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Polysaccharide and its component sugars from natural soil aggregates

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Abstract: Soil aggregates *viz.* termite mounds, ant hill, crab nest, crumbs of forest soil and fertile soil were collected from the experimental farms of Tamil Nadu Agricultural University, Coimbatore. The study estimated the polysaccharides associated component sugars and other binding agents of microbes from the soil aggregates. Termite mound possessed maximum amount of total polysaccharides (55.6 mg/g), that included both water soluble and insoluble. The composition of polysaccharides from different soil aggregates showed majority of fructose, galactose and glucose. Whereas the culture *viz. Beijerinckia* and *Azotobacter* isolated from soil aggregates revealed its component sugars contained majority of glucose, fructose, inositol and galactose.

Key words : Polysaccharides, Soil aggregates, Beijerinckia, Azotobacter.

Introduction

Soil aggregates composed of primary particles and binding agents are the basic unit of soil structure. The ability of soil microorganisms to produce extra cellular polysaccharides in pure culture is well known. The polysaccharides produced by microorganisms are grouped into homo and hetero polysaccharides. Sutherland (1977) reported that both gram negative and gram positive organisms were responsible for polysaccharide production. Bacterial polysaccharides have received considerable attention because of their striking physical properties, immunological reaction, diversity of composition and structure. In bacterial aggregation, the surface and extra cellular polysaccharides are generally implicated. Other polymeric material notably nucleic acid and protein excreted or introduced to the medium by cell lysis have also been shown to play a significant role in bacterial aggregation.

Materials and Methods

The soil aggregates like termite mound, crab nest, ant hill and soil crumbs were collected from the experimental farms of Tamil Nadu Agricultural University, Coimbatore. Bacteria associated with soil aggregates were isolated and characterized. Polysaccharide production and their component sugars produced by the isolated cultures were estimated as per the method of Cheshire (1979). The sugars associated with polysaccharide were quantified by acid hydrolysis, sugars analysis by colorimetry, quantitative separation by paper chromatography and gas chromatographic assays (Cheshire, 1979).

Results and Discussion

Extra cellular polymeric material of biological origin is either due to lytic products or biological excretions and are usually distributed abundantly in natural environment. Polysaccharides were produced both under aerobic and anaerobic conditions. The extra cellular polymers which are products of the microorganism are responsible for attaching to the surface of the soil. The extra cellular polysaccharides produced by the microbes play a significant role as soil binding agents, leading to the formation of soil aggregates or soil crumb. Polysaccharide production by these cultures protect the inner cell against external stresses (Dudman, 1977). The organisms isolated from the soil aggregates in the present study are *Azotobacter* sp. and *Beijerinckia* sp. (Table 1 and 2). They are gram negative, coccal, rod shaped, smooth, glistening and extra cellular polysaccharides producer (ECP). It is well known that bacterial polysaccharides and polyuronides generally present in soil in sufficient concentrations are particularly effective in enhancing aggregation. The accumulation of polysaccharides in soil composed of fine particles improves the rate

Table 1. Enumeration of aerobic bacteria from different gut region of termites

Particulars	Bacteria x 10^4	*Azotobacter* x 10^4	*Beijerinckia* x 10^4
Salivary gland	32.0	19.0	13.2
Foregut	24.6	16.0	11.5
Midgut	19.3	5.4	4.6
Hindgut	26.6	3.2	3.5

SEd 0.29 0.13 0.25
CD 1.28 1.17 1.24
Media Becking's media (Becking, 1974)
Cell morphology was identified under direct microscopic observation
Revealed the gram negative and polysaccharide producing rods
The population of the colonies were expressed in colony forming units (CFU ml^{-1})

Table 2. Characterization of N_2 fixing aerobic bacteria isolated from termites gut

Characteristics Cell shape	*Azotobacter* Rod to oval, often in pairs, smooth glistening	*Beijerinckia* Coccal to rods copious slime smooth glistening
Motility	+	-
Green fluorescent	+	+
Acid production	+	+
Catalase activity	+	+
Cellulase activity	-	-
Cellulose hydrolysis	-	-
Starch hydrolysis	-	-
Dye sensitivity	-	-
Cyst formation	+	-
Gram reaction	G-ve to variable	G-ve

Becking media was used for isolation and characterization, Becking (1974)

