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Gelation of Members of a Family of Branched Anionic Heteropolysaccharides Produced by Certain Strains of *Rhizobium leguminosarum*

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ABSTRACT

The structure of the anionic extracellular slime produced by Rhizobium leguminosarum *biovar* phaseoli *8002 has been shown to be unaltered by variation of the* Sym *plasmid. The polysaccharide is a member of a family of branched-anionic polysaccharides. Rheological studies have demonstrated gelation of members of this family of polysaccharides produced by certain* R. leguminosarum *strains. Gelation was found to be dependent upon the ionic strength and salt composition of the sample. Aqueous preparations of the purified polysaccharides did not gel. Thermoversible gels were formed upon addition of salts to the aqueous preparations. Divalent cations were found to be more effective than monovalent cations in promoting gelation. Possible consequences of the gelation of the extracellular polysaccharides, relating to non-specific adhesion of the bacteria to plant roots, water retention in soils, and soil aggregation and stability, are discussed.*

INTRODUCTION

Bacteria provide a source of extracellular polysaccharides (EPS) with potentially useful rheological properties. Only a few gel-forming bacterial EPS have been discovered. Neutral gel-forming EPS include the linear polymer curdlan (Harada, 1979; Harada & Amemura, 1981) produced by *Alcaligenes faecalis* var. *myxogenes* or certain *Agrobacterium radiobacter* strains, and the branched capsular polysaccharide (Zevenhuizen & van Neerven, 1983) produced by certain *Rhizobium*

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leguminosarum biovars (bv). Anionic gelling EPS include the linear polysaccharide gellan gum (Moorhouse *et al.*, 1981; Jansson *et al.*, 1983; O'Neill *et al.*, 1983; Morris *et al.*, 1984; Moorhouse, 1987) produced by *Pseudomonas elodea* (recently renamed *Auromenas elodea*), and XM6 (Nisbet *et al.*, 1984), a branched polysaccharide produced by an *Enterobacter* NCIB 11870. The capsular polysaccharide produced by *Klebsiella aerogenes* serotype K54 has an identical carbohydrate structure to XM6, but contains single *O*-acetyl substituents on the fucosyl residues of alternate tetrasaccharide repeat units, which inhibit gelation of the polysaccharide (O'Neill *et al.*, 1985; Colquhoun *et al.*, 1989). Alkaline de-esterification of the K54 EPS yields a gelling polysaccharide (Morris & Miles, 1986; Atkins *et al.*, 1987). The final example is a mixture of EPS secreted by *Bacillus polymyxa* (Madden *et al.*, 1986). This mixture can be separated into three fractions: a minor neutral polysacchride, a minor acidic polysaccharide and a major acidic polysaccharide. The major acidic polysaccharide, containing glucuronic acid, glucose, mannose and galactose is believed to be responsible for gelation (Madden *et al.*, 1986).

This article reports studies on the cation-induced gelation of members of a family of branched anionic bacterial heteropolysaccharides secreted as slimes by certain *Rhizobium leguminosarum* strains. A preliminary account of this work has been reported elsewhere (Morris *et al.*, 1989). Possible biological and ecological implications of the gelation process are discussed.

MICROBIOLOGY

The strains of *R. leguminosarum* used in the present studies are listed in Table 1. The reported chemical structures of the EPS produced by the conventional strains of these bacteria are indicated in Table 1 and illustrated in Fig. 1. The chemical structure of the EPS of the modified strain *R. leguminosarum* bv *viciae* 8401 pRL1JI was evaluated in the present study. All bacteria used were grown in Y medium containing (grammes per litre) mannitol (10); K_2HPO_4 (0·22); $CaCl_2.6H_2O$ (0·22); glutamic acid (1·1); $Mg_2SO_4.7H_2O$ (0·1); $FeCl_3.6H_2O$ (0·02); and biotin, thiamine and pantothenic acid each at 750 μg litre^{-1}. The final pH was adjusted to 6·8 with NaOH.

Bacteria were grown in 750 ml of medium in 2 litre flasks, shaken at 150 rpm at 29°C. The cultures were harvested in late exponential phase (usually ~ 72 h). Some bacterial cells were removed by centrifuging the

TABLE 1
Bacterial Strains

Bacterium	*Biovar*	*Strain*	*Structure*[a]
R. leguminosarum	*phaseoli*	8002	1
R. leguminosarum	*phaseoli*	RW4pIJ1427	1
R. leguminosarum	—	8401	1
R. leguminosarum	*viciae*	8401pRL1JI	1
R. leguminosarum	*viciae*	8401pRL1JIpss-1::Tn5pIJ1427	1
R. leguminosarum	*phaseoli*	LPR5	1
R. leguminosarum	*phaseoli*	LRP49	1
R. leguminosarum	*trifoli*	TA-1	1
R. leguminosarum	*phaseoli*	127K87	4

[a]Structures shown in Fig. 1.

culture broths at $23 \times 10^3 g$ for 30 min. The supernatant was then filtered at ambient temperature through two layers of glass fibre paper (Whatman GF/B) and then sequentially through Millipore filters of decreasing pore size (1·2 μm, 0·8 mm and 0·65 μm) to remove any remaining cells. Samples were concentrated ($\times 3$) by rotary evaporation, and the EPS precipitated with 4–5 volumes of industrial alcohol. The precipitate was collected, washed several times with industrial alcohol, redissolved in distilled water and then freeze dried. This sample was called *crude EPS*.

