Innovative polysaccharides as matrices for encapsulation of lactic acid bacteria and antimicrobial peptides

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BlueRemediomics

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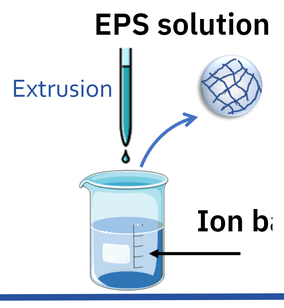
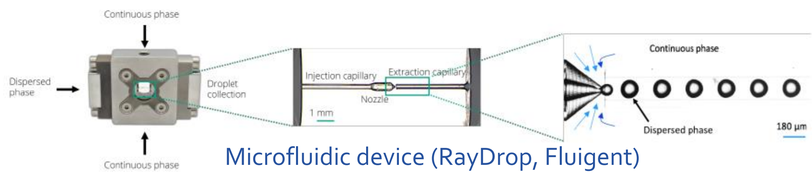
Introduction

- Encapsulation of lactic acid bacteria (LABs) is an innovative process for food biopreservation and biomedical field. LABs produce bacteriocins that inhibit undesirable and pathogenic bacteria.
- Polysaccharides can be used to make microgels; their physico-chemical and biological properties are mainly based on their structural characteristics such as osidic composition, anionic nature, molecular weight.
- The depths of the ocean remain an untapped reservoir of new organisms and compounds. Only a few exopolysaccharides (EPS) have been described up to now from deep-sea bacteria.

Aim

→ Isolate new EPS from Ifremer's bacterial culture collection and study gel formation through two processes: extrusion and emulsification (capillary microfluidics) in ion bath (Ca^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Mn^{2+}).

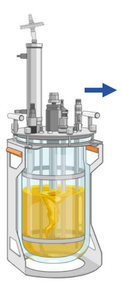




Microfluidic device (RayDrop, Fluigent)

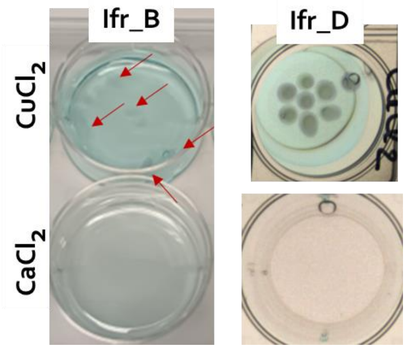
Screening of exopolysaccharides

Culture on Zobell
Glucose 30 g/L
25°C, 150 rpm, 48h



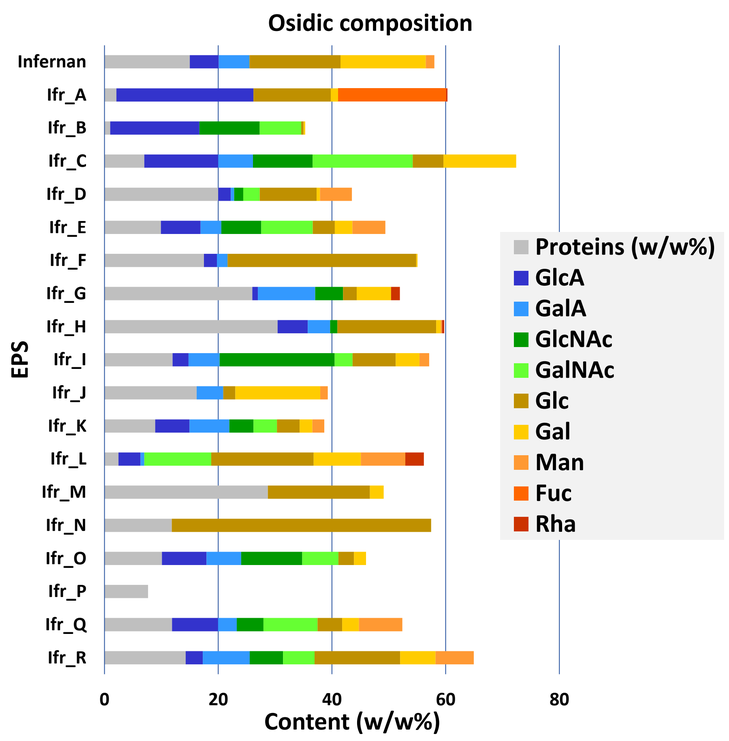
Centrifugation
Filtration
Ultrafiltration
Lyophilization
→ Biochemical analysis

