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Research Note

Gelation of the Extracellular Polysaccharide Produced by *Agrobacterium rhizogenes*

ABSTRACT

It has been shown that the extracellular polysaccharide (EPS) produced by Agrobacterium rhizogenes *will form thermoreversible gels. This EPS belongs to a family of polysaccharide structures all of which have the same backbone structure substituted with different side chains. The EPS produced by* Rhizobium meliloti *IFO 13336 also belongs to this family of structures and T. Harada (*Biochem. Soc. Symp., **48** (1983) 97*) has reported gelation of this polysaccharide. Thus it is possible that gelation is a common feature of this family of structures. Possible biological and ecological consequences of gelation are discussed.*

INTRODUCTION

Biovars of the soil bacterium *Rhizobium leguminosarum* nodulate and fix nitrogen in specific host varieties of legumes. The anionic extracellular polysaccharides (EPS) produced by these bacteria form a family of structures based on the same backbone structure substituted with different side chains (Morris *et al.*, 1989, 1990). Under appropriate conditions members of this family form transparent thermoreversible gels and gelation has been proposed as a non-specific attachment mechanism for the initial adhesion of individual bacterial cells to plant root tips (Morris *et al.*, 1989). EPS gelation may also be important in the binding of *Rhizobium* to soil particles and hence influence soil aggregate stability, soil structure and water retention (Morris *et al.*, 1989, 1990).

Figure 1 shows a second family of related EPS structures produced by the soil bacterial species *Rhizobium* and *Agrobacterium*. This article reports preliminary rheological studies on a member of this family of EPS structures: the EPS produced by *Agrobacterium rhizogenes*. *A. rhizogenes* was grown in Y medium which is related to the glutamic acid–mannitol–salts medium used by Zevenhuizen (1986), but contains higher concentrations of salts. The medium contained (per litre):

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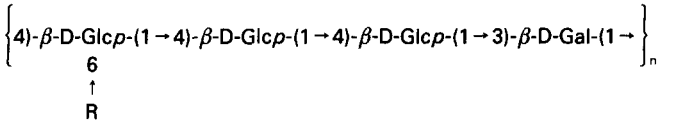
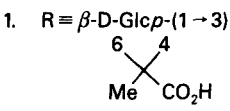
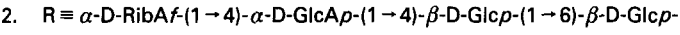
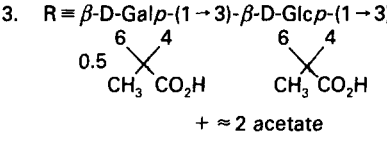


1. 

R. meliloti[a]
A. rhizogenes[a]
A. radiobacter[a]
A. tumefacians[a]
Alcaligenes faecalis var. *myxogenes* 10C3[a]

2. 

R. meliloti IFO13336[b]

3. 

R. leguminosarum bv. *trifolii* AHU1134[c]
R. leguminosarum bv. *phaseoli* AHU1133[c]
R. leguminosarum bv. *lupini* KLU[c]

Fig. 1. Structures of the extracellular acidic heteropolysaccharides produced by certain *R. leguminosarum*, *Agrobacterium* and *Alcaligenes* species. The polymers are substituted with succinyl esters and are known collectively as succinoglycans. The structures are as reported by [a]Harada *et al.* (1979); Hisamatsu *et al.* (1980), [b]Amemura *et al.* (1981), [c]Amemura and Harada (1983).

mannitol (10 g), K_2HPO_4 (0·22 g), $CaCl_2.6H_2O$ (0·22 g), glutamic acid (1·1 g), $MgSO_4.7H_2O$ (0·1 g), $FeCl_3.6H_2O$ (0·02 g) and biotin, thiamin and pantothenic acid (750 μg). The pH of the medium was adjusted to 8. Bacteria were grown at 29°C in 750 ml of medium contained in 2 litre flasks, shaken at 150 rpm. Cultures were harvested in the late phase of exponential growth (~ 72 h). Bacterial cells were removed by centrifugation (23×10^3 *g*, 30 min), decanting the supernatant and filtering it through two layers of glass fibre paper (Whatman GF/B), then sequentially through Millipore filters of decreasing pore size (1·2, 0·8 and 0·65 μm). The solution was concentrated (× 3) and the EPS precipitated with industrial alcohol. The precipitate was washed with industrial alcohol, redissolved in water and freeze dried. Gels were prepared by dissolving the EPS in hot water (90°C), pouring the solution into plastic moulds and allowing the samples to cool to room temperature (25°C).

