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Stabilization of *Lactiplantibacillus plantarum* WCFS1 during freezing, freeze-drying, and storage using legume wastewater extracts

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Title

Stabilization of *Lactiplantibacillus plantarum* WCFS1 during freezing, freeze-drying, and storage using legume wastewater extracts

Keywords galacto-oligosaccharides, legumes, lactic acid bacteria, stabilization

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Abstract

The soaking and cooking wastewaters obtained from the treatment of legumes before consumption represent a form of environmental pollution and loss of valuable resources. These wastewaters are rich in galacto-oligosaccharides (GOS) that are known for their prebiotic potential. They can also be used to stabilize and protect bacteria during preservation processes (freezing and freeze-drying) and during storage, increasing their shelf-life. In this work, the cryoprotective potential of chickpea and lentils wastewaters was assessed towards *Lactiplantibacillus plantarum* WCFS1, a probiotic lactic acid bacteria strain.

Dry and soaked chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* M.) seeds were cooked for 30 min, obtaining GOS-rich wastewaters. Their carbohydrate composition was determined with High Pressure Liquid Chromatography with Refractive Index detection.

Lactiplantibacillus plantarum WCFS1 was grown in de Man, Rogosa, and Sharpe (MRS) medium, and was then used for fermentation in a 5 L bioreactor. 10 g of GOS protective solution was added to 5 g of biomass and the mixtures were frozen at -80 °C. They were then lyophilized for 48 h. Protected dried cells were then kept 1 week in storage at 25 °C. Culturability measurements (CFU/mL) in MRS agar and acidifying activity assessments (dtpH1.5) by CINAC method were performed before freezing and after freezing, lyophilization, and storage. Sucrose and a commercial FOS mixture (Beneo Orafiti) were used as reference materials.

CFU/mL values decreased in all samples in similar proportion after each step and, consequently, the dtpH1.5 values increased accordingly, however GOS extracts were capable of preserving bacterial activity throughout all cryoprotection steps.

This work demonstrates GOS wastewaters from legume processing have the potential to be included in the industrial production of probiotic lactic acid bacteria for its stabilization during storage minimizing waste production and environmental impact and maximizing economic gain.

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