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Isolation and Characterization of Lipopolysaccharides from Cell Wall of the Cyanobacterium *Oscillatoria redekei*

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Lipopolysaccharides were isolated from the filamentous cyanobacterium *Oscillatoria redekei* by hot phenol-water and purified by ultracentrifugation. Chemical analyses demonstrated that they were extracted into the phenol phase of phenol-water extracts. Nearly 38% of LPS consisted of polysaccharides. Lipid-A, although it could not be separated by mild acid hydrolysis from the polysaccharide moiety, indicated the presence of b-hydroxy palmitic and b-hydroxy myristic acids and glucosamine. The lipopolysaccharides were free of heptose and 2-keto-3-deoxy octonate. Protein and phosphorus content of the lipopolysaccharides were low.

Key Words- Cyanobacterium Filament Lipopolysaccharide Phosphorus Protein Purification.

According to Drews & Weckesser (1982) the fine structure of cell wall of cyanobacteria corresponds to that of Gram-negative bacteria. There are significant similarities in the chemical composition and morphological organization of the cell walls of these organisms. Like the cell wall layers of gram-negative bacteria, an outer membrane and the peptidoglycan layer has been observed in the cell wall of cyanobacteria. However, in the latter it is thicker than with gram-negative bacteria. Recently high molecular weight materials similar to O-antigens (lipopolysaccharides = LPS) of gram-negative bacteria have been isolated from the cell walls of various strains of cyanobacteria i.e. *Synechococcus* (Schmidt *et al.*, 1980[a]), *Synechosystis* (Schmidt *et al.*, 1980[b]), *Anabaena* PCC 7118(Weckesser et al., 1974), *Agmenellum* (Buttke & Ingram, 1975) and *Phormidium* (Mikheyskya *et al.*, 1977). No lipopolysaccharides, however was detected in *Anabaena flos-aquae* A-37(Wang & Hill, 1977); the materials extracted in both the aquous phase and phenol phase were only polysaccharides. In this communication, the lipopolysaccharides of a filamentous non-heterocystous cyanobacterium *Oscillatoria redekei* Van Goor have been isolated and chemically characterized.

MATERIALS & METHODS The cells of *Oscillatoria redekei* Van Goor were grown in nitrogen supplemented BG 11 medium (Rippka *et al.*, 1979) under continuous light (3000 - 4000 lux). The cells were harvested after 15 days and collected by centrifugation.

Isolation of lipopolysaccharides The cells were lyophilized and lipopolysaccharides were extracted by hot phenol-water procedure (*Flow diagram*) and purified by ultracentrifugation (105,000 x g, 4 h, three times). After dialysis, to remove the nucleic acid contaminants, the water phase supernatant and sediment were treated with a-amylase and RNA ase (24 h, 37 C) followed by repeated washing. The sediment, extracted into phenol phase was further extracted with chloroform-methanol (2:1; v/v 22 C) to make it free from contaminating phospholipids.

Chemical analyses Neutral sugars, liberated by 0.5 N H_2SO_4 (100 C,4 h) were identified by thin layer chromatography (solvent-ethyl acetate:pyridine:water = 12:5:4; staining-anilinium hydrogen pthalate) and quantitatively determined as alditol acetate derivatives by GLC (ECNSS-M, 3 % on Gas Chrom-Q, 100-200 mesh). For detection of uronic acids and 2-keto-3-deoxy octonate, the samples were hydrolysed in 1N H_2SO_4 (100°C,4 h) and separated by high voltage paper electrophoresis in pyridine: formic acid: acetic acid: water (2:3:20:180,v/v) at pH 2.8. Amino sugars, liberated by hydrolysis in 6N HCl at 100°C for 16 h, were identified by high voltage paper electrophoresis and quantitatively determined in an automatic amino acid analyser (Durrum, model D-500). Fatty acids liberated by hydrolysis in 4N HCl at 100 C for 6 h were identified as their methyl esters by gas-liquid chromatography (Casterwax, 2.5 % on chromosorb G, 80-100 mesh and EGSSX, 15% on Gaschrom P, 100-200 mesh). Phosphorus and protein was estimated according to Lowry *et al.*(1954) and Lowry *et al.*(1951), respectively.