Purified EPS (*pure EPS*) was prepared from the crude extract by selective precipitation of the anionic EPS with cetyl trimethyl ammonium bromide (CTAB) using the method described by Robertsen *et al.* (1981) and Scott (1965). A 500 ml aqueous sample (0·2%, w/w) of crude EPS was prepared and sodium sulphate added to a final concentration of 10 mM. EPS was precipitated with 50 ml of a 3% (w/w) CTAB solution (~ 2 h, 37°C). After filtration the precipitate was washed first with distilled water (3×600 ml), redissolved in 400 ml of 10% (w/w) NaCl, precipitated with acetone, redissolved in 400 ml of 10% (w/w) NaCl, dialysed against 1% (w/w) NaCl and then distilled water, and finally freeze dried.

CARBOHYDRATE CHEMISTRY

Neutral sugars were released by a Saeman hydrolysis and analysed as their alditol acetates by gas chromatography (Selvendran & Dupont, 1984). Uronic acid was determined colorimetrically by a modification (Selvendran & Dupont, 1984) of the method described by Blumenkrantz

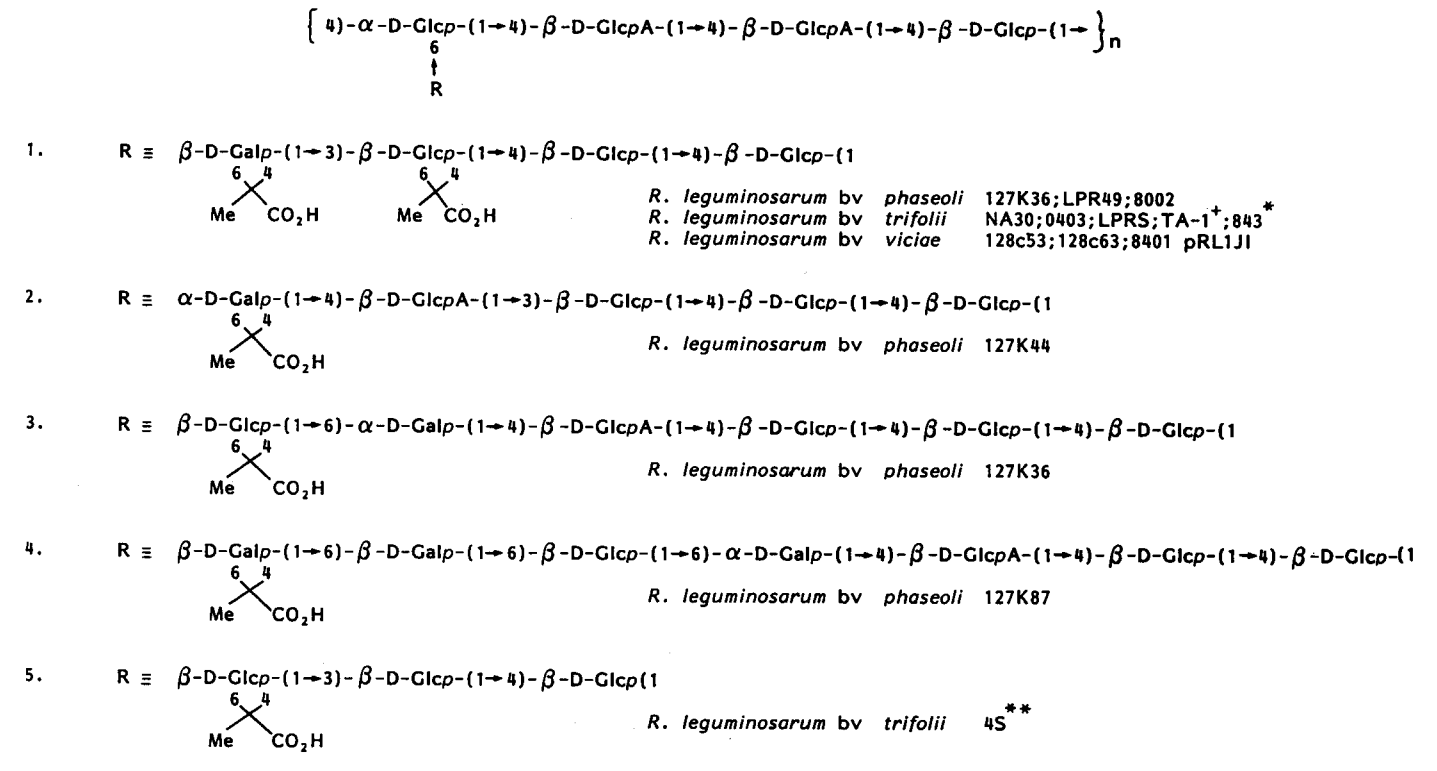

Fig. 1. Structures of the extracellular acidic polysaccharides produced by certain *R. leguminosarum* species. The structures are as reported by McNeill *et al.* (1986) except for *Hollingsworth *et al.* (1988) and Kuo and Mort (1986) and ** Amemura *et al.* (1983).

and Asboe-Hansen (1973). The HPLC method of Cheetham and Punruckrong (1985) was used to determine pyruvate and acetate substitution. Inter-sugar linkages were determined by methylation analysis using a modification of the Hakomori method (Ring & Selvendran, 1978). Individual, partially methylated, alditol acetates were identified by their retention times relative to a standard 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl glucitol, and from their mass spectrometric fragmentation patterns (Jansson *et al.*, 1976). Quantitative analysis was achieved using the molar response factors given by Sweet *et al.* (1975).

