# Valorization of Agro-Industrial By-Products and Food Waste for Sustainable Biomass and Functional Ingredient Production


## Grigorios Nelios and Yiannis Kourkoutas

*Laboratory of Applied Microbiology and Biotechnology, Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece.*

*Email: ikourkou@mbg.duth.gr*


## Introduction

The growing need for sustainable and low-cost biotechnological processes has increased interest in the use of ago-industrial by-products and food waste. These materials are often discarded but can be used as alternative substrates for microbial biomass production, supporting circular economy approaches [1]. At the same time, maintaining the stability and viability of beneficial microoganisms remains a major challenge for their use in food and feed products [2]. In this study, agro-industrial by-products and food waste were evaluated for biomass production and cell immobilization of *Lacticaseibacillus rhamnosus* OLXAL-1 strain with the aim of developing functional food ingredients aligned with a sustainable production approach.

## Materials and methods

The wild-type strain *L. rhamnosus* OLXAL-1 was selected based on its documented *in vitro* functional properties, antagonistic activity against pathogens and spoilage microorganisms, resistance to simulated gastrointestinal conditions, and compliance with EFSA safety requirements [3]. A range of agro-industrial by-products and food waste, including expired fruit juices, dairy by-products, and formulated mixtures, were evaluated as alternative growth media. Biomass production, maximum specific growth rate (*μmax*), and viable cell counts were determined and compared with conventional laboratory growth media.

For cell stabilization, natural supports derived from agricultural residues and fiber-rich food matrices were tested as immobilization supports. Immobilization efficiency was assessed by determining the viable immobilized cell load. The effect of freeze-drying on the viability of free and immobilized cells was subsequently examined.

## Results

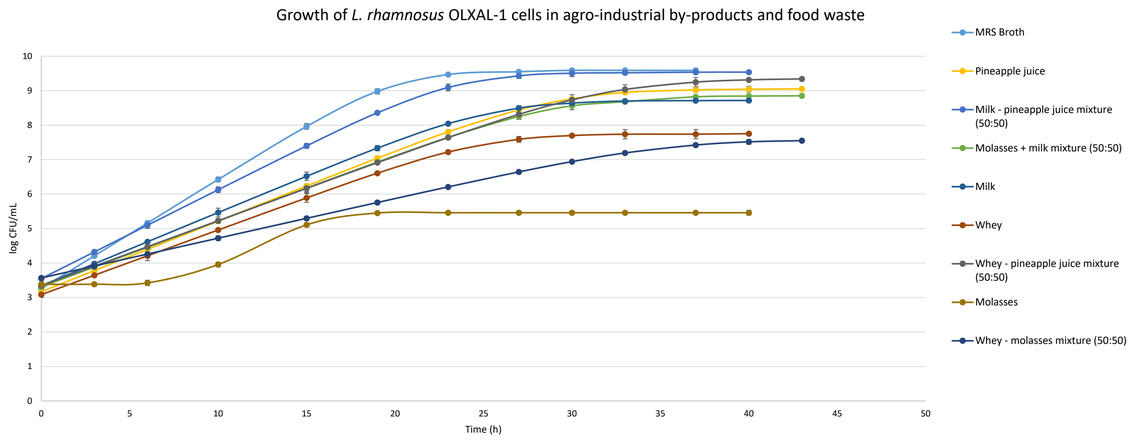
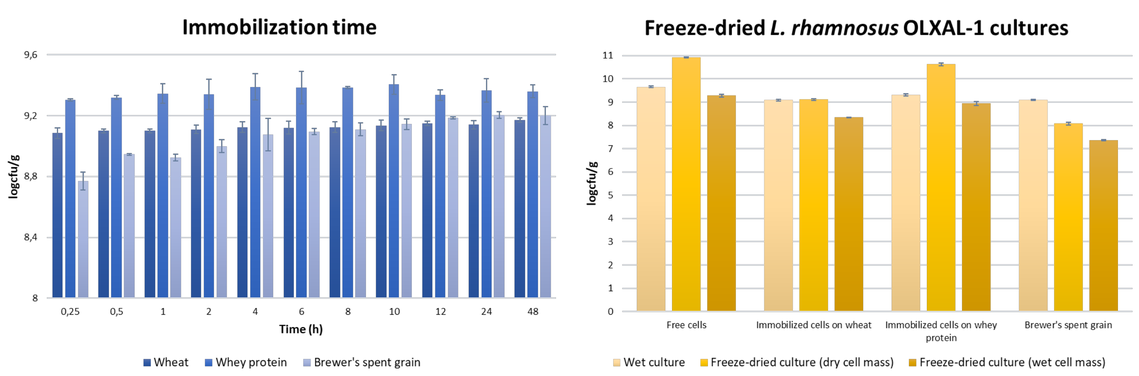



**Figure 1.** Cell concentrations (log CFU/mL) of *L. rhamnosus* OLXAL-1 during growth in agro-industrial residues and food waste compared with synthetic food grade media.