RESULTS & DISCUSSION The cells after extraction following the method of Westphal *et al.*(1952) and purification by ultracentrifugation, sedimentation were obtained both in aqueous phase and phenol phase. Table-1 shows the distribution of the extracted materials between the two phases and their chemical analyses for carbohy-

Flow Diagram

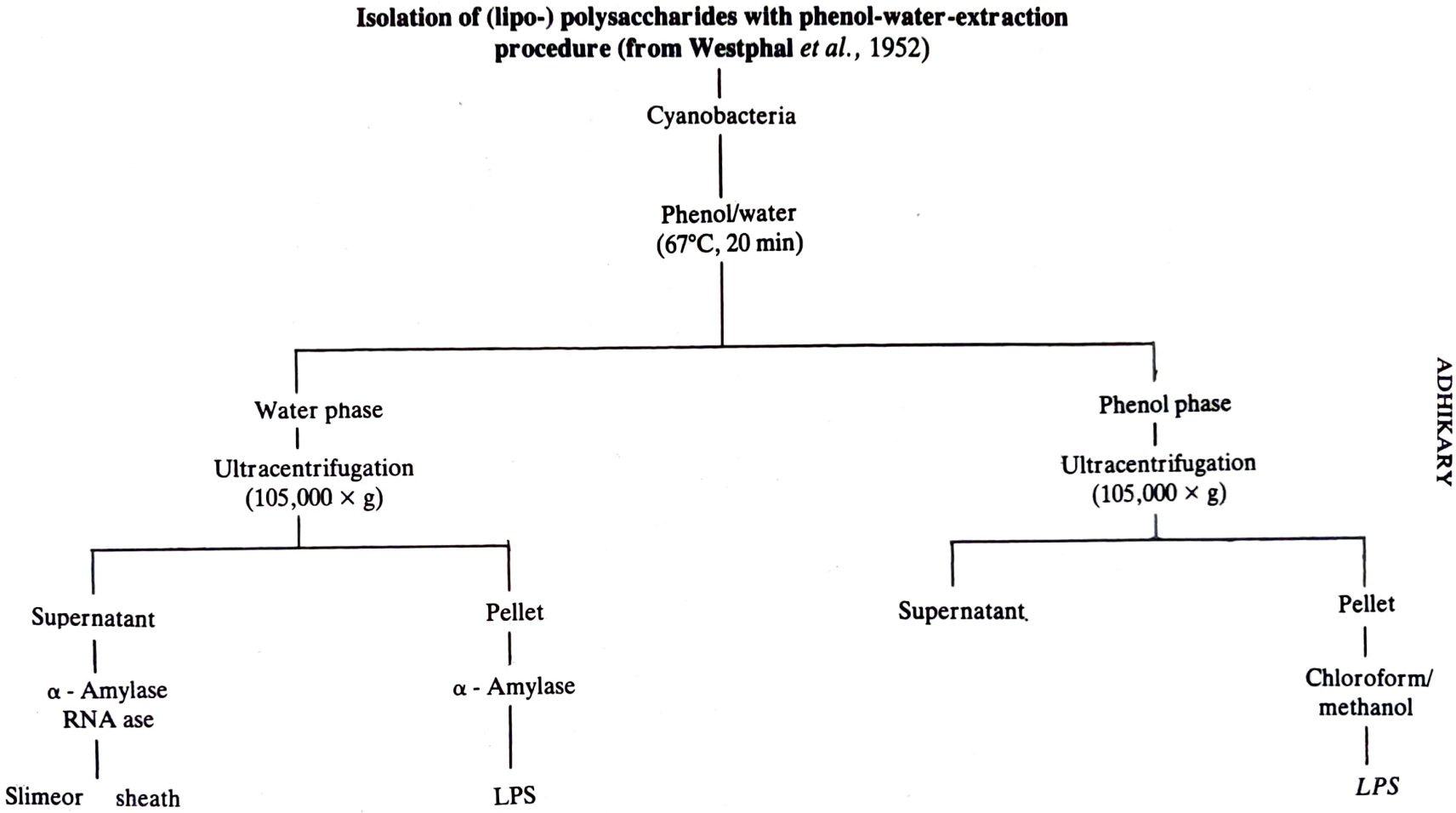

Table 1 Yield of (lipo-) Polysaccharide (sediment at 105,000 × g, 4h) from *O. redekei* at Different Isolation Steps by phenol/water procedure.

Isolation step	Yield (Per cent cell mass dry weight)
Hot Phenol-water aquous phase supernatent	2.3
Hot Phenol-water aquous phase pellet	0.7
Hot Phenol-water phenol phase pellet	0.9

Table 2 Chemical Composition (% of dried material) of the phenol/water Extractable Materials at Different Isolation Steps of *O. redekei*

Component		w.ph supt.	w.ph pellet	Ph. pH pellet
Rhamnose		11	11.6	2
Fucose		7	0.5	8
Ribose		3	-	-
Arabinose		-	-	8
Mannose		17	4	1
Galactose		-	-	2
Glucose		-	-	17
Unknown sugar - X		-		6
2-Keto-3-deoxy octonate		-	-	-
Total sugars		31	16	33
Glucosamine		0.2	0.8	2.5
Phosphorus		1.1	1.0	0.9
Fatty acids	C_{13}	0.019	0.031	0.023
	C_{14}	0.023	0.036	0.036
	C_{16}	0.105	0.088	0.111
	C_{18}	0.019	0.026	0.06
	C_{20}	0.032	-	0.02
