Selection of Promising Exopolysaccharides-Producing Starter Cultures for Gluten-Free Sourdough

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Introduction

Celiac disease is an autoimmune enteropathy that affects the small intestine and is caused by the gliadin fraction of wheat gluten and other alcohol-soluble proteins (prolamines) of barley and rye in genetically predisposed subjects. Although the market of gluten-free (GF) products has been increased in the last few years, their improvement is still a challenge for the food industry. Considering the GF bakery products, some of them can result in unappetizing taste with high-fat content. These inconveniences can be minimized or solved by using sourdough fermentation.

Sourdough is a stable culture of lactic acid bacteria (LAB) and yeast in a mixture of flour and water. In particular, GF sourdough represents a rich source of naturally occurring LAB strains and wild yeasts which play important roles in food fermentation. This is mainly due to their potential in improving functional, technological, and probiotics properties contributing to safe and more tasty food. Moreover, exopolysaccharide (EPS)-producing strains can contribute to improving the sensory and rheological quality of the different GF products as well [1].

This study aims to isolate and characterize the microbial pool from a GF sourdough made with rice flour and to evaluate the ability of selected LAB strains to produce EPS for producing an innovative starter culture.

Methodology

GF sourdough samples

↓

Homogenization in Stomacher x 2 min
(90 mL 0.9% saline solution with 10 g of samples)

↓

Serial dilutions

↓ Growth on selective agar media (MRS and YPDA)

Purification and isolation of 110 strains

Phenotypical characterization
- Gram staining
- Catalase test
- Fermentative test
- Morphology observation

Molecular characterization [2]
- Extraction of gDNA
- Species-specific PCR
- $(GTG)_5$-PCR fingerprinting
- Sanger sequencing of 16S rRNA gene
- Identification of 25 LAB

EPS characterization
- Preliminary screening of ropiness/slimy colonies for EPS production
- Extraction of EPS following the protocol by [3]
- Analysis of the high yield producing strains (PP_34, LP_42, and LP_50) by:
  - Fourier-transform infrared spectroscopy (FT-IR)
  - Scanning electron microscopy (SEM)
  - X-ray diffraction (XRD)

Results

Molecular and phenotypical characterization

Among the 110 isolated strains tested for the production of EPS only three LAB with the highest production yield were selected and characterized. The results are shown in table 1.

Table 1. Results of the molecular characterization of the three strains and their EPS production yield.

Code	Species	EPS yield (g/L)	Morphology	Gram staining	Catalase test	Fermentative test
PP_34	*Pediococcus pentosaceus*	1.64	Cocci	Positive	Negative	Positive
LP_42	*Lactiplantibacillus plantarum*	1.14	Rod	Positive	Negative	Positive
LP_50	*Lactiplantibacillus plantarum*	4.88	Rod	Positive	Negative	Positive

XRD

XRD analyses of EPS-like compounds produced by *P. pentosaceus* PP_34 and *L. plantarum* LP_42 are presented in the figures 1a and 1b, respectively. The EPS produced by both strains showed a single large diffraction peak at 21°.

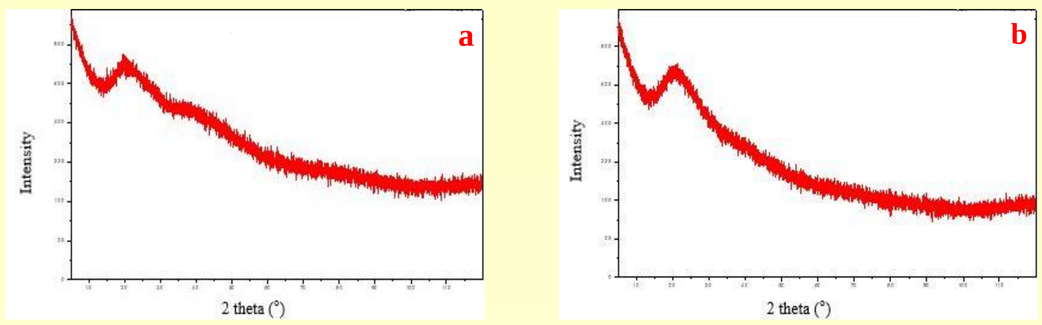

Figure 1. XRD analysis of EPS produced by (a) *P. pentosaceus* PP_34 and (b) *L. plantarum* LP_42.