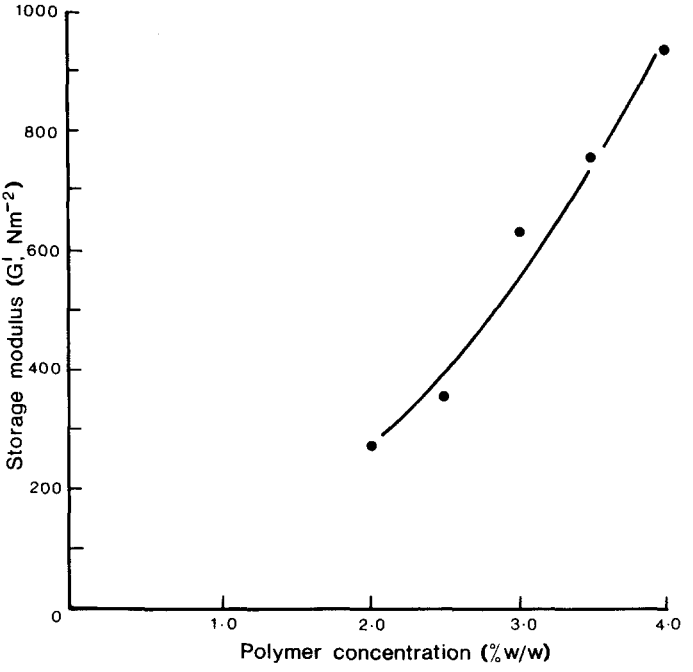


Fig. 2. Rheological data for *A. rhizogenes* EPS gels. Dependence of the storage modules (G′ — measured at 1 Hz, peak strain 0·05) on polymer concentration.

Rheological measurements were made on gels which had been aged for ~16 h. Gels were measured using a parallel plate configuration on an Instron 3250 rotary rheometer. Table 1 shows the frequency dependence of the storage modulus (G′) and the loss modulus (G″) for a 4% (w/w) EPS gel measured at a strain of 0·05. The predominance of G′ (G′ > G″ and thus the low phase angle) and the flat frequency response are characteristic behaviour of a strong gel network. Figure 2 shows the dependence of G′ (measured at 1 Hz, 0·05 strain) on polymer concentration. Further studies are needed in order to determine the role of salt composition of the medium and succinyl content of the polymer upon gelation.

The EPS structure produced by *R. meliloti* IFO 13336 also belongs to this family of structures (Fig. 1). Harada (1983) has reported gelation of this polysaccharide. The work of Harada (1983) together with the present studies suggest that gelation may be a common feature of this family of polysaccharides. Thus these EPS may fulfil a similar role to that suggested (Morris *et al.*, 1989, 1990) for the EPS family produced by *R. leguminosarum*. Gelation of the EPS structures shown in Fig. 1 may provide a non-specific attachment mechanism for *Rhizobium* and *Agro-*

TABLE 1
Rheological Data on an *A. rhizogenes* EPS Gel[a]

Frequency (Hz)	*Storage modulus* (Nm^{-2})	*Loss modulus* (Nm^{-2})	*Phase angle* ϕ *(deg)*
0·10	884	157	10·1
0·14	908	157	9·8
0·19	934	155	9·4
0·27	960	152	9·0
0·37	986	149	8·6
0·52	1013	150	8·4
0·72	1040	146	8·0
1·00	1069	145	7·7
1·39	1095	144	7·5
1·93	1124	142	7·2
2·68	1155	142	7·0
3·72	1183	141	6·8
5·18	1219	138	6·5
7·20	1260	139	6·3
10·00	1318	139	6·0

[a]Polymer concentration 4% (w/w), peak strain 0·05, temperature 25°C.

bacterium cells in the early stages of root nodulation and nitrogen fixation or the formation of crown gall tumours. In addition, gelation of the EPS may aid soil particle aggregation and thus soil stability and water retention. There is independent evidence for the binding of soil bacteria to clay particles (Santoro & Stotzky, 1968; Lynch & Bragg, 1985). Furthermore, Fehrmann and Weaver (1978) obtained electron microscope pictures illustrating the role of EPS in the binding of *R. meliloti* to silt particles. The EPS produced by common strains of *R. meliloti* has the same structure as the EPS produced by *A. rhizogenes* (Harada *et al.*, 1979; Hisamatsu *et al.*, 1980).

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