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Peptidoglycan diversity and anti-inflammatory capacity in *Lactobacillus* strains

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ABSTRACT

Lactobacillus species are potential probiotic bacteria for humans because of their capacity to improve certain biological functions in the host's immune system. In this study, we focused on three peptidoglycans (PGNs) derived from different *Lactobacillus* strains and investigated each PGN's anti-inflammatory capacity. Each PGN was analyzed using HPLC, MALDI–TOF/TOF MS and FTIR. All three PGNs displayed a β-1,4-linked *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) structure with some modifications in the polypeptides at the end of the MurNAc residue. In a new insight, we found that PGNs inhibit the release of inflammatory cytokines in LPS-induced RAW 264.7 cells; a capacity that may be related to the TLR-4 pathway. The goal for exploring PGN diversity in *Lactobacillus* strains is to better understand the potential use of *Lactobacillus* PGNs in food and pharmaceutical applications.

1. Introduction

Traditional probiotic strains of lactic acid bacteria (LAB) have a long history of safe use in many bioprocessed food environments made of meat, milk and vegetable products. Recently, LAB has been studied intensely for its human health promoting capacity, since these microorganisms have the capacity to improve some biological functions in the host (Dogi, Galdeano, & Perdigon, 2008). Given its surface area and an environment of nutritional abundance, gastro-intestinal tract (GIT) is the preferred site of colonization for LAB (Lomer, Thompson, & Powell, 2002). Due to the genetic diversity and complexity of probiotic bacteria, the binding of probiotic bacteria to intestinal epithelial cells (IECs) in the mono-stratified epithelium of the GIT is believed to have lasting beneficial health effects, including the exclusion of pathogens, immunomodulation, and the production of beneficial bacterial molecules (O'Hara et al., 2006).

The human gut microbiota has been estimated to consist of at least 400 different species, and it is likely that each species varies in its ability to influence immune homeostasis (Suau et al., 1999). Inflammatory bowel disease (IBD) results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host. IBD comprises two types of chronic intestinal disorders: Crohn's disease (CD) and ulcerative colitis (UC) (Shivananda et al., 1996). Because of strain-specific variability, and clinical and therapeutic heterogeneity in CD and UC, it cannot be assumed that a given probiotic is equally suitable for all individuals (Fergus, 2000). Therefore the effects of probiotics are expected to be diverse and include modulation of the gut immune system through interaction

Abbreviations: PGNs, peptidoglycans; MurNAc, *N*-acetylmuramic acid; GlcNAc, *N*-acetylglucosamine; LAB, lactic acid bacteria; GIT, gastro-intestinal tract; IECs, intestinal epithelial cells; IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; TLRs, toll-like receptors; NOD2, nucleotide oligomerization domain 2.

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with gut epithelial cells and immune cells via toll-like receptors (TLRs) and nucleotide oligomerization domain 2 (NOD2) (Cario, 2005).

LAB are known to have a wide range of effects on the immune system and increasing evidence suggests the possibility of novel LAB-based treatment strategies. Research has found that some macromolecules of LAB are essential ingredients in the pathogenesis of IBD (Fasano & Shea-Donohue, 2005). PGN is a constituent of the bacterial cell wall, and is shed as bacteria divide (Vollmer & Bertsche, 2008). PGN is an indispensable molecule of LAB, is suggested to be important to a probiotic bacterium (Adam, Bharti, & Naidu, 2012). The PGN network in the bacterial cell wall comprises glycan chains of β-1,4-linked MurNAc and GlcNAc cross-linked by short peptide stems of L- and D-amino acids anchored to the lactyl moiety of MurNAc (Schleifer & Kandler, 1972). Research has also found that *lactobacillus* strain-specific anti-inflammatory capacity is correlated with the PGN structure (Fernandez, Pot, & Grangette, 2011). PGN can be detected beyond mucosal surfaces, and their receptor can be expressed in tissues and cells that are far from the niches where bacteria reside. Macrophages, similar to other cells, exhibit heterogeneity in their behavior. They appear to be important during the resolution of inflammation and repair of the intestinal mucosa that occurs during disease remission (Mahida, 2000).

The majority of the LAB used for probiotic purposes belong to the genus *Lactobacillus* and *Bifidobacterium* strains (Reuter, 2001). Strain diversity and the complexity of their interplay with the immune system warrant a careful selection process before using any LAB in clinical trials. Therefore, it is necessary to conduct appropriate *in vitro* studies to characterize and compare the immune modulating capacity of different LAB strains to select the best probiotic strains for use in new clinical studies.

It is understood that there is no universal strain or species that can provide a complete range of benefits. Therefore, the current work focused on screening anti-inflammation activities of three available *Lactobacillus* strains: *Lactobacillus acidophilus* (*L. acidophilus*), *Lactobacillus rhamnosus* (*L. rhamnosus*) and *Lactobacillus casei* (*L. casei*). Molecular science-based screening and characterization assays were used to explore the three PGN's diversity and anti-inflammatory capacity.

