

Isolation and Characterization of Endotoxin in Psychrophiles

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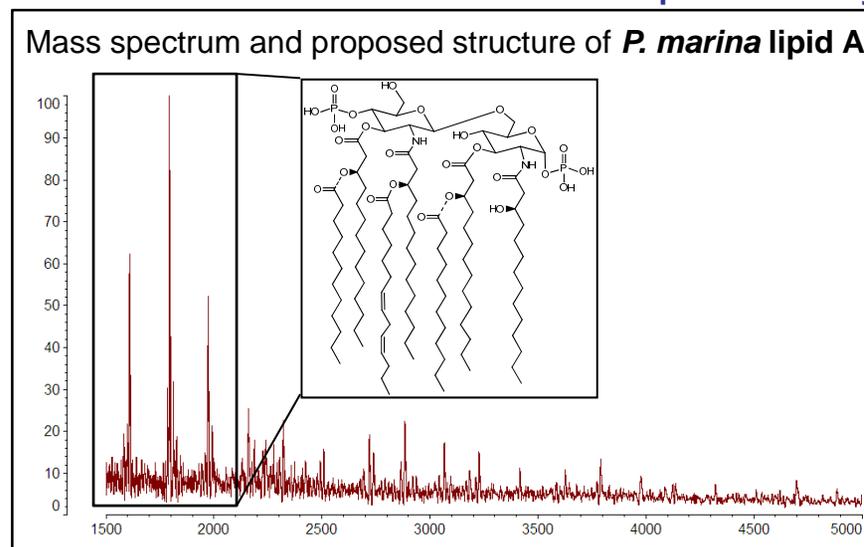
Abstract

This research project focuses on the structure of endotoxin (lipopoligosaccharide, or LOS) and its hydrophobic anchor lipid A in psychrophilic bacteria. Psychrophiles are bacteria that can survive and multiply at low temperature, and it is reasonable to suspect that the composition of the cellular membrane of these cold-tolerant bacteria differs from that of mesophiles in order to maintain membrane fluidity. In particular, this work examines LOS and lipid A in *Psychrobacter cryohalolentis* and *Psychromonas marina*. Preliminary MALDI-TOF mass spectrometry experiments using purified solvent-extracted LOS suggest this bacterium has a mix of penta-acyl and hexa-acyl lipid A containing unsaturated secondary chains, in agreement with previous students' work with lipid A purified by different means. In addition, molecular forms containing oligosaccharide residues can be seen in the LOS MALDI. Additional structural understanding of the lipid and oligosaccharide portions of the molecule will require additional experiments to be undertaken in the second semester, including sugar and fatty acid GC-MS compositional analysis.

Results

P. cryohalolentis and *P. marina* LOS fractions were isolated by two-phase hot phenol-water extraction and purified by dialysis, ultracentrifugation, and differential precipitation. The purified oligosaccharide and saccharolipid structures were then analyzed by mass spectrometry (MALDI-TOF MS). Structural analysis of these data is consistent with lipid A (saccharolipid) molecular masses seen in earlier direct isolations, with *P. cryohalolentis* lipid A in a cluster centered on 1601 daltons and *P. marina* lipid A showing predominant forms at 1612 and 1794 daltons. However, the LOS isolate shows additional forms as well; *P. marina* displays a strong peak corresponding to a third (hepta-acyl) lipid at 1977 daltons. Furthermore both species show significant glycosylation, with hexose repeating units extending out into the 4-5 kilodalton region. Elucidating the identity and structural location of these acyl chains and sugars will require further compositional analysis using GC-MS methods.

Graphic Summary



Relevance

Bacterial endotoxin (lipid A, LOS) is an important structural component of the Gram-negative cell wall and one of the most medically important toxins in Gram-negative bacterial infection. While direct weaponization of endotoxin itself is unlikely, this molecule is central to both the damaging effects of known infections and the ability of mammals to mount an effective immunological defense. This 1/C MIDN SC495/6 project aims to purify endotoxin from a psychrophile that grows in marine environments, and characterize the structure of this material. This is a necessary first step toward understanding the biological and immunological role and importance of endotoxin in these organisms. Preliminary work supports our hypothesis that evolutionary changes in the structure of lipid A play a role in the cold-tolerance of this organism, and this work will expand current understanding of the structural and functional diversity of endotoxin.