RHEOLOGY AND PHYSICAL CHEMISTRY

Rheological studies were carried out using an Instron 3250 mechanical spectrometer (Instron, High Wycombe, UK). Gels were investigated using a flat plate configuration and liquids studied using a cone and plate arrangement (cone angle 2·4°). Optical rotation data were recorded at a wavelength of 405 nm in a 10 cm cell using a Jasco Dip 360 optical polarimeter (Jasco, Japan Spectroscopic Co., Tokyo, Japan). Circular dichroism spectra were measured using a Jasco J-600 spectrometer (Jasco, Japan Spectroscopic Co., Tokyo, Japan). Gel 'melting temperatures' were measured in the following way: gels were prepared in sealed glass containers, immersed in a water bath and heated. At regular temperature intervals the glass containers were inverted. The 'melting-point' was taken to be the temperature at which the sample first flowed upon inversion.

RESULTS

R. leguminosarum bv *viciae* 8401pRL1JI is nearly isogenic with the wild type *R. leguminosarum* bv *phaseoli* 8002 and was derived from it by removal of the *'phaseoli' Sym* plasmid pRP2JI (Lamb *et al.*, 1982) and subsequent insertion of the *'viciae' Sym* plasmid pRL1JI by conjugation (Downie *et al.*, 1983; Borthakur, 1986).

Initial experiments were carried out using the crude EPS from *R. leguminosarum* bv *viciae* 8401pRL1JI. These experiments were designed to test whether alteration of the *Sym* plasmid, led to changes in the amount or structure of the EPS produced by the bacteria.

The parent strain *R. leguminosarum* bv *phaseoli* 8002 should produce three types of extracellular polysaccharide: these consist of low molecular weight cyclosophorans, an NaOH soluble tightly bound branched

neutral capsular polysaccharide (CPS), and a water soluble anionic branched hetero-polysaccharide (EPS) (Zevenhuizen, 1984, 1986). In view of the growth conditions, and the method of extraction, the crude EPS should consist solely of the anionic extracellular slime. Comparative studies showed that the yield of crude EPS was the same for the parent strain and the derived strains. Rheological measurements on the crude EPS were used as an approximate qualitative method for assessing changes in molecular weight distribution or changes in EPS chemical structure. Data obtained for the EPS of the modified strain and the parent strain are shown in Figs 2 and 3. Crude EPS was dispersed in water at high temperatures (90°C). At concentrations above 2% (w/w) the aqueous samples formed thermo-reversible gels upon cooling to room temperature. Figure 2 shows typical data obtained for a 3% (w/w) aqueous crude EPS sample. Gelation is indicated by the frequency independent values of the storage (G') and loss (G'') moduli and the predominance of the storage modulus ($G' \gg G''$). Figure 3 shows data on the concentration dependence of the storage modulus for the modified strain and the parent strain which demonstrate similar properties for the modified and the parent strain. However, the unusual result was the observation that the crude EPS fraction gelled. Review articles dealing with the properties of the polysaccharides produced by *R. leguminosarum* (Zevenhuizen, 1984) normally emphasise the gel-forming ability of the CPS and the water solubility of the EPS. A possible explanation

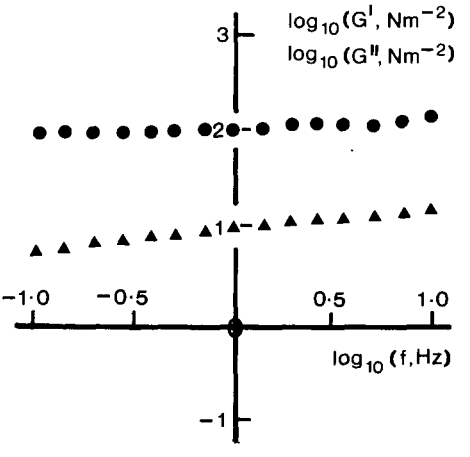

Fig. 2. Rheological data measured for a *R. leguminosarum* bv *viciae* 8401 pRL1JI pss-1::Tn5 pIJ1427 crude EPS gel. Polymer concentration 3% (w/w). Data were obtained using a flat-plate configuration at a peak strain of 0·05. ●, Storage modulus (G'); △, loss modulus (G''), f is the frequency of oscillation; temperature = 25°C.

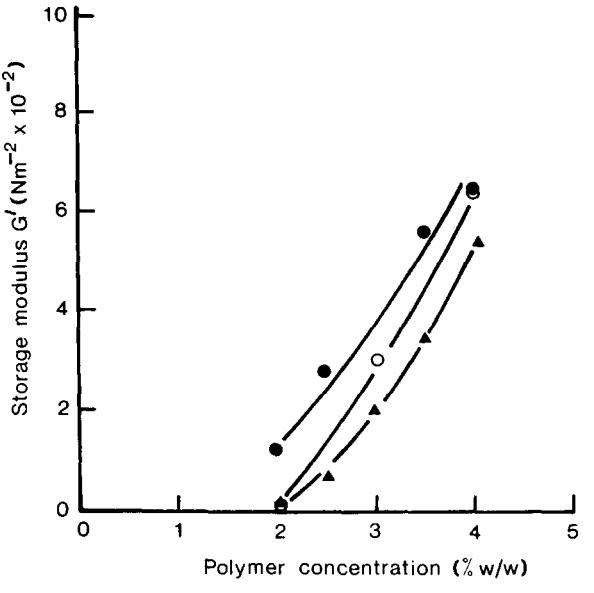

Fig. 3. Dependence of storage modulus (G'), measured at 0·2 Hz, peak strain 0·05, plotted as a function of polymer concentration for crude EPS gels: ●, *R. leguminosarum* bv *phaseoli* RW4 pIJ1427; ○, *R. leguminosarum* bv *viciae* 8401 pRL1JI pss-1::Tn5 pIJ1427; △, the parent strain *R. leguminosarum* bv *phaseoli* 8002. Samples measured using a flat plate configuration; temperature = 25°C.

for the gelation of the crude EPS might be due to its contamination with small amounts of CPS. Accordingly, the crude EPS was fractionated into neutral and acidic fractions by CTAB precipitation and the compositions of the neutral and acidic (pure EPS) fractions were determined. Structure 1 (Fig. 1) shows the expected structure for the EPS and Figs 4(a) and 4(b) show the expected structures for the CPS and cyclosophorans.

