Isolation and Characterization of Exopolysaccharide from *Limosilactobacillus fermentum* FS15.2

Intiranee Suwanrueng and Apinun Kanpiengjai*

Division of Biochemistry and Biochemical Innovation, Department of Chemistry, Faculty of Science, Chiang Mai University

Corresponding author email: apinun.k@cmu.ac.th

ABSTRACT

Prebiotics are non-digestible food ingredients that resist absorption in the gastrointestinal tract but beneficially affect the host by balancing the microbiota within the large intestine, and activity of beneficial microbiota in the colon, thereby improving host's digestive health. Besides prebiotic oligosaccharides, exopolysaccharides (EPS) are alternative potential prebiotics. Lactic acid bacteria are the major EPS-producing microorganisms which play a role in the manufacturing of fermented food products. *Limosilactobacillus* (*L.*) *fermentum* FS 15.2 has been previously characterized as a probiotic lactic acid bacterium. It produces a viscous broth, thereby being presumptively assumed as EPS, when grown in the deMan Rogosa and Sharpe (MRS) medium. This research aimed to isolate and characterize exopolysaccharides (EPS) produced from *L.* fermentum, as a prebiotic. Preliminarily, *L. fermentum* FS 15.2 was grown in MRS broth containing 50 g/L of glucose. After 48 h of cultivation, culture supernatant was harvested for further precipitation and isolation of EPS using absolute ethanol. The EPS yield was recorded at 45.71 mg/50 mL culture as determined by the phenol-sulfuric acid method. It was found that MRS broth containing 50 g/L of sucrose and 1% NaCl was found as an optimal medium for EPS production. *Limosilatobacillus fermentum* FS15.2 produced the highest EPS content of 126.78 mg/50 mL culture at 48 h of cultivation. The extracted EPS showed high resistance to simulated gastrointestinal digestion as its residual EPS ranging from 96–97% and 87–88% were retained after the simulated gastric and intestinal condition, respectively. In addition, this EPS was partially fermentable by eight strains of probiotic lactic acid bacteria. In summary, EPS possesses primary properties of prebiotics however, other properties are recommended to further explore.

BACKGROUND

Exopolysaccharides (EPS) are extracellular macromolecules excreted as tightly bound capsule or loosely attached slime layer in microorganisms.

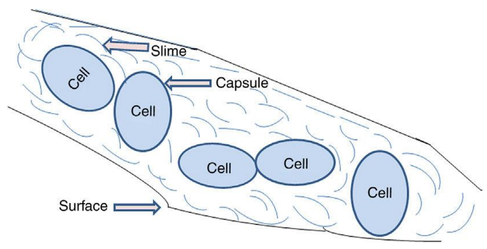


Figure 1 Forms of Exopolysaccharides (EPS) (Jindal & Singh Khattar, 2018)

Limosilactobacillus (L.) fermentum FS 15.2

↓

Exopolysaccharide (EPS)

↓

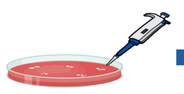
Prebiotics ??



Figure 2 Biological and functional activities exerted by EPS produced by LAB (Jurášková et al., 2022)

METHODS

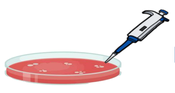
1. Preliminary EPS production by *L. fermentum* FS 15.2



MRS-glucose 50 g/L, 400 mL → Incubation at 37 ºC for 48 h

Culture broth:
- Isolation of EPS
- Measurement of OD_{600}
- Determination of RS (DNS method) (Miller et al., 1959)

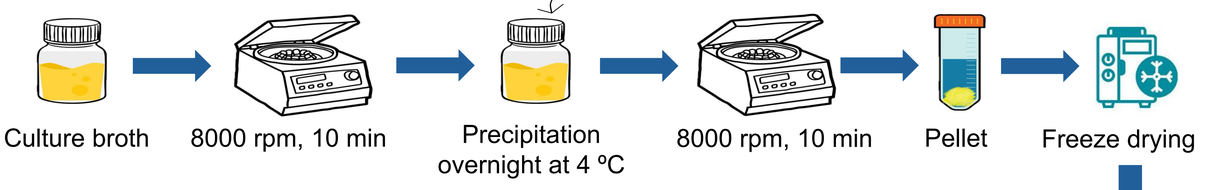
2. Effect of sucrose and NaCl concentrations on EPS production



MRS-sucrose + NaCl 0%, 0.5%, 1%, 2% and 4% → Incubation at 37 ºC for 48 h

Culture broth:
- Isolation of EPS
- Measurement of OD_{600}
- Determination of RS (DNS method)

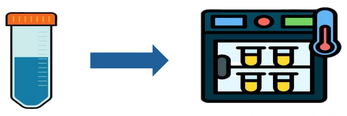
3. Isolation of EPS from cell culture



Culture broth → 8000 rpm, 10 min → Precipitation overnight at 4 ºC → 8000 rpm, 10 min → Pellet → Freeze drying → **EPS powder**

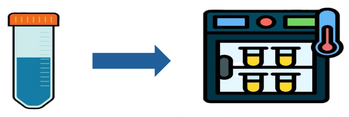
4. Digestibility

Gastric phase



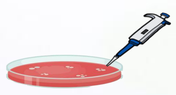
SGF solution + EPS → Incubation at 37 ºC for 2 h

Intestinal phase



SIF solution + Gastric chyme-EPS → Incubation at 37 ºC for 3 h

5. Fermentation



Probiotics: FS2.2, FS15.2, FS41.1, FS46.2, FS48.1,FS49.2, CG403, CG501 → MRS-EPS, 5 mL → Incubation at 37 ºC for 48 h

