

Structural studies of a cell wall polysaccharide from the probiotic strain *Lactobacillus farciminis* CIP 103136

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Lactobacillus farciminis is a Gram positive bacteria first isolated from meat products [1] and kimchi, known as health promoting Korean food [2]. It was previously demonstrated that treatment with *L. farciminis* CIP 103136 exerts a beneficial anti-inflammatory effect against colonic inflammation and prevents stress-induced visceral hypersensitivity in rats [3], [4].

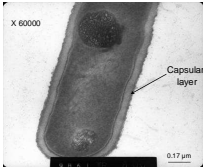


Fig. 1. TEM observation of *L. farciminis* cell

Microscopic observation of *L. farciminis* CIP 103136 cells indicated the presence of an outside capsular layer (Fig. 1)

In order to understand the rationale of the probiotic activities of this bacterium, we studied its polysaccharide (PS) composition.

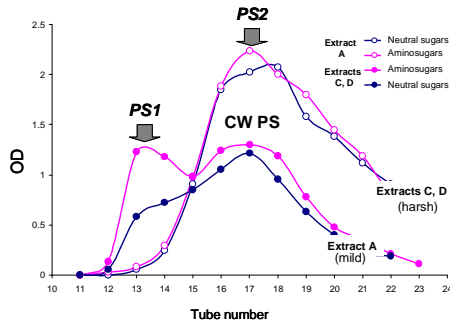


Fig. 2. Typical elution profiles of deproteinated extracts on a Sephadex G-50 column (2.6 x 90 cm)

PS were extracted from bacterial cells sequentially by increasingly strong treatments: autoclaving (A), cold 5% TCA (B), and hot 0.01 M and 0.1 M HCl (C, D). Extract A afforded a mixture of two PSs, designated as PS1 (less than 2% of total PS) and PS2. Hot acid extracts C and D contained exclusively PS2 (~95% of total PS, Fig. 2). This allowed us to consider PS2 as a cell wall associated PS (CW PS).

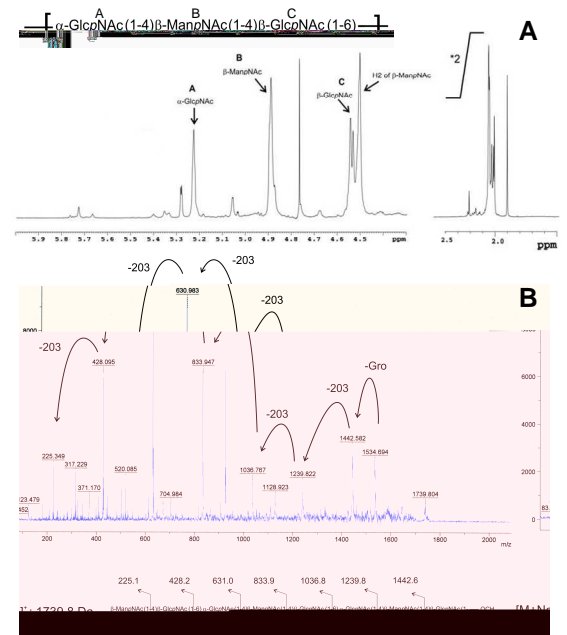
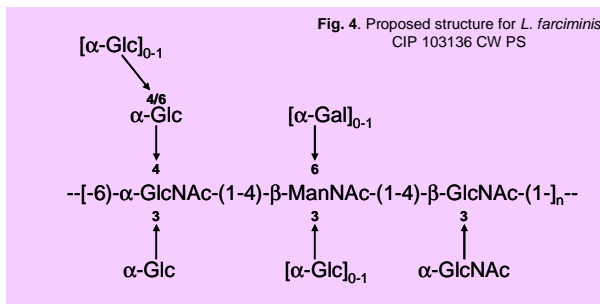


Fig. 3. NMR (A) and MALDI-TOF-TOF MS (B) spectra of a product of Smith degradation of CW PS

Monosaccharide and methylation analysis of CW PS indicated the presence of GlcNAc and ManNAc; as well as terminal and 4/6 linked Glc and Gal. The PS was subjected to Smith degradation, which led to a complete elimination of Hex residues. The structure of this product was studied by 2D NMR spectroscopy and mass spectrometry, and was shown to be composed of linear trisaccharide repeating units (Fig. 3).

2D NMR studies and methylation analysis of the native CW PS led to the following structure (Fig. 4) :



In vitro biological studies of immuno-modulatory properties of the CW PS are in progress.

While the poly-NAC-hexosamine backbone was surprisingly reminiscent of that found in cell wall PS of *Bacillus anthracis* and *Bacillus cereus* [6], [7], the original hexose substitution pattern makes the structure of *L. farciminis* 103136 CW PS novel and unique.



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