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].

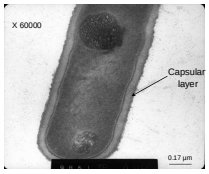

Fig. 1. TEM observation of *L. farciminis* cell

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.

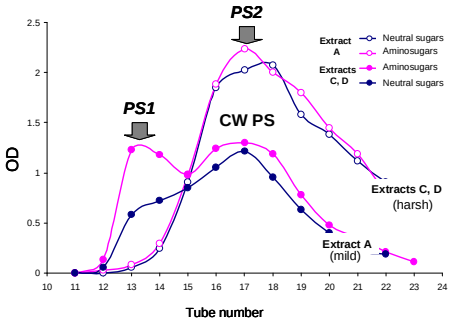

Fig. 2. Typical elution profiles of deproteinated extracts on a Sephadex G-50 column (2.6 x 90 cm)

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).

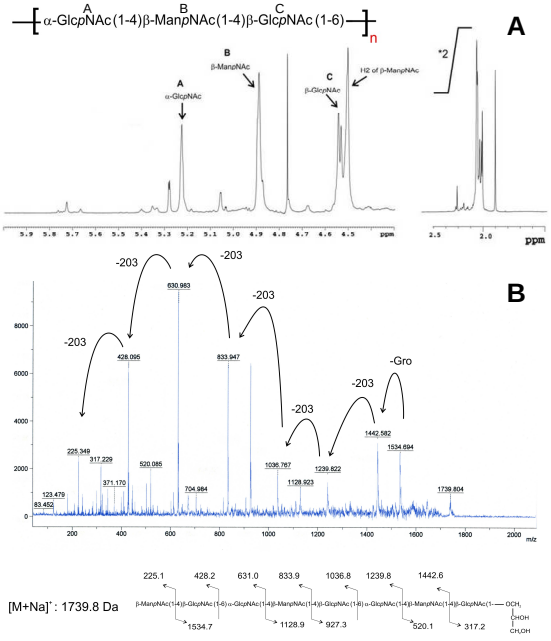

Fig. 3. [1]NMR (A) and MALDI-TOF-TOF MS (B) spectra of a product of Smith degradation of CW PS

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) :**

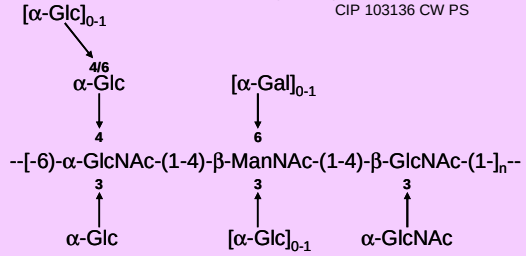

Fig. 4. Proposed structure for *L. farciminis* CIP 103136 CW PS

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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