Results of the sugar analysis, methylation analysis and measurements of the uronic acid content of the pure EPS for the modified strain and the parent *R. leguminosarum* bv *phaseoli* 8002 were found to be consistent with structure 1 (Fig. 1) first reported by Robertsen *et al.* (1981). Pyruvate determinations corresponded to two substituents per repeat unit (~11% (w/w)). Sugar analysis of the neutral fraction revealed the presence of glucose but no detectable mannose or galactose. This demonstrates the absence of CPS (Fig. 4(a)) as a contaminant in the crude EPS. The total yield of neutral polysaccharide was also very low: 11·5 g of crude EPS yielding ~7·5 g of pure EPS and ~0·4 g of neutral polysaccharide. Methylation analysis of the neutral fraction revealed (1→2) Glc consistent with the presence of a small quantity of cyclosophoran (Fig. 4(b)).

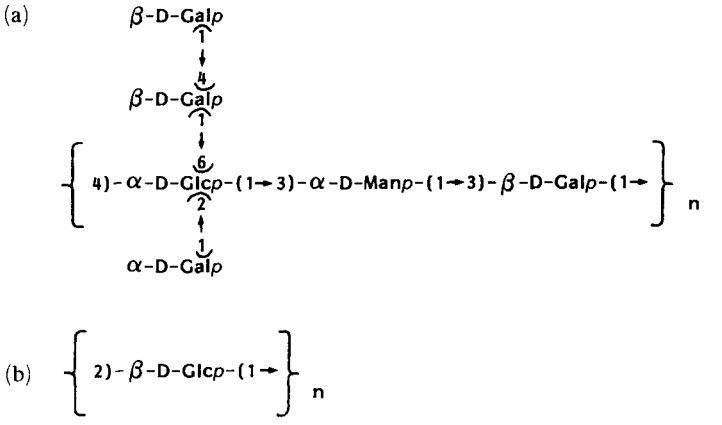

Fig. 4. Chemical structures of the capsular polysaccharide (a) and the cyclosophorans (b) produced by certain *R. leguminosarum* species.

Unlike the crude EPS, the purified EPS did not gel. This is consistent with the results of other workers (Zevenhuizen, 1984). Furthermore, mixing the neutral and acidic fractions did not restore gelation, and the neutral fraction alone did not gel. Both observations are consistent with the absence of CPS. Thus, these data suggested that the component responsible for gelation had been removed during the dialysis step in the purification and fractionation process. Microprobe analysis of the crude EPS and the pure EPS powders, using a Phillips PSEM 501B scanning electron microscope (Phillips, Cambridge, UK) linked to a LINK 860 series 2 EDS system (Link systems, High Wycombe, UK), revealed the presence of inorganic salts in the crude EPS sample, with a major component being $CaCl_2$ which was present in the 'microbial' growth medium. The possibility that the presence of this excess salt could cause gelation was explored. An aqueous crude EPS sample (0·2% w/w) was deionised and reexamined: the aqueous crude EPS sample was dialysed against water to remove excess salt, ion-exchanged into the acidic form, (Dowex 50W-X8 resin), in order to remove any bound cations, converted into the sodium salt form by neutralisation with 0·1 M NaOH, and finally freeze dried. The removal of excess salt was checked by microprobe analysis on the freeze dried powder. Following the removal of the salt from the crude EPS, it was found that the polysaccharide sample had lost its ability to form a gel (Fig. 5(a)). Addition of $CaCl_2$ to the deionised crude EPS restored the ability to form thermoreversible gels (Fig. 5(b)). Salts of other Group IIA divalent cations led to gelation (Table 2) and the addition of salts of monovalent (Group IA) cations at a similar ionic strength also induced gelation. Figure 5(c) and Table 2 show typical data