	$C_{22:1}$	0.012	-	0.178
	β-$C_{14}OH$	0.034	0.015	0.219
	β $C_{16}OH$	0.042	0.055	0.378
Total fatty acids		0.286	0.251	1.025
Uronic Acids		-	-	-
Protein		0.25	0.02	0.18

- = absent

Table 3 Summary of the Composition of Lipo-Polysaccharides from *Synechococcus* PCC 6907[1] PCC 6908[2], *Synechosystis* PCC 680 [3], PCC 6807 4, *Anabaena variabilis* 5 *O. redekei* 6 *O. rubescens*7 and *Rhodopseudomonas capsulata* 8

Components	Organisms							
	1	2	3	4	5	6	7	8
3-O-methyl pentose			+					
2-O-methyl hexose			+	+				
3-O-methyl hexose		+						
4-O-methyl hexose		+					+	
6-O-methyl heptose							+	
L-acofriose				+				
Rhamnose	++				+			
Fucose	+	+	+	+	+	+	++	+
Mannose	+	++	+	++	+	+	+	
Galactose	+	+	+	+	+	+	+	
Glucose	+	+	++	+	++	++	+	++
Tyvelose	+							
Heptose				+				
2-Keto-3-deoxy octonate		+						
Uronic acids				+				+
Glucosamine	+	+	+	+	+	+	+	+
Unknown amino sugars	+	+						
Fatty acids C_{14}	+			+		+	+	+
C_{16}	+	+	+	+	+	+	+	
C_{18}	+	+		+		+	+	
ß-C_{14}OH	+	+	+	+	+	+	+	+
ß-C_{16}OH	+	+	+	+	+	+	+	
Unknown B-OH fatty acids	+	+	+	+				
Phosphorus	+	+	+	+	+	+	+	+

1 & 2 Schmidt *et al.*, 1980 a; 3 & 4, Schmidt *et al.*, 1980 b; 5, Weckesser *et al.*, 1974; 6, Adhikary (present communication); 7, Adhikary (unpublished); 8, Omar *et al.*, 1983.