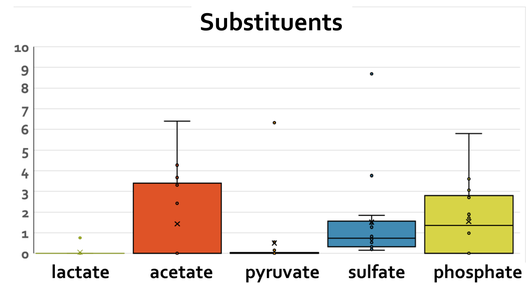
Screening of gelling by extrusion



- Stable beads formed in the copper bath by extrusion with Ifr_B and Ifr_D
- But Ifr_B and Ifr_D too viscous for microfluidics

Chemical analysis of EPS





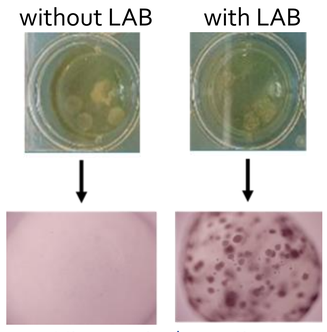
- 20 new strains were screened for EPS production. Infernan is our lab reference (Roger et al., Carbohydr Res, 2004)
- Uronic acids and anionic substituents are crucial for ionic gelling.

Encapsulation of LAB in polysaccharide macroscopic beads and microgels

Infernan, a lab reference EPS, was firstly depolymerised to enhance its gelling properties with calcium and then mixed with alginate to obtain a strong gel through ionic cross-linking in $CaCl_2$ bath. *Carnobacterium divergens* V41 producing an antimicrobial peptide, diversin, was then encapsulated.

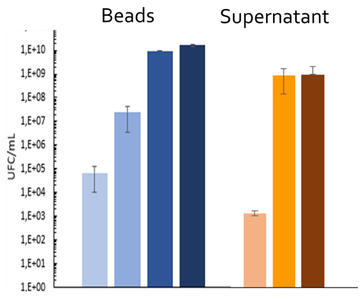
Extrusion (macroscopic beads)

Control without LAB | Beads with LAB

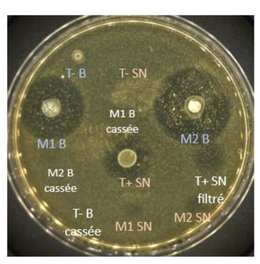


Microscopic observation: beads ~4 mm in diameter

Enumeration of LAB in beads at 0, 6, 24 and 48 hours



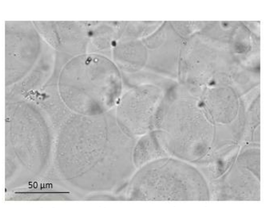
Inhibition of *Listeria innocua* by LAB encapsulated in beads at 24h



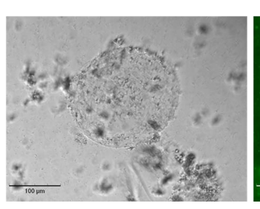
Emulsification by microfluidics (microgels)

Microscopic observation of infernan-alginate microgels

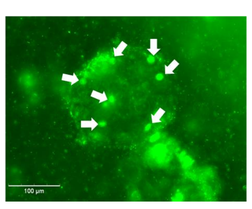
Control | **With encaspulated LAB at 48h**



Monodispersed gels: 60 µm ± 13 µm (n=50)



Microgels



Live LAB cells (SYTO9)

- Exponential growth of LAB inside beads incubated in TSB medium
- Slow release of LAB outside microgels.
- No inhibition with control.
- Inhibition of *Listeria* strain by encapsulated LAB and released solution.

Mixed infernan-alginate beads and microgels provide a favorable environment for bacteria, stimulating both their proliferation and their ability to secrete bacteriocins.

Future work

- Screen other EPS for gelling
- Cross-linking with different types of ions and ion mixtures, co-gelling with alginate if necessary
- Decrease molecular weight to improve gelling
- Detection of bacteriocin (tricine PAGE, growth inhibition quantification in liquid broth)
- Applications in food preservation (challenge tests)

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