- Measurement of OD_{600}
- Measurement of pH

RESULTS

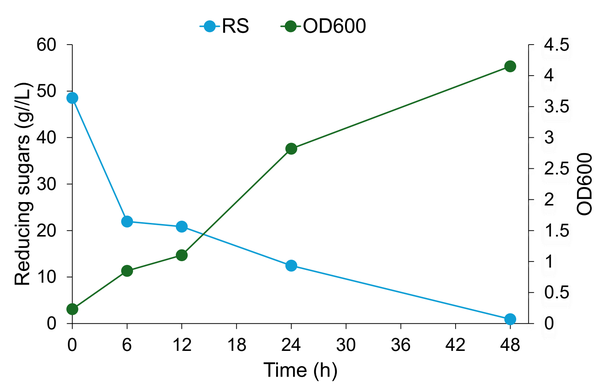


Figure 3 Time-course of cell growth and reducing sugar concentration during cultivation of *L. fermentum* FS15.2 in MRS broth at 37 °C.

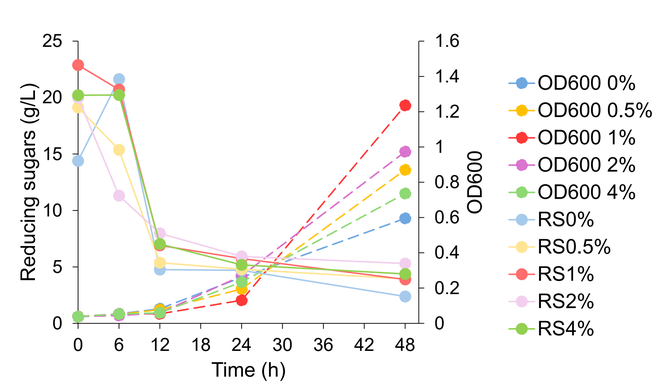


Figure 4 Time-course of cell growth and reducing sugar concentration of *L. fermentum* FS15.2 during growth in different culture conditions.

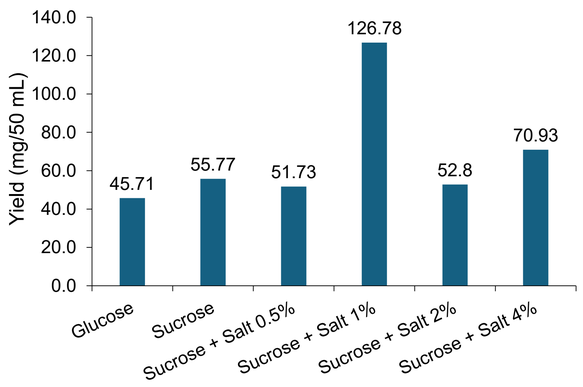


Figure 5 Comparison of EPS yields (mg) under different conditions.

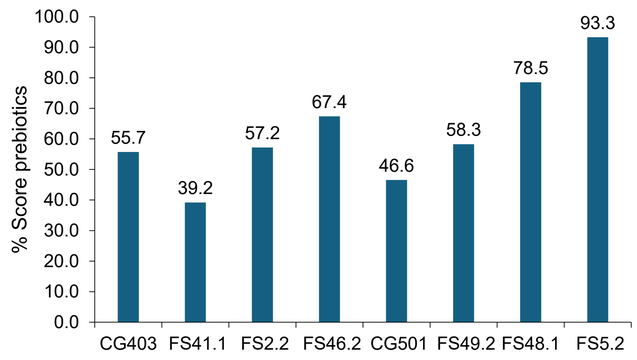


Figure 7 Evaluation of prebiotic scores towards 8 probiotic strains utilizing EPS as the sole carbon source.

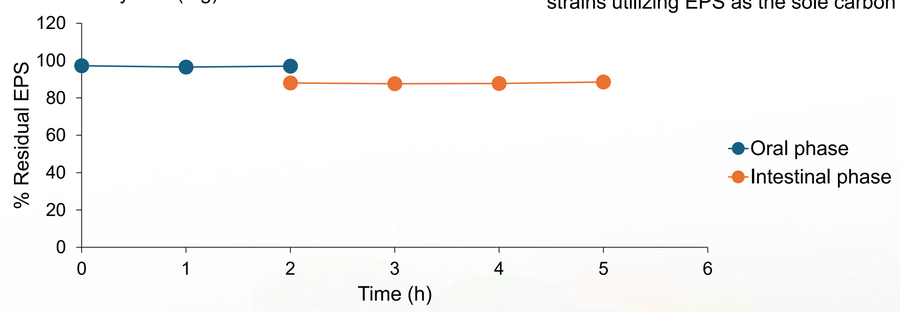


Figure 6 %Residual EPS after in vitro simulated gastric and intestinal conditions

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CONCLUSION

- The highest EPS yield of 126.78 mg/50 mL was achieved using sucrose as the sole carbon source supplemented with 1% NaCl
- The EPS exhibited high gastrointestinal stability, with residual percentages of 96 – 97% and 87– 88% in the gastric and intestinal phases, respectively
- The fermentation of EPS by FS15.2 yielded the highest prebiotic score at 93.3%, indicating its superior prebiotic potential toward its own producer strain

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- Jurášková, D., Ribeiro, S.C., & Silva, C.C.G. (2022). Exopolysaccharides Produced by Lactic Acid Bacteria: From Biosynthesis to Health-Promoting Properties. Foods, 11(2): 156