Table 3. Water soluble and water insoluble polysaccharides from soil aggregate

Sample	Water soluble polysaccharides mg g^{-1}	Water insoluble polysaccharides mg g^{-1}	Total soluble polysaccharides mg g^{-1}
Soil crumbs	32.9	1.8	34.7
Red soil	33.2	1.9	35.1
Black soil	32.8	1.9	35.1
Acid lateritic	49.8	5.8	55.6
Termite mound	48.2	5.4	52.6
Ant hill	36.4	2.6	39.0
Crab nest (Red)	36.2	2.8	39.0
Crab nest (Black)	32.8	2.1	34.9
Fertile soil (Al)	32.1	2.1	34.2

Residue hydrolysate reducing sugar as glucose

of water transfer by enhancing soil aggregation and permeability. The distribution of water-soluble and water insoluble polysaccharides extracted with dimethyl sulphoxide from different sources of soil aggregates are presented in Table 3. Water soluble polysaccharides are more in acid lateritic soil and ant hill (49.8 and 48.2 mg g^{-1}) whereas water insoluble polysaccharides were more in acid lateritic soil and termite mound (5.8 & 5.4 mg g^{-1}). The polysaccharides isolated from soil are generally very complex and are a mixture of linear and flexible polymers (Martin, 1971). Finch *et al.* (1971) reported that if appreciable amount of soil polysaccharides were of microbial origin, then the microbial materials have a high degree of resistance to decomposition. Gaur and Rao (1975) also found a positive relationship between the uronic acid content of bacterial polymers and their stabilizing effect of *Azotobacter*. Production of polysaccharides and its component sugars by microorganisms isolated from the aggregates and component of natural soil aggregates are presented in the Table 4 & 5.

It was observed that glucose was absent in all the sample studied except *Beijerinckia*. Microbial synthesized or converted sugar, glucose was present in termite mound, ant hill, crab nest and fertile soil. Thus the available carbohydrate from the soil is converted to required sugars and then polymerized by the organisms. Hence, the culture isolated from the aggregate were tested for their polysaccharide production. Bacteria are known to contain nearly 60-80 per cent of the cell dry weight as polysaccharides. Martin (1971) reported that soil polysaccharide contributed to soil aggregate stability. The isolated *Azotobacter* sp. and *Beijerinckia* sp. produced extracellular polysaccharides and they contain mainly glucose and galactose. The amount of ECP production by the organism ranged between the extractable polysaccharides and aggregation. Polysaccharides are linear or branched with a variety of functional group such as hydroxyl and methoxyl group. Hence, the extracellular polysaccharide produced by the culture contains heteropolymers which act as binding agents, a primary particle and basic unit for the stable soil aggregates. By methylation process, the hydrolysis of polysaccharide disrupt the ionic linkages between the soil and polymeric components hence it makes the soil as a stable aggregates.

The present study explored that the relationship of soil polysaccharide and their component sugars linked to other binding agents present in the soil for stable soil aggregation thereby improve the soil structure, nutrient status and reduces the soil erosion.

Table 4. Composition of polysaccharides in different soil aggregates

Samples	Component sugars
Soil crumbs	Fructose, Galactose, Glucose, Arabinose, Xylose
Acid lateritic	Fructose, Galactose, Arabinose
Termite mound	Fructose, Galactose, Mannose, Glucose
Ant hill	Fructose, Galactose, Mannose, Arabinose, Glucose
Crabnest 1	Rhamnose, Fucose, Mannose, Glucose
Crab nest 2	Fructose, Rhamnos, Mannose, Glucose
Fertile soil	Fructose, Glucose, Galactose, Xylose, Arabinose
Forest soil	Galactose, Xylose, Arabinose, Glucose

Table 5. Production of polysaccharides and its component sugars

Organisms	mg/g of carbon	Component sugars
Azotobacter chrococcum	603	M, F, G, Mn
Azotobacter vinelandii	820	M, G, Gl
Beijerinckia 1	813	I, M, F, Gl
Beijerinckia 2	820	I, F, M, Mn, G, Gl
Beijerinckia 3	640	I, F, Mn, G, Gl

M - Mannose; F - Fructose; Mn - Mannitol; G - Glucose; Gl - Galactose; I - Inositol

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