 mL slabs of gel were prepared in Petri dishes (80 x 10 mm). Gels were stained by addition of 25 ppm methylene blue before gelation so their dissolution could be easily visualized. Gels of higher strength were prepared by proportionally increasing gellan and cation concentrations. The gellan repeating unit (Mol Wt 646)) contains one glucuronic acid (carboxylic acid residue), and the standard gels all possessed a molar ratio of carboxyl to divalent cation of 3.00.

Gellan dissolution experiments: Disks of 14 mm diameter were removed from 30 mL gellan slabs with cork borers. They were 6 mm deep and weighed 0.63 g. In experiments to optimize conditions for gellan solubilization, individual disks were vortexed in a 12-tube vortex evaporator (Buchler, Fort Lee, NJ) in 10 volumes (6.3 mL) of the sequestrants sodium citrate, sodium hexametaphosphate (FMC, Philadelphia, PA) or EDTA, in 50 mL polycarbonate tubes. Sequestrant solution concentrations from 1 to 20 mM were tested at several pH values. Times of dissolution were monitored visually.

Root and microbial culture: Carrot roots, transformed by the T-DNA of the Ri plasmid of *Agrobacterium rhizogenes*, were cultured in gellan (0.4%) prepared with M medium (Bécard and Fortin, 1988). After one week of growth, root cultures were axenically solubilized in 15 min. at 30°C in 5 volumes of sterile-filtered (0.22µm) sodium citrate (10 mM, pH 6.0) or in sodium hexametaphosphate (1 mM). Then, the roots were transferred onto fresh M medium and after four weeks of culture, the growth (dry weight) of transferred and non-transferred (control) roots were compared to the weight of roots before the solubilization treatments. Data reported was based upon five root explants per treatment. *Pseudomonas syringae* and *Nectria haematococca* (imperfect stage *Fusarium solani*) and *Rhizopus delemar* were cultured in 0.4% gellan prepared with Tryptone Bacto Yeast Extract Broth (Difco Laboratories, Detroit, MI). The gellan media for the microbial cultures were solubilized as described above for the root cultures. Microorganisms were then subcultured onto fresh medium to further assess their viability, and their regrowth was monitored visually.

RESULTS AND DISCUSSION

Citrate was first tested for chelation solubilization of gellan because it is widely distributed in nature and recovery of viable tissues might be more likely from it than from synthetic alternatives. Sodium citrate buffer effectively solubilized gellan gels, whether they had been prepared with Mg^{2+} Ca^{2+}, or mixtures of counterions such as are present in plant root culture nutrient media. Figure 1 shows that both 0.4% and 0.75% gellan gels (Mg^{2+} counterion) are most rapidly solubilized near pH 6. Anionic polysaccharides other than gellan, including alginic and pectic acids, and *iota*-carrageenan are also known to gel upon addition of divalent cations such as Mg^{2+} and Ca^{2+} (Glicksman, 1983). Sequestration of Ca^{2+} from alginate gels was shown to be most effective at pH 6, which is consistent with our results. The optimum citrate concentration for solubilization is about 10 mM for gels of both strength (Figure 2), except that 6 mM is optimal for 0.75% gels at 25°C. Times of dissolution were similar regardless of the type of counterion mixture or the cation/gellan ratio.

The other chelators tested were EDTA and sodium hexametaphosphate. EDTA was much less effective in dissolving gellan gels than was sodium citrate, but at ambient temperature, sodium hexametaphosphate dissolved the gels in about one-half the time. Relative rates of dissolution of firm 1.0% gellan gels (Mg^{2+} counterion) in sodium citrate buffer (10 mM, pH 6.0) and sodium hexametaphosphate (1 mM had been established as optimal concentration) are shown in Figure 3. At

Figure 1. Effect of pH of sodium citrate (10 mM) on time to dissolution of gellan at 25°C.

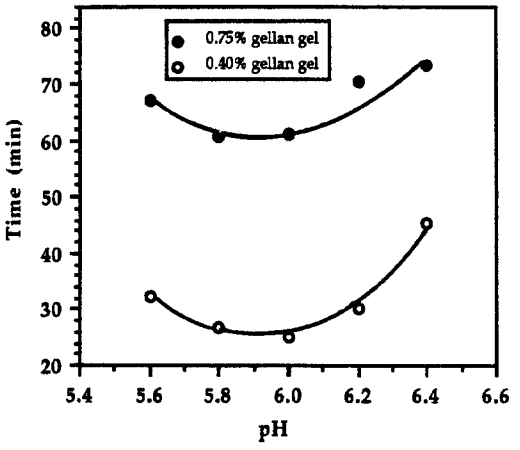


Figure 2. Effect of sodium citrate buffer concentration and temperature on time to dissolution of gellan.

