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Comparative exoproteome analyses of *Lactobacillus* spp. reveals species- and strain-specific proteins involved in their extracellular interaction and probiotic potential

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ABSTRACT

Due to their health-promoting effects, the probiotic applications of lactobacilli have been investigated. Aside from their basic cellular functions, the extracellular activities of probiotic lactobacilli influence their establishment and interaction with the host. Many extracellular proteins play important roles in bacterial colonization and survival in their host's gastrointestinal environment. In this study, we compared the exoproteome of three lactobacillus strains isolated from the gastrointestinal tracts of pigs and chickens, namely, *Lactobacillus mucosae* LM1, *L. johnsonii* PF01, and *L. johnsonii* C1–10. Extracellular proteins collected in the mid-logarithmic growth phase were identified and quantified using a Q Exactive™ Orbitrap mass spectrometer (MS). Of 99 total extracellular proteins, 83% belonged to *L. mucosae* LM1; *L. johnsonii* PF01 strains had fewer extracellular proteins. Enolase, which is involved in the glycolysis pathway and has moonlighting functions in the adhesion of probiotic bacteria, was found in the core exoproteomes of the three strains. The most abundant proteins of each strain were phosphoketolase in *L. mucosae* LM1, aggregation-promoting factor in *L. johnsonii* PF01, and levansucrase in *L. johnsonii* C1–10. The observed differences between these three exoproteomes in terms of cellular and molecular function elucidate the extracellular activities of these isolates.

1. Introduction

Lactobacilli are common in both the proximal and distal regions of the porcine digestive tract, colonizing soon after birth (Valeriano, Balolong, & Kang, 2017; van Baarlen, Wells, & Kleerebezem, 2013). Other than their ability to produce lactic acid, prevent microbial spoilage, and facilitate the treatment and prevention of enteric infections and related diseases, some lactobacilli have been used as probiotics due to their ability to colonize and survive in their host's gastrointestinal tract (Lebeer, Vanderleyden, & De Keersmaecker, 2008; Reid, 1999). This ability has also been instrumental in the role of lactobacilli in modulating the immune response of their host (Ashraf & Shah, 2014; Danielsen et al., 2007; Sánchez, Bressollier, & Urdaci, 2008; Valeur, Engel, Carbajal, Connolly, & Ladefoged, 2004; Weiss et al., 2010).

One key factor that determines the colonization ability of each probiotic is their complement of exported proteins. Some of extracellular proteins play important roles in bacterial adhesion and interaction with the host intestinal surface (Maldonado Galdeano & Perdigon, 2006; Sánchez, Urdaci, & Margolles, 2010). Evaluation of the adhesion abilities of lactobacilli for probiotic applications has led to the discovery of proteins that function at the extracellular level. These proteins include mucin-binding proteins, proteins that associate with the cell surface, and cytoplasmic proteins without signal peptides or surface retention domains (Beck et al. 2009; Espino et al., 2015; Johnson et al. 2015; Kelly et al. 2005; Sánchez et al., 2008). However, these proteins are specifically at the cell wall or the surface layer (Johnson, Selle, O'Flaherty, Goh, & Klaenhammer, 2013; Espino et al., 2015; Celobioglu and Svensson, 2017). Few studies have exclusively investigated the extracellular proteomes of lactobacilli.

We previously characterized two lactobacilli, *Lactobacillus johnsonii* PF01 and *L. mucosae* LM1 (Lee, Pajarillo, Kim, Chae, & Kang, 2013; Valeriano, Parungao-Balolong, & Kang, 2014), and discovered their potential as probiotics (Table 1). Both strains, isolated from pig, showed higher adhesion to porcine mucin than does the *L. rhamnosus* GG

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Table 1
Preliminary characterization of lactobacilli isolates.

Species	Strain	Source	Adhesion to Porcine Mucin	Bile Tolerance (CFU/mL)	Whole Genome Sequence (GenBank Accession)	Proteome Database (UniProt)	References
Lactobacillus johnsonii	PF01	Pig feces	79.5%	10^{5-6} at 0.5% bile for 5 h	CP024781.1	UP000003032	Ahn et al. (2002), Lee et al. (2013), Valeriano et al. (2016)
Lactobacillus mucosae	LM1	Pig feces	82.6%	10^{5-6} at 0.3% bile for 1.5 h	CP011013.1	UP000003645	Pajarillo, Kim, Lee, Valeriano, and Kang (2015), Valeriano et al. (2016)
Lactobacillus johnsonii	C1-10	Chicken intestine	–	–	–	–	–

–, Not yet known; CFU, colony forming unit.