2. Materials and methods

2.1. Strains and cells

L. acidophilus, *L. rhamnosus* and *L. casei* were obtained in our laboratory and cultured in de Man–Rogosa–Sharpe (MRS) media. Mouse macrophage cells (RAW 264.7) were kindly provided by Xiang Cao (Nanjing Normal University, China) and incubated at 37 °C in 5% CO_2. HT-29 cells were purchased from Boster Biotechnology (Wuhan, China).

2.2. Regents

Fluorescein isothiocyanate (FITC) was purchased from the Sigma-Aldrich Company (Shanghai, China). Fetal Bovine Serum (FBS) was obtained from Shanghai ExCell Biology, China. Enzyme-linked immunosorbent assay (ELISA) kits were obtained from Nanjing Jiancheng Bioengineering Institute, China. Anti-TLR4 and PE-conjugated rat IgG_{2A} control was purchased from R&D systems, USA. Fura-3/AM, LysoTracker probes (Molecular Probes) were purchased from Beyotime Institute of Biotechnology, China. Other reagents were all of analytical reagent quality.

2.3. Soluble PGN analysis by HPLC

PGNs isolated from *Lactobacillus* strains were described in our previous study (Wu, Pan, Guo, & Zeng, 2013). Samples containing soluble PGN were separated with an Agilent HPLC system. An Aminex HPX-87H column (300 × 7.8 mm) was purchased from Bio-Rad (Shanghai, China). The column was equilibrated with 5 mM

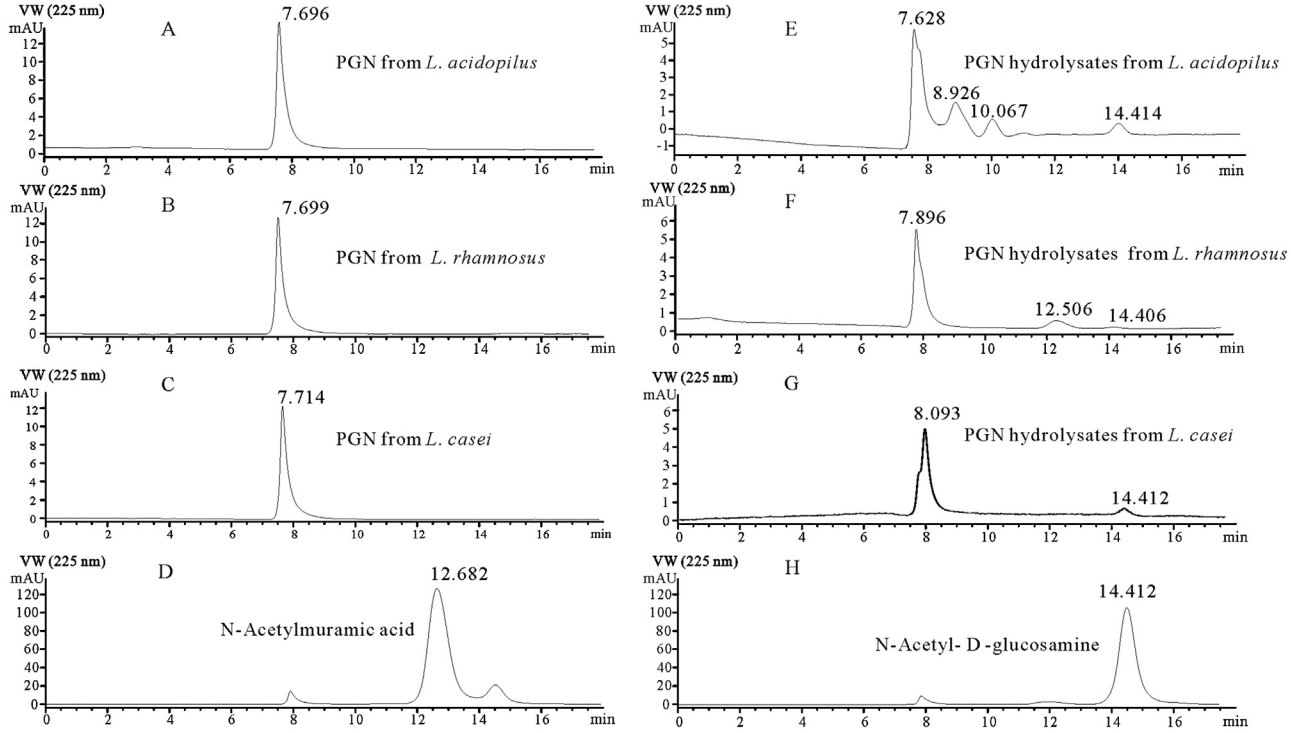

Fig. 1. HPLC profiles of PGN and PGN hydrolysates. (A) PGN of *L. acidophilus*. (B) PGN of *L. rhamnosus*. (C) PGN of *L. casei*. (D) NAM on Aminex HPX-87H column. (E) PGN hydrolysates of *L. acidophilus*. (F) PGN hydrolysates of *L. rhamnosus*. (G) PGN hydrolysates of *L. casei*. (H) NAG on Aminex HPX-87H column.