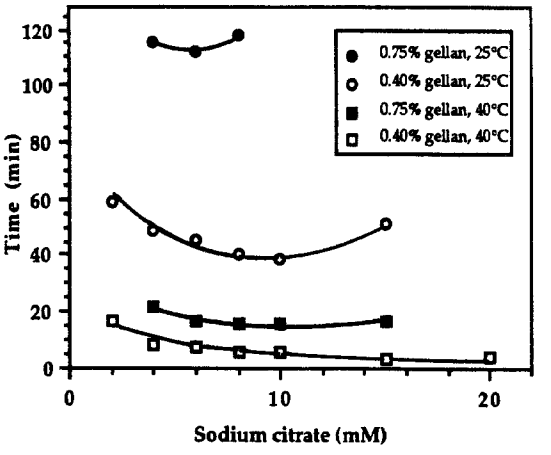


Figure 3. Decrease in mass of 1.0% gellan gel with time at 25°C.

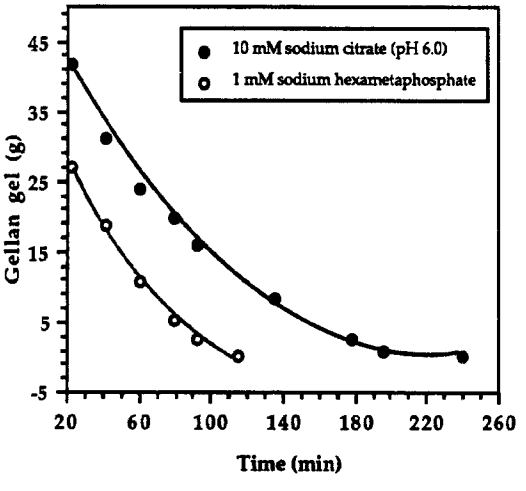
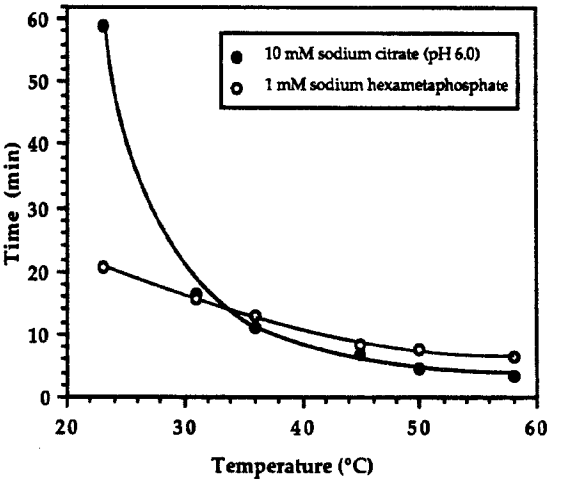


Figure 4. Effect of temperature on time to dissolution of 0.75% gellan.



ambient temperature sodium hexametaphosphate was found to solubilize gellan more rapidly than citrate regardless of gel strength or the type of counterion mixture. A solvent/gel ratio of 10:1 (v/w) was used in the experiment represented in Figure 3. We have found that the rate of dissolution of gellan increases with this ratio, even though at 2:1 there is already a 10-fold molar excess of citrate over Mg^{2+}.

The effect of temperature on gellan solubilization is quite pronounced. The time for dissolution in citrate relative to the time in sodium hexametaphosphate rapidly decreases with temperature, and from about 32°C, the dissolution time is less in citrate than in sodium hexametaphosphate (Figure 4). At 75°C, a 5.0 gm slab of 1.0% gellan (Mg^{++} counterion) in 5 volumes of citrate (10 mM, pH 6.0) dissolved in 9.2 min. whereas 47 min. was required in sodium hexametaphosphate (1 mM). The pH of a 1 mM solution of sodium hexametaphosphate drops from 6.6 at 25°C to less than 6.0 at 15 min. in an autoclave at 121°C, suggesting that is hydrolyzed to smaller fragments, which possibly have less capacity to sequester cations.

P. syringae, N. haematococca, R. delemar, and transformed carrot roots were all still alive after isolation from culture in gellan by chelation solubilization. The microorganisms demonstrated their viability by growing rapidly in fresh medium, even after 1 h exposure to the solubilization medium. The roots in cultures solubilized by citrate treatment after one week of growth and recultured in gellan increased in weight from 43 mg to 190 mg, while those that had not been recultured (control) increased to 223 mg. Those which had been solubilized by sodium hexametaphosphate increased to just 120 mg. The slightly lower rate of growth after solubilization with citrate when compared to the control may be simply due to the root transfer process itself. Sodium hexametaphosphate however, seemed to have a slight toxic effect, leading to the 46% drop in growth.

CONCLUSIONS

Gellan gels, regardless of strength or type of counterion, are most effectively solubilized by cation chelation with sodium citrate (10 mM, pH 6.0) at temperatures above 30°C. The time required for solubilization in sodium citrate decreased more rapidly with temperature than was the case when sodium hexametaphosphate (1 mM) was used. In addition, viable bacteria, fungi, and roots could be isolated from gellan and recultured with continued viability. The availability of this procedure will allow studies on tissues recovered from culture that were not previously possible.

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