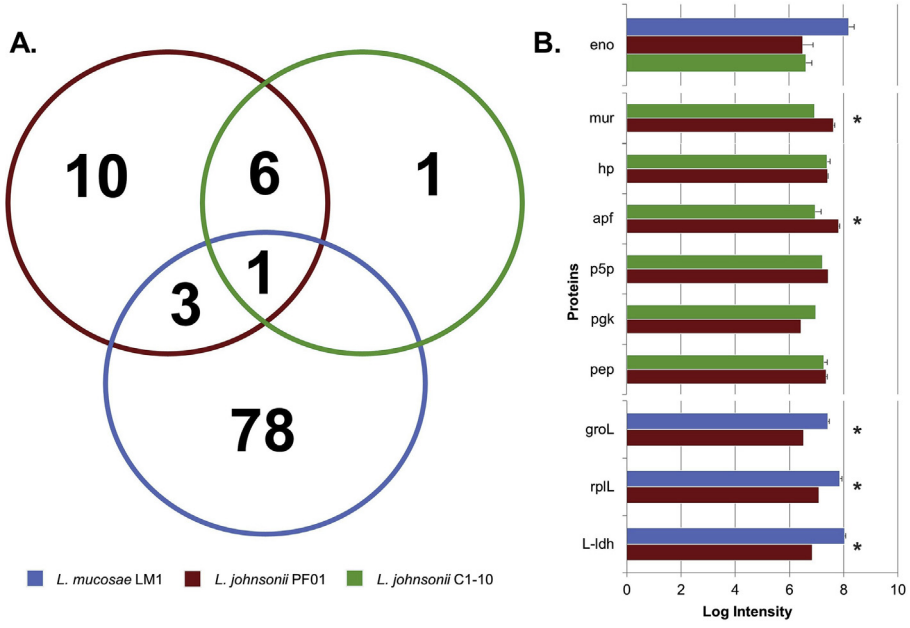

Fig. 1. Extracellular proteins of the lactobacilli isolates were detected in the mid-logarithmic growth phase. (A) The number of extracellular proteins in the three strains. (B) The relative expression level of the proteins found at least two strains, presented as $\log_{10}$ intensity values: *eno*, enolase; *mur*, N-acetylmuramoyl-L-alanine-amidase; *hp*, hypothetical protein; *apf*, aggregation promoting factor; *p5p*, pyridoxamine 5′-phosphate oxidase; *pgk*, phosphoglycerate kinase; *pep*, peptidase; *groL*, molecular chaperone GroEL; *rplL*, 50s ribosomal protein L7/L12; *L-ldh*, L-lactate dehydrogenase. *Significant difference in expression level between strains ($p < 0.05$). Error bars represent standard error of mean.

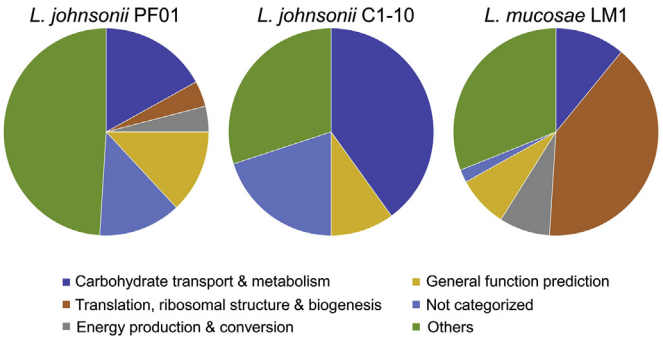

Fig. 2. Cluster of Orthologous Groups-based functional classification of extracellular proteins from *Lactobacillus johnsonii* PF01, C1-10, and *L. mucosae* LM1. At the mid-logarithmic phase, proteins for carbohydrate metabolism are higher for *L. johnsonii* strains whereas *L. mucosae* LM1's exoproteome is mostly composed of proteins for translation, ribosomal structure, and biogenesis.

probiotic (Valeriano et al., 2014). These strains were also proven to adhere to porcine intestinal epithelial cells (IPEC-J2) and to human epithelial colorectal adenocarcinoma cells (Caco-2) well (Valeriano, Bagon, Balolong, & Kang, 2016). In addition, the molecular interactions between LM1 and IPEC-J2 cells have recently been investigated based on their global proteomes, revealing that proteins such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and elongation factor Tu are induced by their interaction (Pajarillo, Kim, Valeriano, Lee, & Kang, 2017).

Thus, we characterized the extracellular proteomes of three *Lactobacillus* strains, including two *L. johnsonii* species and one *L. mucosae* species, using an MS-based approach, to understand their interaction with the extracellular environment. Comparative analyses of the three exoproteomes, leading to identification of their common and unique extracellular proteins, will further our understanding of the probiotic potential of these strains.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Lactobacillus spp. strains previously isolated from pig (*L. johnsonii* PF01, and *L. mucosae* LM1) and chicken (*L. johnsonii* C1-10) were used in this study. All strains were grown in de Man-Rogosa Sharpe (MRS) broth (Difco, France) at 37 °C in anaerobic conditions. Seed cultures for each strain were prepared in 10 mL broth and incubated for 24 h. Fresh seed cultures of the three strains were inoculated into 100 mL MRS broth (1% v/v) and incubated at 37 °C for 24 h. Samples were taken at 2 h intervals for the first 12 h and viable cells were quantified through serial dilution and spread plating into MRS agar plates. The time points identified as when strains were at mid-logarithmic growth phase were used for sample collection and then extracellular protein preparation. Three replicates taken at the same sampling time were prepared for each strain.

2.2. Extracellular protein sample preparation

Cultures of the three lactobacillus strains were inoculated separately