++ = major component; + = present.

drates, fatty acids, amino sugars, phosphorus and protein are shown in Table-2. About 55% of the total materials extracted was found in aqueous phase supernatant, accounting for 2.25% of the dry weight of the cell mass. Only negligible amount of glucosamine and long chain fatty acids were detected (Table-2). These are the major components of the lipopolysaccharide fraction of bacteria and cyanobacteria (Weckesser *et al.*,1979). The aqueous phase supernatant contained 31% carbohydrates and was composed of predominantly mannose (17 %) and rhamnose (11%). In addition, fructose and ribose were present. Since *O redekei* produces sheath around its filaments, probably these materials were extracted into the water phase supernatant and composed of various sugars with traces of protein and fatty acids. The materials extracted into water phase pellet contained 16% sugars and lesser amounts of glucosamine, phosphorus, proteins and fatty acids. Presence of negligible amounts of long chain fatty acids and glycosamine indicated that the materials extracted into water phase sediment were not similar in composition as the bacterial lipopolysaccharides (Weckesser *et al.*,1979). The materials extracted into the phenol phase contained 38% carbohydrates, 2.5% glucosamine and 1% fatty acids. In addition, 1% phosphorus and traces of proteins were present. The major sugar component was glucose and in addition mannose, rhamnose, fucose, arabinose and a fast running sugar were identified.. The fatty acid component of the phenol phase sediment contained long chain fatty acids (ß-hydroxy myristic acid and ß-hydroxy palmitic acid) which are normally the typical components of the lipid-A fraction of the lipopolysaccharides of bacteria and cyanobacteria (Weckesser *et al.*,1979). However, 2-keto-3-deoxy octonate (KDO) and heptoses were not detected in the phenol phase as well as in the aqueous phase material. The separation of the polysaccharide moiety (degraded polysaccharide) from the lipid-A moiety was not achieved by treatment of the lipopolysaccharide fraction, isolated into the phenol phase sediment, with 1% acetic acid (100 C, 3 h). Similar observation was made by Weckesser *et al.* (1974) in their work with *A variabilis*. They suggested the possibility that a stronger chemical bond might be present in the material extracted into the phenol phase linking the lipid portion to the polysaccharide moiety.

Since the first hint of the presence of lipopolysaccharides in *Anacystis nidulans* (Weise *et al.*,1970), a number of cyanobacteria belonging to Chroococcacea, Oscillatoria and heterocystous sub-groups have been investigated for lipopolysaccharides in the outer membrane (Weckesser *et al.*,1979). There was no uniformity in LPS of various cyanobacteria in one particular phase sediment and were extracted either into phenol phase or into water phase depending on the strains (Schmidt *et al.*, 1980 ab: Weckesser *et al.*,1974; Wang and Hill, 1977). The comparison of the chemical composition of LPS from *O.redekei* with the LPS from other cyanobacteria strains (Schmidt *et al.*,1980 a,b; Weckesser *et al.*, 1974; Adhikary, unpublished) and the LPS from *Rhodopseudomonas capsulata* (Omar *et al.*,1983) are presented in Table-3. The macromolecular composition of LPS of cyanobacteria showed a marked variation among themselves (Table-3). The sugar composition and major sugar component of the LPS vary among the cyanobacterial strains. Though the lipid-carbohydrate complex extracted by phenol-water method from various cyanobacteria resemble bacterial LPS in physical appearance and biological activity (Buttke & Ingram,1975;Weise *et al.*,1970), there were marked differences in chemical composition. The two sugars, L-glycero-D-monoheptose and KDO, both the components of bacterial R-core region, were absent in most of the cyanobacterial strains. The lipid component of LPS of the most of the cyanobacteria was low. Although lipid-A was detected in the LPS from *O redekei* and other cyanobacteria (Weckesser *et al*, 1979), it was not split off from the polysaccharide moiety upon mild acid hydrolysis. In *Anabaena flos-aquae* A.37 (Wang & Hill, 1977) the phenol-water extractable materials were polysaccharides without any lipid. Thus the general structural construction of the cyanobacterial lipopolysaccharides cannot be demonstrated at the present time.

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