**Table 1.** Maximum cell concentration (log CFU/mL) and maximum growth rate – $\mu_{max}$ ($h^{-1}$) of *Lacticaseibacillus rhamnosus* OLXAL-1 strain in agro-industrial residues, food waste and synthetic food grade media.

| Growth medium | Initial cell concertation (log CFU/mL) | Maximum cell concentration (log CFU/mL) | Maximum growth rate - $\mu_{max}$ ($h^{-1}$) |
|---|---|---|---|
| MRS Broth | 3.45±0.07 | 9.58±0.04 | 0.317 |
| Synthetic food grade medium - Procelys | 3.42±0.04 | 9.68±0.03 | 0.334 |
| Synthetic food grade medium - Sensient | 3.42±0.05 | 9.53±0.03 | 0.320 |
| Pineapple juice | 3.23±0.04 | 9.05±0.08 | 0.205 |
| Milk – pineapple juice mixture (50:50) | 3.42±0.08 | 9.54±0.06 | 0.258 |
| Molasses – milk mixture (50:50) | 3.10±0.14 | 8.85±0.04 | 0.191 |
| Milk | 3.38±0.04 | 8.72±0.02 | 0.212 |
| Whey | 3.05±0.07 | 7.75±0.04 | 0.188 |
| Whey – pineapple juice (50:50) | 3.39±0.08 | 9.34±0.03 | 0.188 |
| Molasses | 3.41±0.05 | 5.46±0.05 | 0.244 |
| Whey – molasses mixture (50:50) | 3.38±0.04 | 7.59±0.07 | 0.115 |




(a) (b)

**Figure 2.** Determination of (a) the optimal time for cell immobilization on agro-industrial residues; (b) immobilized cell loads (logcfu/g) after freeze-drying.

(a) (b) (c)

**Figure 3**. Freeze-dried immobilized *Lacticaseibacillus rhamnosus* OLXAL-1 cells on (a) brewer's spent grain, (b) wheat, and (c) whey protein.

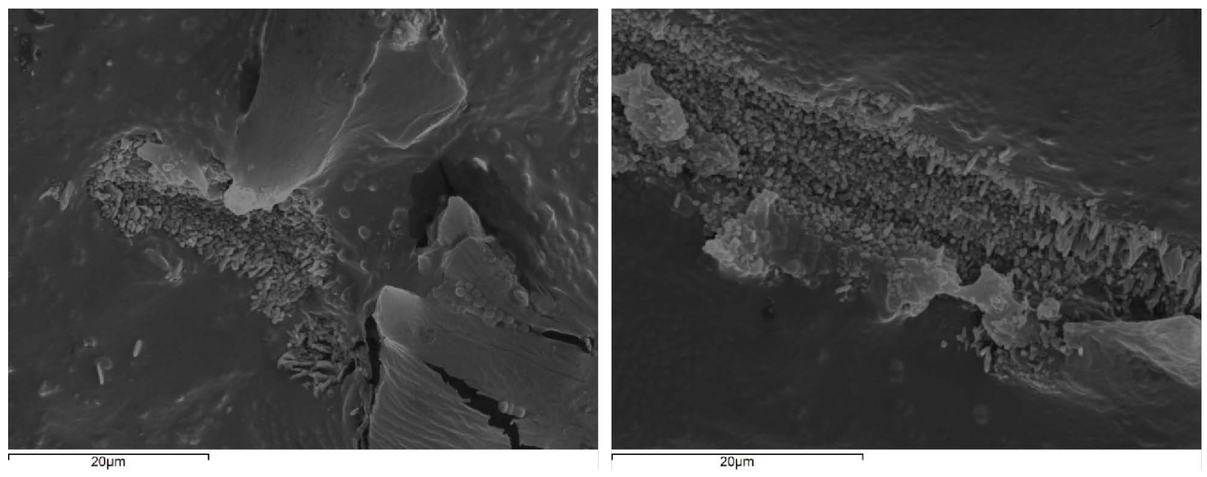



**Figure 4**. Scanning electron microscopy (SEM) images of immobilized *L. rhamnosus* OLXAL-1 cells on BSG (left) and wheat (right).

## Conclusions and perspectives

- High *L. rhamnosus* OLXAL-1 cell counts (> 9 logcfu/mL), similar to commercial MRS Broth and synthetic food-grade media, were achieved when pineapple juice, whey-pineapple juice mixture (50:50) and milk-pineapple juice mixture (50:50) were used as cultivation media.
- Expired milk – pineapple juice mixture exhibited the highest maximum growth rate – $\mu_{max}$ ($P < 0.05$), among the tested growth media consisting of agro-industrial wastes and food residues.
- Cell populations of freeze-dried immobilized *L. rhamnosus* OLXAL-1 cultures on whey protein, wheat and brewer's spent grain were recorded at levels > 8 logcfu/g.
- Agro-industrial residues and food waste proved effective microbial growth cultivation substrates and immobilization carriers of probiotic cells.
- Freeze-dried immobilized probiotic cell cultures on whey protein, wheat and brewer's spent grains were considered as promising "readily-marketable" functional ingredients to be incorporated in food or animal feed products.

## References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