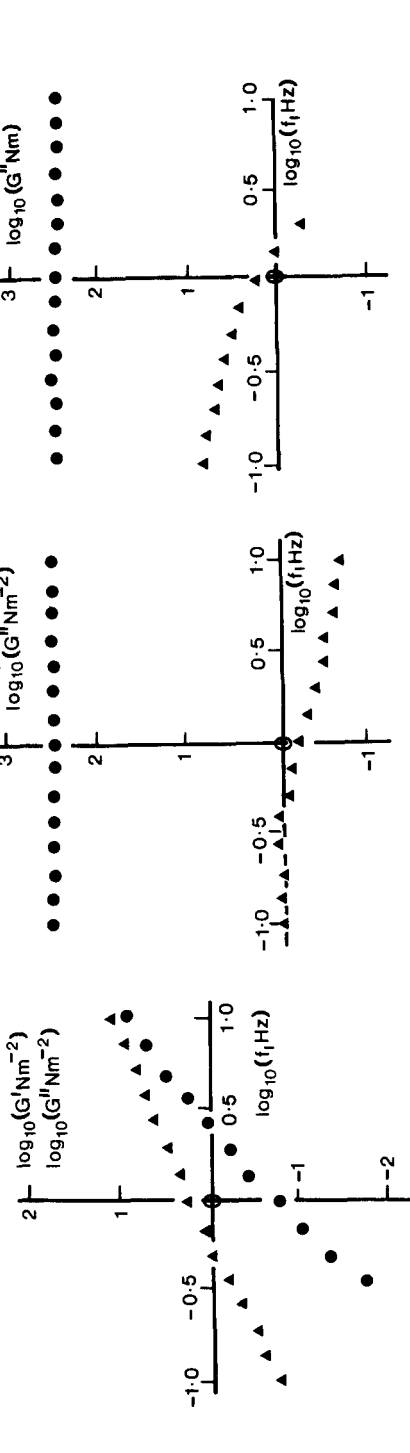

Fig. 5. Rheological data obtained for *R. leguminosarum* bv *viciae* 8401 pR1JI deionised crude EPS (a) 3% (w/w) deionised crude EPS aqueous sample, (b) 3% (w/w) deionised crude EPS gel containing 0·4 M $CaCl_2$, and (c) 3% (w/w) deionised crude EPS gel containing 1·6 M NaCl. Gels were studied using a flat plate configuration — peak strain 0·05. Liquids were measured with a cone and plate arrangement (cone angle 2·4°). ●, Storage modulus (G'); △, loss modulus; (G''); f is the frequency of oscillation; temperature = 25°C.

TABLE 2
Ionic Effects Upon Gelation[a]

Salt	*Molarity (M)*	*G' (Nm^{-2})*	*G'' (Nm^{-2})*	*ϕ (degrees)*
$MgCl_2$	0·4	459	3	3·5
$CaCl_2$	0·4	326	3	0·5
$SrCl_2$	0·4	341	7	1·1
$BaCl_2$	0·4	304	3	0·5
LiCl	1·6	132	19	8·2
NaCl	1·6	314	2	0·3
KCL	1·6	130	16	7·1
CsCl	1·6	226	26	6·5
RbCl	1·6	175	20	6·6
—	—	0·18	2	83

[a]Data measured on 3% (w/w) *R. leguminosarum* bv *viciae* 8401 pRL1JI deionised crude EPS samples with and without added salt. Gels measured using a flat-plate assembly, liquids measured with a cone and plate (2·4° cone angle). Values of G' (storage modulus), G'' (loss modulus) and ϕ (phase angle) measured at a frequency of 1 Hz.

obtained upon addition of NaCl to deionised crude EPS. Addition of trivalent cations such as Fe^{3+} usually caused flocculation but may cause gelation, under the correct conditions of polymer concentration and salt concentration. Measurements of the melting points of the gels showed that increasing the electrolyte concentration raised the melting point of the gel. Figure 6 shows data obtained upon addition of NaCl to purified EPS. The apparent saturation at high ionic strength may reflect an inhomogeneous gel structure at high electrolyte concentrations. At high ionic strength it becomes progressively more difficult to adequately disperse the samples even at elevated temperatures. In future studies it may be preferable to prepare gels by dialysis of aqueous samples of crude deionised EPS against suitable electrolytes.

The structure of the EPS produced by the modified strain and the parent is a member of a family of structures shown in Fig. 1. A number of other *R. leguminosarum* strains also produce the same EPS structure (Table 1, Fig. 1). Growth of these strains on Y medium should also yield gelling crude EPS. Table 3 shows data measured for gels prepared from crude EPS obtained from cultures of *R. leguminosarum* bv *phaseoli* LPR5; *R. leguminosarum* bv *phaseoli* LPR49 and *R. leguminosarum* bv *trifolii* TA-1. Despite the identical structures of the EPS, and the identical growth conditions, there are marked differences in the rheological properties of the curde EPS gels (Figs 2 and 3, Table 3) obtained from

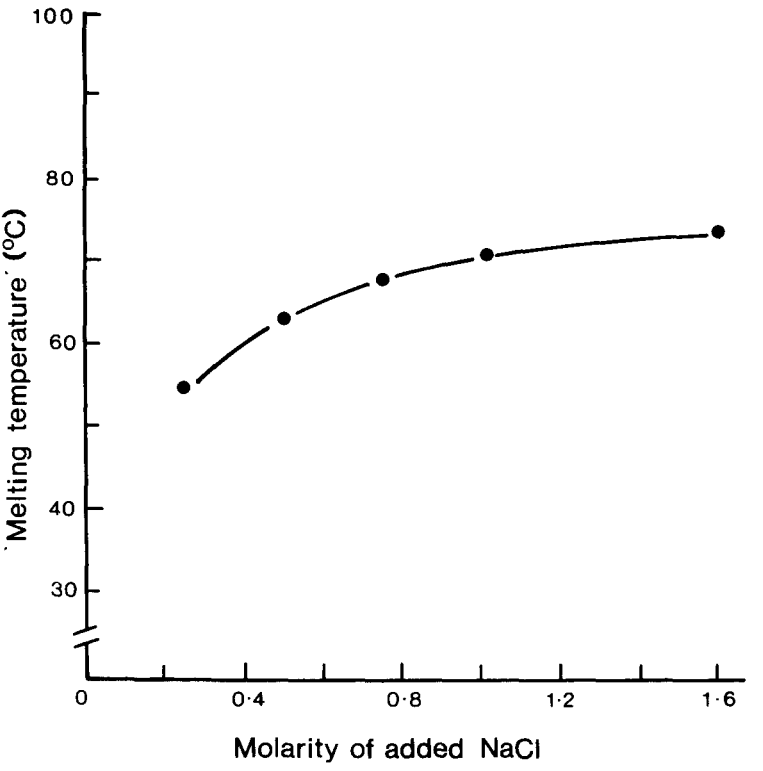

Fig. 6. Effect of added NaCl on the 'melting point' of 3% (w/w) *R. leguminosarum* 8401 pR1JI deionised crude EPS gel. In the absence of NaCl the sample is fluid at room temperature (25°C).

different bacterial sources. These differences probably reflect different EPS yields which in turn result in different EPS/salt ratios in the crude EPS preparations.

