Evaluation of diversified bioprocessing schemes for biosurfactants production from *Lactobacillus* strains using cheese whey

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Biosurfactants or microbial surfactants constitute a group of amphiphilic molecules, comprising of both a hydrophobic (e.g. long-chain fatty acid, hydroxyl fatty acid) and a hydrophilic moiety (e.g. carbohydrate, amino acid, peptides, phosphate, alcohol).
Microbial surfactants

- Biodegradable-Environmentally benign
- Surface active properties
- Moderate to low toxicity
- Numerous applications

Biosurfactants

Biosurfactant production

Bacteria, yeast and fungi

Bioremediation-
Environment

- Soil washing
- Pharmaceuticals
- Food industry
- Cosmetic formulations
- Agriculture

However...

Industrial production is hindered

- High cost of production-cost of raw materials
- Pathogenic strains-restrict food applications
- Characterisation of the produced structures
- Low productivities
- Downstream separation
Biosurfactants

- Surface activity properties
- Effective Critical micelle concentration
- Stability to several factors: pH, temperature, salt concentrations

Significant characteristics specifically for the food industry

Potential applications the food industry

- Antimicrobial agents
- Biofilm formation inhibition
- Emulsifying agents
- Antioxidant properties
- Novel food formulations

Could replace the chemically derived counterparts
Biosurfactants market

In 2018, the global biosurfactants market was > $1.5 billion

Global market size and growth forecast by product type

Market share and forecast by applications

Misailidis, N., Petrides, D. Intelligen, Inc.

Biosurfactants

Cost competitive BS production

✗ High cost of production-cost of raw materials

億 Utilisation of renewable resources
(e.g. agro-industrial waste and by-product streams)

- Molasses
- Waste frying oil
- Glycerol
- Winery by-products
- Lignocellulosic biomass
- Cheese whey

Annual Operating Cost Breakdown of a plant producing rhamnolipids via fermentation with *Pseudomonas* strain

Misailidis, N., Petrides, D. Intelligen, Inc.
Biosurfactants

Cost competitive BS production

✗ Pathogenic strains-restrict food applications

≤ Identification of novel GRAS strains (e.g. lactobacilli strains)

≤ Isolation of strains found in the microbiota of fermented foods
  (e.g. dairy industry)

☐ Establishing novel end product formulations with increased added value will
  mediate the sustainability of BS production

☐ Integration in biorefinery concepts within the concept of circular bio-economy
Twofold approach of this study:

A. Identification of potential biosurfactant producers selected from lactobacilli isolated from several sources

- Four different culture collections were employed

Screening for BS producers

- Ten (10) strains were selected after the screening

Evaluation of bioprocessing strategies

- Microtiter fermentations
- Shake flasks
- Bioreactor studies
Renewable resources for BS production

B. Implementation of cheese whey as a low-cost fermentation feedstock

Cheese whey: by-product stream of the dairy industry

Mainly water, but also:
- lactose (66–77%, \text{w/w})
- protein (8–15%, \text{w/w})
- minerals salts (7–15%, \text{w/w})

\( \sim 9 \text{ L of whey are obtained for every } 1 \text{ kg of cheese produced} \)

*Cheese whey-integrated biorefining approaches within circular economy*

Lappa et al., Foods 2019, 8, 347.
Experimental design for BS production

LAB culture on commercial medium and cheese whey→ Biosurfactant evaluation

- Surface tension measurements
- Blood agar test- Haemolytic activity
- Oil displacement test
- Emulsification index ($E_{24}$, $E_{48}$)
- Lactose consumption

LAB BS production→ Extracellular or cell-bound

Supernatant and PBS-extracts were tested→ Selection of potential BS producers

Several methods for screening of LAB strains
Experimental design for BS production

- Selection of potential BS producers

A. Shake flask fermentations

B. Microplate experiments

C. Bioreactor fermentations

Effect of pH and incubation temperature (°C)

