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The structure of the lipid A component of *Sphaerotilus natans*

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Abstract. The lipopolysaccharide of *Sphaerotilus natans* afforded a ladder-like pattern of bands in sodium deoxycholate-polyacrylamide gel electrophoresis, indicating the presence of a S-form lipopolysaccharide. The chemical analysis showed neutral sugars (rhamnose, glucose, L-glycero-D-manno-heptose), 3-deoxy-octulosonic acid (Kdo), amino compounds (glucosamine, glucosamine phosphate, ethanolamine and ethanolamine phosphate), and phosphorus. The lipid A fraction contained saturated and unsaturated capric, lauric, and myristic acids, and 3-hydroxy capric acid (3-OH-10:0). Its chemical structure was consisting of a glucosamine disaccharide, glycosidically substituted by a phosphomonoester, and substituted at C-4′ by a pyrophosphodiester esterified with ethanolamine. The amino groups of both glucosamines are acylated by 3-hydroxy capric acids and these in turn are substituted by saturated and unsaturated capric, lauric, and myristic acids. Hydroxyl groups of the backbone disaccharide at C-3 and C-3′ were also esterified by 3-hydroxy capric acid, those at C-4 and C-6 were unsubstituted. The latter provides the attachment site for Kdo.

Key words: *Sphaerotilus natans* – Lipopolysaccharide – Lipid A – Laser desorption mass spectrometry – DOC-PAGE – 3-Hydroxycapric acid – *Proteobacteria*

Lipopolysaccharides are amphiphilic macromolecules which are localized in the outer leaflet of the outer membrane of almost all Gram-negative bacteria (Rietschel et al. 1987; Mayer et al. 1989b). They represent the O-antigens and endotoxins of these bacteria and participate in several distinct membrane functions (Rietschel et al. 1987; Lüderitz et al. 1982; Nikaido 1970). Lipopolysaccharides have a common general architecture, being composed of a hydrophilic polysaccharide region, consisting of O-chain and R-core, and a hydrophobic lipid moiety, the lipid A (Rietschel et al. 1987; Lüderitz et al. 1982). The latter represents the endotoxic principle of lipopolysaccharides (Rietschel et al. 1987; Lüderitz et al. 1982; Galanos et al. 1972; Shiba et al. 1984; Shiba and Kusumoto 1984) and constitutes the most conservative part of the molecule, at least for *Enterobacteriaceae* (Rietschel et al. 1987). The structures of lipid A of many *Enterobacteriaceae* and phototrophic bacteria have been reported. Distinct variations in the backbone structure and its substitutions have been found especially with non-enterobacterial species (Mayer et al. 1990). Differentiation in lipid A structure led in many cases to deviations in biological activities (Galanos et al. 1977; Lüderitz et al. 1986; Brade et al. 1988; Rietschel et al. 1984b; Tharanathan et al. 1985).

Lipid A composition and structure are in many instances reflecting the phylogenetic relatedness of distinct species as determined by 16 S rRNA homologies within the *Proteobacteria* (Mayer et al. 1989a; Weckesser and Mayer 1988; Stackebrandt et al. 1988). *Sphaerotilus natans*, a sheated chlamydobacterium, is phylogenetically closely related to the phototrophic non-sulfur purple bacterium *Rhodocyclus gelatinosus*. Both are belonging to the β-1 subclass of *Proteobacteria* (Stackebrandt et al. 1988; Woese et al. 1984). The chemical structure, the serological properties, as well as the endotoxic activity of lipolysaccharides of *R. gelatinosus* strains have been determined (Galanos et al. 1977; Tharanathan et al. 1985; Weckesser et al. 1975). The present study describes the structural elucidation of the lipid A component of *S. natans* revealing structural similarities to that of *R. gelatinosus*.

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Abbreviations: Kdo, 3-deoxy-D-manno-octulosonic acid; 3-OH-10:0, 3-hydroxy capric acid; DOC-PAGE, deoxycholate-polyacrylamide gel electrophoresis; GC-MS, gas chromatography/mass spectrometry; LD-MS, laser desorption mass spectrometry; LPS, lipopolysaccharide; PS, polysaccharide

Materials and methods

Bacterial strain and isolation of lipopolysaccharide

Sphaerotilus natans ATCC 13338, obtained from the American Type Culture Collection (Rockville, MD, USA), was cultivated