In order to examine whether gelation was a common feature of the family of structures shown in Fig. 1 the behaviour of the crude EPS produced by *R. leguminosarum* bv *phaseoli* 127K87 (structure 4 in Fig. 1) was also examined. This EPS structure contains the largest branch and the longest repeat unit within the family of presently known structures. Figure 7 illustrates gelation of this crude EPS sample. These data suggest that gelation is likely to be a common feature of this family of polysaccharides.

Optical rotation or circular dichroism can be used to probe ion binding or molecular conformational changes accompanying the gelation of polysaccharides (Rees *et al.*, 1982). Figure 8 shows the change in optical rotation accompanying the melting of a 3% (w/w) purified EPS gel pre-

TABLE 3
Rheological properties of *R. leguminosarum* EPS

	R. phaseoli LPR5			*R. phaseoli LPR49*			*R. trifolii TA-1*		
Frequency (Hz)	*G' (Nm^{-2})*	*G'' (Nm^{-2})*	*ϕ (deg.)*	*G' (Nm^{-2})*	*G'' (Nm^{-2})*	*ϕ (deg.)*	*G' (Nm^{-2})*	*G'' (Nm^{-2})*	*ϕ (deg.)*
0·10	74	0·78	0·6	70	1·09	0·9	80	1·26	0·9
0·14	74	0·78	0·6	70	1·10	0·9	80	1·40	1·0
0·19	74	0·78	0·6	70	1·35	1·1	81	1·55	1·1
0·27	74	1·04	0·8	71	1·35	1·1	81	1·84	1·3
0·37	75	1·31	1·0	71	1·72	1·4	81	2·13	1·5
0·52	75	1·56	1·2	71	2·23	1·8	82	2·56	1·8
0·72	75	1·97	1·5	71	2·74	2·2	82	2·73	1·9
1·00	76	2·39	1·8	72	3·26	2·6	83	3·33	2·3
1·40	76	3·05	2·3	72	3·78	3·0	83	4·22	2·9
1·93	76	3·20	2·4	73	4·86	3·8	85	4·00	2·7
2·68	78	4·21	3·1	74	5·97	4·6	85	4·77	3·2
3·73	78	4·93	3·6	75	7·39	5·6	86	5·74	3·8
5·18	80	5·45	3·9	77	8·23	6·1	87	6·25	4·1
7·20	81	6·53	4·6	80	10·61	7·6	89	7·29	4·7
10·00	84	7·93	5·4	83	12·5	8·6	91	7·81	4·9

G' — storage modulus, G'' loss modulus, ϕ phase angle, 3% gels, strain 0·05.

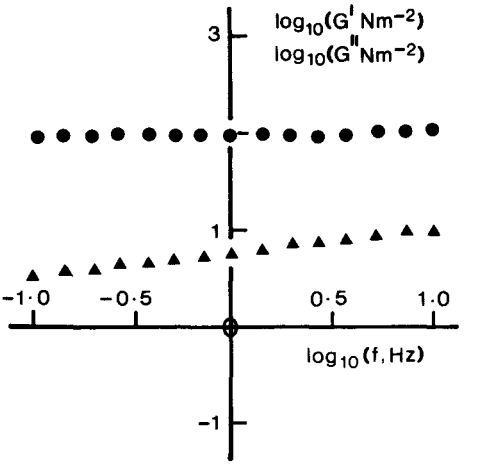

Fig. 7. Rheological data measured for *R. leguminosarum* bv *phaseoli* 127K87 crude EPS gel. Polymer concentration 3% (w/w). Data measured using a flat-plate assembly at a peak strain of 0·05. ●, Storage modulus (G'); △, loss modulus (G''); f is the frequency of oscillation; temperature = 25°C.

pared in the presence of 0·5 M NaCl. The 'melting point' of the gel is indicated by an arrow in Fig. 8. The optical rotation change upon heating involves a change in both magnitude and sign. The net change upon heating is positive. In the absence of added NaCl the 3% (w/w) purified EPS did not gel and the optical rotation varied monotonically with increasing temperature (Fig. 8). In the absence of added salt, and at a polymer concentration of 0·5% (w/w), there was only a monotonic variation of optical rotation upon heating. Raising the salt concentration to 0·5 M NaCl induced a change in optical rotation (Fig. 8) with a similar transition temperature to that observed at 3% (w/w) polymer concentration. Figure 9 shows the change in optical rotation observed at room temperature for a 0·5% (w/w) purified EPS sample upon increasing the concentration of added NaCl. Figure 10 shows the near ultraviolet circular dichroism spectrum of the purified EPS (0·5% (w/w) polymer concentration, 0·5 M NaCl) at room temperature and the changes observed in the spectrum upon heating.

DISCUSSION AND CONCLUSIONS

The present studies suggest that altering the *Sym* plasmid of *R. leguminosarum* bv *phaseoli* 8002 did not lead to alterations in the chemical structure of the EPS. These observations support the suggestion (Robertsen *et*