Effect of selected nitrogen sources and micronutrients ($\mu$, $h^{-1}$)

Controlled pH and temperature
Shake flask fermentations

- Cheese whey was the sole fermentation substrate
- Initial lactose: ~20 g/L, pH: 4, 5, 6.8 and T: 25, 30, 37 and 40 °C

Lactose consumption was significantly low and fermentation was prolonged

$E_{24}(\%)$ was higher at pH 5 and pH 6.8 and T:37 and 40 °C

pH 4 and T:25 °C did not sustain microbial growth

Low Lactic Acid (LA) and Total Dry Weight (TDW, gL⁻¹) production
<table>
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<tr>
<th>Strain</th>
<th>T&lt;sub&gt;f&lt;/sub&gt; (h)</th>
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<th>Lactic acid (g/L)</th>
<th>TDW (g/L)</th>
<th>E&lt;sub&gt;24&lt;/sub&gt; PBS extracts (%)</th>
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Microplate experiments

- Cheese whey was supplemented with several nitrogen sources
- Yeast extract, peptone, beef extract and their combinations

The combination of yeast, peptone and beef extract indicated higher specific growth rates
Cheese whey was supplemented for further experiments

Medium D: yeast extract 4 gL⁻¹, peptone 10 gL⁻¹, beef extract 8 gL⁻¹
Medium F: yeast extract 4 gL⁻¹, 5.3 gL⁻¹ ammonium citrate, peptone 10 gL⁻¹
Microplate experiments

- Cheese whey was also supplemented with specific micronutrients: Ca, Mg, Mn, and two combinations of micronutrients.
- Initial lactose: \(\sim 20\) g/L, pH: 6.8 and T: 37 °C

![Absorbance vs. Fermentation Time](image)

- The addition of Ca improved microbial proliferation, followed by Mg
Shake flask fermentations

- Cheese whey was supplemented with yeast extract, peptone and beef extract
- Initial lactose: ~20 g/L, pH: 5, 6.8 and T: 30, 37 and 40 °C

In most cases pH 5 led to lower BS production
T:40 °C inhibited BS secretion in some strains

Bioreactor studies were performed with less strains to further study the fermentation conditions
Bioreactor fermentations

*Lacticaseibacillus rhamnosus CECT 278*

Significant reduction occurs the first hours
Bioreactor fermentations

*Limosilactobacillus fermentum* ACA DC 183

Cheese Whey with yeast extract and peptone

- **Lactose**
- **Surface Tension**
- **Lactic acid**
- **TDW**

Cheese Whey
Bioreactor fermentations

*Limosilactobacillus fermentum* ACA DC 183

**Cheese Whey**

Maximum reduction in ST occurs the first hours of fermentation when TDW increases two-fold

Crude BS increase along with biomass increase
Preliminary BS characterisation

Downstream of BS

Extraction of cell bound BS with PBS ➔ Centrifugation ➔ Dialysed with membranes ➔ Freeze Drying ➔ Crude BS extract ➔ Characterisation

Surface tension measurements

Ninhydrin

protein-based BS molecule

60-70% protein content
20% carbohydrates

Protein and Carbohydrates content
Bioreactor fermentations

*Limosilactobacillus fermentum* ACA DC 183

**Critical Micelle Concentration (CMC)**

- **CMC**: 0.08 mg/mL

**Biosurfactant concentration (mg/L)**

**Surface Tension (mN/m)**

**Antimicrobial activity against fungal strains**
Future studies

Evaluation of bioprocessing strategies

Target: To further increase biomass production within the first hours

- Evaluate aeration conditions (facultative anaerobic strains)
  Trigger biomass production instead of lactic acid
- Evaluate repeated fed-batch
- Stability studies and antimicrobial activity to identify potential applications
- Detailed characterisation of produced BS
- Investigate emulsion stability and surface tension for cosmetic/food formulation
Thank you for your attention

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