SEM

SEM showed cracks and irregular microstructures and in particular EPS produced by *L. plantarum* LP_42 showed rough and a more inhomogeneous surface morphology (Figure 2b).

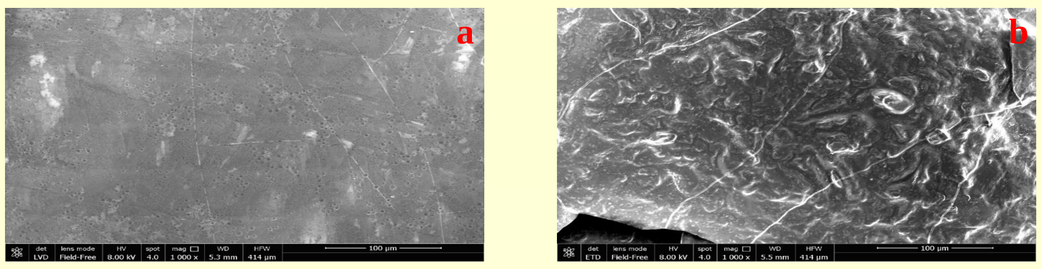

Figure 2. Surface SEM images of EPS produced by (a) *P. pentosaceus* PP_34 and (b) *L. plantarum* LP_42.

FT-IR

The FT-IR spectra of EPS produced by *L. plantarum* LP_50 and *P. pentosaceus* PP_34 showed characteristic peaks at 900-1100 cm^{-1} and 3000-3500 cm^{-1} (Figure 3) [4].

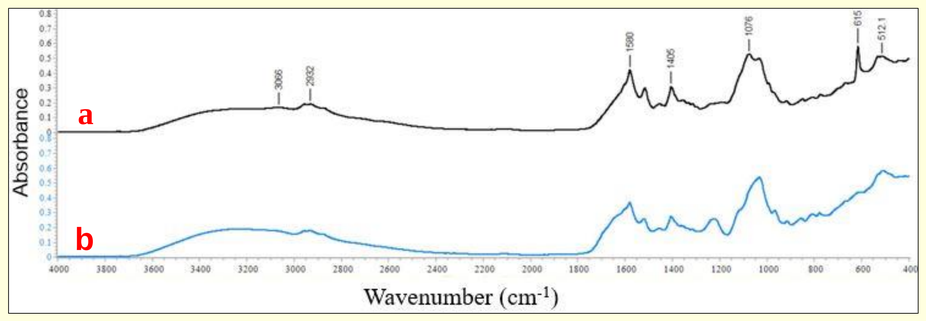

Figure 3. FT-IR spectra of (a) *P. pentosaceus* PP_34 and (b) *L. plantarum* LP_50.

Conclusion

The natural microbial biodiversity of sourdoughs and the ability of these microorganisms for producing EPS or EPS-like compounds can be successfully exploited for the production of both traditional and gluten-free baked products. In this preliminary study, a total of 110 LAB isolated from GF sourdough. Among them, high EPS yield was only obtained by three strains which were identified as *Pediococcus pentosaceus* and *Lactiplantibacillus plantarum*. The preliminary analysis performed on these two strains are in accordance with the previous literature and confirmed the presence of EPS with irregular surface microstructure and the typical EPS peaks obtained by the FTIR and XRD analysis. However, further studies are required in order to improve the extraction protocol and to define the composition of these EPS-like compounds.

References

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