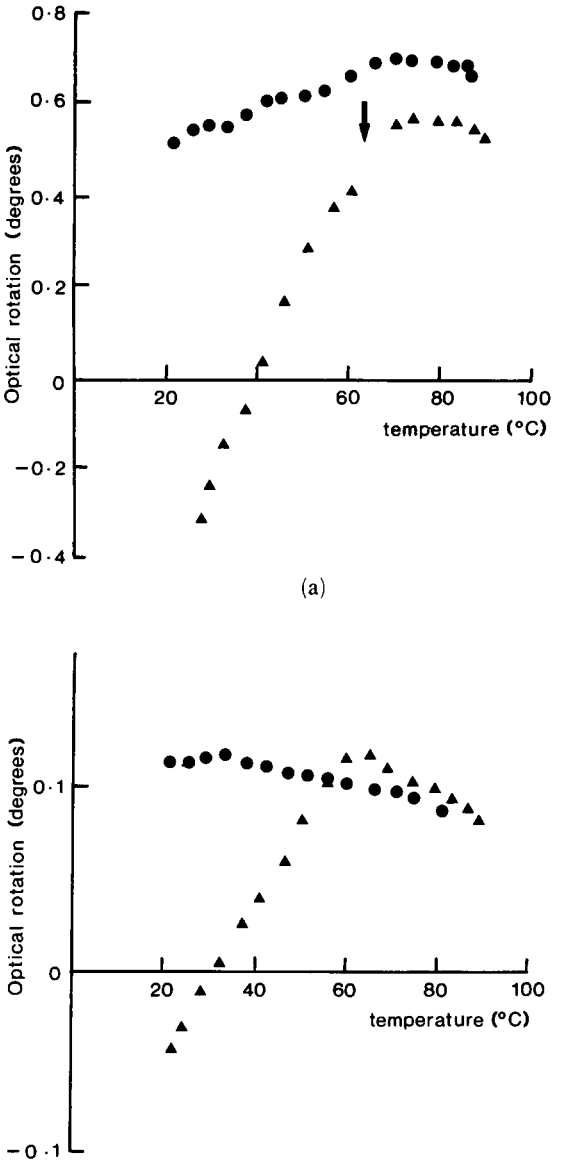

Fig. 8. Optical rotation data obtained for *R. leguminosarum* bv *viciae* 8401 pRL1JI purified EPS (a) Optical rotation change observed upon heating a 3% (w/w) sample: ●, in the absence of added salt — the sample remained fluid at low temperature; △, in the presence of 0·5 M NaCl — the sample gelled at low temperatures. (b) Optical rotation change observed upon heating a 0·5% (w/w) sample: ●, in the absence of added salt; △, in the presence of 0·5 M NaCl. The sample remained fluid in both cases. The melting temperature of the 3% (w/w) gel is shown by an arrow in (a).

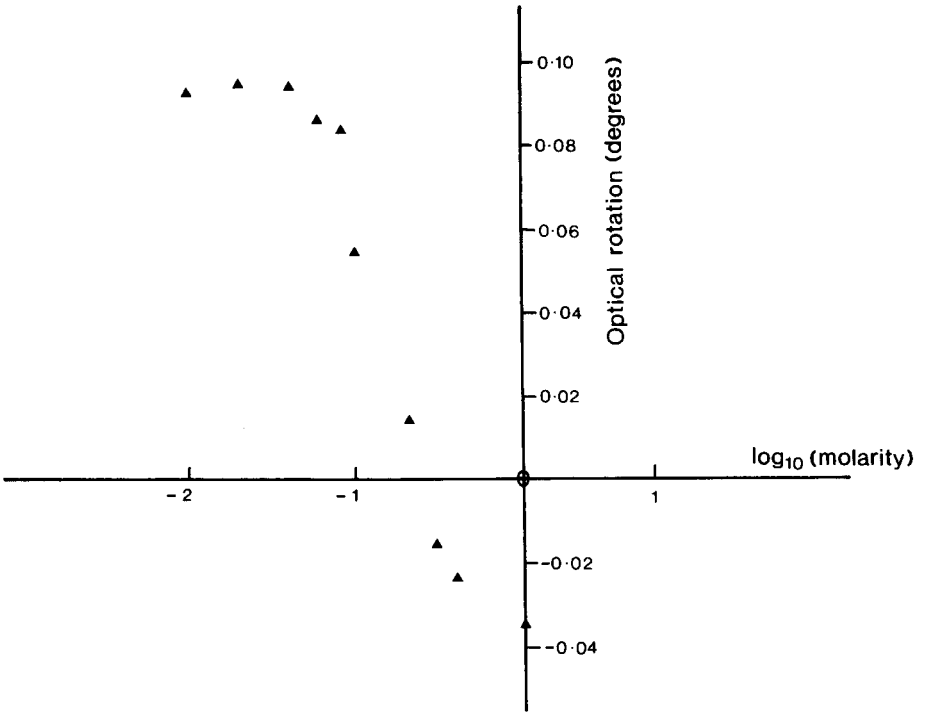

Fig. 9. Plot of optical rotation versus molarity of added NaCl, for a 0·5% (w/w) preparation of *R. leguminosarum* by *viciae* 8401 pRL1JI purified EPS. Temperature = 25°C.

al., 1981; McNeill *et al.*, 1986) that EPS is unlikely to play an important role in *specific* bacterium–plant host recognition.

The present studies suggest that the family of EPS structures (Fig. 1) produced by certain *R. leguminosarum* strains will gel in the presence of added salt, and that salts containing divalent cations are more effective at causing gelation than salts containing monovalent cations. The results shown in Table 2 suggest that ionic size may influence gelation but further studies are needed to substantiate such a conclusion. The culture broths obtained from bacteria producing these EPS structures grown in a supplemented salts medium, could be used directly as gelling agents. These samples will yield opaque thermoreversible gels. Transparent thermoreversible gels can be prepared from crude EPS and derived from such bacteria grown in a supplemented salts medium. Deionised crude EPS, or pure EPS, may be used for the preparation of cold-setting gels: cations can be introduced into aqueous samples by dialysis or slow-release methods. By varying the ionic strength, salt composition and

