Intra and inter generic homology in polysaccharide structure of *Rhizobium* and *Alcaligenes*.

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**Abstract.** A study was conducted on the structure of extracellular, water-soluble polysaccharides from 5 different strains of *Rhizobium* viz. *R. trifolii* J60 and *R. meliloti* strains J7017, 202, 204 and 207. All these polysaccharides were found to contain glucose and galactose in the approximate molar ratio of 7:1. Methylation analysis revealed these polysaccharides to contain (1 → 3), (1 → 6), (1 → 4), (1 → 4, 1 → 6)-linked D-glucose residues, (1 → 3)-linked D-galactose and non-reducing terminal D-glucose attached to pyruvate. These polysaccharides were also found to be acylated by both acetyl and succinyl residue. This structure was found to be similar to that of succinoglycan, a succinic acid-containing water-soluble, extra-cellular polysaccharide elaborated by *Alcaligenes faecalis* var. *myxogenes* 10C3. This similarity in structure of polysaccharides from two different species of *Rhizobium* and also the polysaccharide produced by *Alcaligenes* has been discussed.

**Keywords.** *Rhizobium*, *Alcaligenes*, polysaccharides, structure, homology, methylation analysis.

**Introduction**

Symbiotic bacteria belonging to the genus *Rhizobium* play an important role in the nutrition of leguminous plants by fixing atmospheric nitrogen in the root nodules. The ability to form nodules has been found to be highly host specific for different *Rhizobium* species (Fahraeus and Ljunggren, 1968). Some reports suggest the involvement of bacterial capsular polysaccharides in the Rhizobial host specificity (Fahraeus and Ljunggren, 1968; Bjorndal *et al.*, 1971). Hence the structural analysis of these polysaccharides is a matter of interest.

**Materials and methods**

**Strains**

*R. trifolii* J60 was obtained from Prof. Y. Maruyama of the University of Tokyo, and *R. meliloti* strains J7017, 204, 202 and 207 were obtained from Prof. M. Yatazawa of Nagoya University, Nagoya, Japan.

**Production of polysaccharides**

Succinoglycan, an acidic, water-soluble polysaccharide elaborated by *Alcaligenes faecalis* var. *myxogenes* 10C3 was supplied by Dr. A. Amemura of Osaka University,
Osaka, Japan. *Rhizobium* polysaccharides were produced in a chemically defined medium (Amemura and Harada, 1971) supplemented with 0.1% yeast extract, contained in 500 ml Erlenmeyer flasks (each containing 100 ml medium) and incubating for 7 days at 30°C on a gyratory shaker.

The water soluble polysaccharides were separated from the culture medium by centrifugation and precipitation with acetone and cetylpyridinium chloride as described by Misaki et al. (1969). The dried polysaccharides were redissolved in water (to 0.1% concentration), dialyzed for two days against distilled water and freeze-dried.

**Quantitative analysis of sugars**

Polysaccharides were hydrolyzed, individually, in a sealed tube with 1 ml of 90% formic acid for 6 h at 100°C. The hydrolysate was converted to alditol acetates and analyzed (Björndal et al., 1967) in a Shimadzu GC7A gas Chromatograph, fitted with a flame ionization detector and a column (4mm × 200 cm) of 3% ECNSS-M on Gas-chrom Q at 190°C. Xylose was used as an internal Standard.

**Quantitative analysis of organic acids**

Pyruvic acid in water-soluble polysaccharides was assayed by the method of Koepsell and Sharpe (1952). Acids in the form of esters were assayed by the colorimetric method of McComb and McCready (1957). Succinic acid and acetic acid were assayed by high speed liquid chromatography as described by Hissamatsu et al. (1978).

**Optical rotation**

Optical rotation was measured in a Perkin-Elmer polarimeter using a 0.25 % polysaccharide solution in water.

**Preparation of deacylated polysaccharides**

A 0.1% solution of the polysaccharide was stirred in 100 mM KOH at 20°C followed by dialysis and freeze drying as suggested by Sloneker and Jeanes (1962).

**Methylation analysis of polysaccharides**

Methylation of native and depyruvylated (prepared by the method of Chaudhari et al., 1973) polysaccharides was done by the Hakomori (1964) technique followed by gas chromatography using an OV 275-GEX 1150 column. The overall method and scheme for methylation was essentially that reported by Hisamatsu et al. (1980).

**Results and discussion**

A study was carried out on the extra-cellular, water-soluble polysaccharides of 13 different strains belonging to three species of *Rhizobium*. Out of these, polysaccharides of 5 strains viz. *R. trifolii* J60, *R. meliloti* strains J7017, 202, 204 and 207 showed similar structure and are reported.

*R. trifolii* J60 and *R. meliloti* strains J7017, 202, 204 and 207 when grown in the chemically defined medium of Amemura and Harada (1971) supplemented with 0.1% yeast extract gave 580, 220, 330, 355 and 340 mg yields of polysaccharides respectively per 100 ml medium.