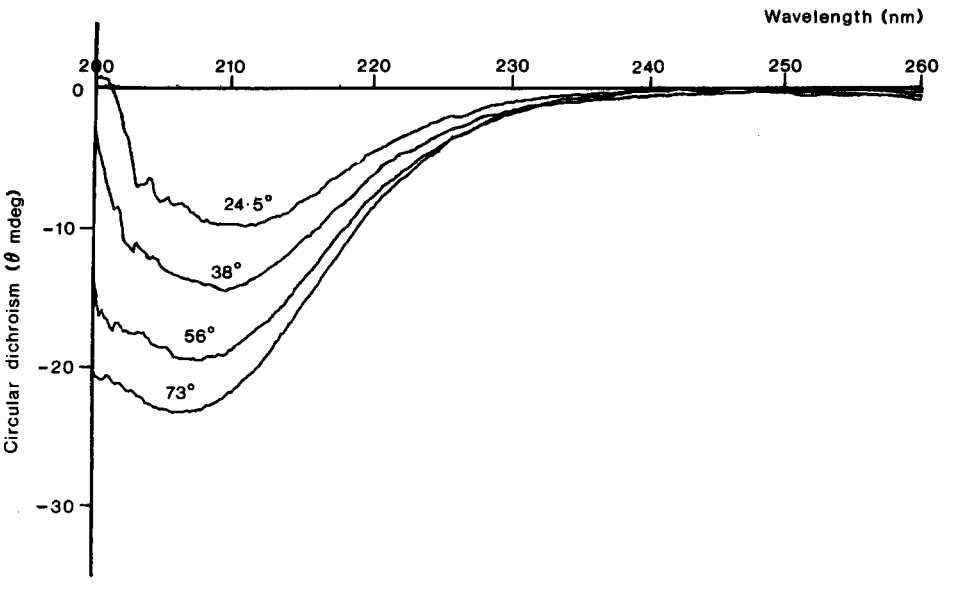

Fig. 10. Circular dichroism spectra for a 0·5% (w/w). *R. leguminosarum* bv *viciae* 8401 pRL1JI purified EPS sample. Data recorded at four temperatures upon heating. The temperature range studied covers the optical rotation transition observed in Fig. 8. Cell pathlength 0·1 cm.

polymer concentration it should be possible to produce both thermoreversible and thermoirreversible gels. At low ionic strengths it should be possible to adjust the 'melting temperature' of the gel to within the vicinity of body temperature.

The optical rotation data shown in Fig. 10 for pure EPS (structure 1 in Fig. 1) suggests that the gelation process may involve a conformational transition of the polysaccharide. The broadness of the optical rotation changes upon heating may arise due to several contributions from different regions of the molecule. The absorption band at 210 nm is negative and will contain contributions from the carbonyl $n \rightarrow \pi$ transitions associated with the D-glucuronic acid residues in the backbone and pyruvate substituents on the side chains. Upon heating the area under the absorption band increases, producing a negative contribution to the change in optical rotation at visible wavelengths. The net positive change in optical rotation when heating (Fig. 9) implies a predominant contribution from far ultraviolet absorption bands at wavelengths below 200 nm. Thus changes in the geometry of the backbone and/or the sidechain may be associated with the gelation process. The involvement of a backbone conformational change would be consistent with the gelation of this

whole family of structures irrespective of the length and composition of the sidechain.

Gelation of the EPS structures may be of biological importance, *R. leguminosarum* species possess the ability to invade, induce nodule formation and fix nitrogen in specific host plant roots. The molecular mechanisms involved in this symbiosis are still largely unexplained. Experimental data (Sanders *et al.*, 1978; Bauer *et al.*, 1979; Napoli & Albersheim, 1980) suggests that EPS is involved in the early stages of the interaction. Studies by Albersheim and coworkers (Robertsen *et al.*, 1981; McNeill *et al.*, 1986) and the studies reported here suggest that EPS are not *specific* host recognition determinants. Smit *et al.* (1987) have proposed a two-stage mechanism for the binding of *R. leguminosarum* cells to plant roots. They show that the initial attachment of individual bacteria to the plant root surface involves a calcium dependent adhesion, although other Group IIA cations may suffice. The second stage is suggested to involve colony formation via the production of cellulose fibrils (Deinema & Zevenhuizen, 1971; Smit *et al.*, 1987) leading to cap formation. Gelation of the EPS produced by the bacteria as an extracellular slime in the vicinity of the root tips, due to calcium (or other salts) present in the soil, may aid the initial binding of individual bacteria, providing a possible *non-specific* adhesion mechanism.

If the mineral content of soils is sufficient to induce gelation of extracellular EPS slimes then the gelation process may have ecological implications in terms of water retention in soils and soil aggregate stability. Such phenomena would suggest additional industrial applications for bacterial cultures as soil inoculates. The role of microorganisms in controlling soil aggregate stability has recently been reviewed by Lynch and Bragg (1985). It has been known since the time of Cato, 239–149 BC, that the 'ploughing in' of lupins and beans improves, soil structure (Gati, 1982). It has been demonstrated that soil bacteria can absorb to the surface of pure clays (Santoro & Stotzky, 1968; Stotzky & Bystricky, 1969). More specifically Fehrmann and Weaver (1978) have observed the absorption of *R. meliloti* to silt particles. These workers concluded that polysaccharides produced by the bacteria were responsible for the absorption and the subsequent aggregation of the silt particles (Fehrmann & Weaver, 1978).

These initial studies on the gelation of a family of EPS produced by the soil bacteria *R. leguminosarum* suggest interesting industrial applications for the EPS and possible biological and ecological roles for the gelation process. It is hoped that these studies will promote further investigations on the applications and mechanisms of the gelation of these families, and possibly other related families, of bacterial EPS.

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