Alginate lyase production from native alginate degrading microorganisms

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Introduction

- South Africa's west coast is abundant in *Ecklonia maxima* (sea bamboo) and *Laminaria pallida* (split fan kelp) – **Brown Seaweeds**
- **Alginate lyases (AL)** are promising enzymes for use in application of brown seaweeds in the food, agricultural, and pharmaceutical industries
  - Enzymatic hydrolysis of alginate into alginate oligosaccharides – bioactive lower MW products
- Need to isolate and identify native AL producing microorganisms

Screening and Isolation

✓ Screened abalone gut (*Haliotis midae*), sea urchin gut (*Parechinus angulosus*), sea bamboo (*Ecklonia maxima*), and sand flea (Genus: *Orchestia*)

✓ Isolated microorganisms were confirmed for extracellular alginate lyase expression.
- Grams iodine plate assay

✓ Identified using 16S and ITS rRNA

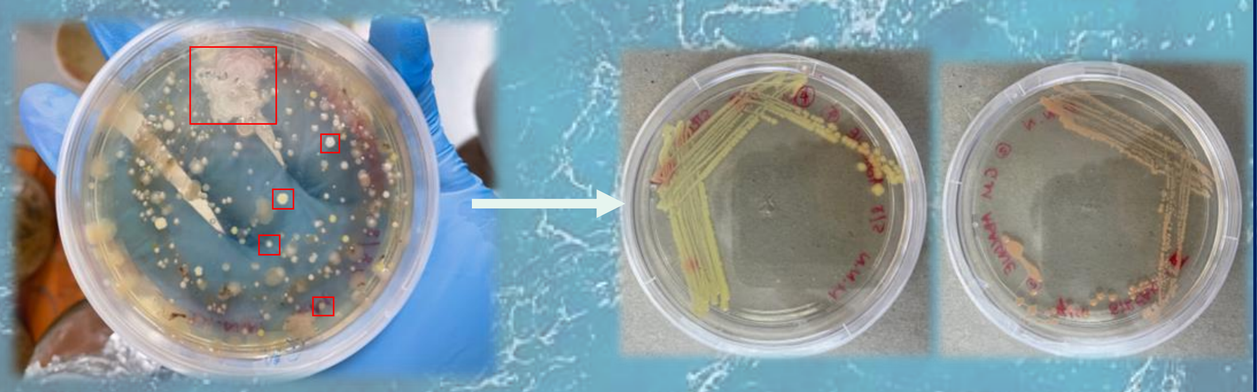

Figure 1: Different colonies streaked to purity.

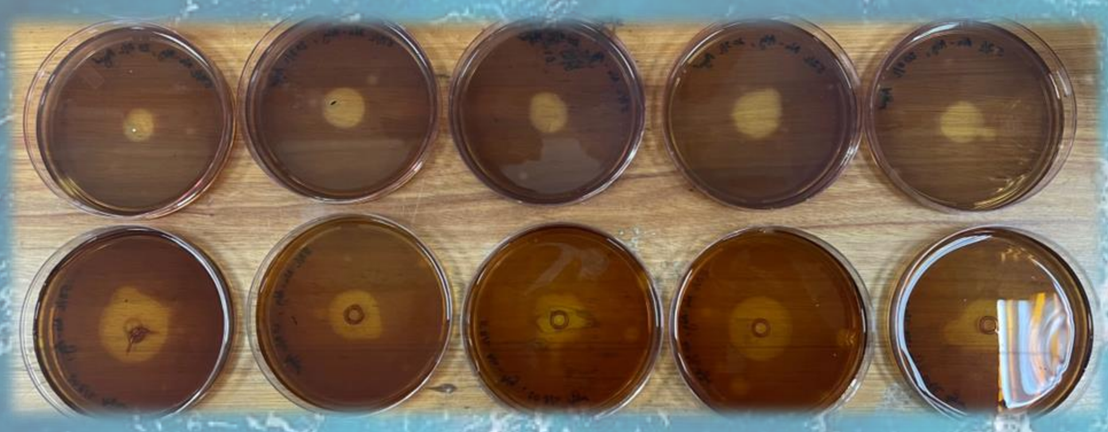

Figure 2: Plate assay indicating alginate lyase activity.

✓ Genus of identified isolates confirm for alginate lyase production
- Bacteria - *Pantoe* sp. (1/7), *Lysinibacillus* sp. (1/7), *Stappia* sp. (1/7), *Bacillus* sp. (3/7)
- Yeast – *Rhodotorula* sp. (1/7)

Selection & AL production

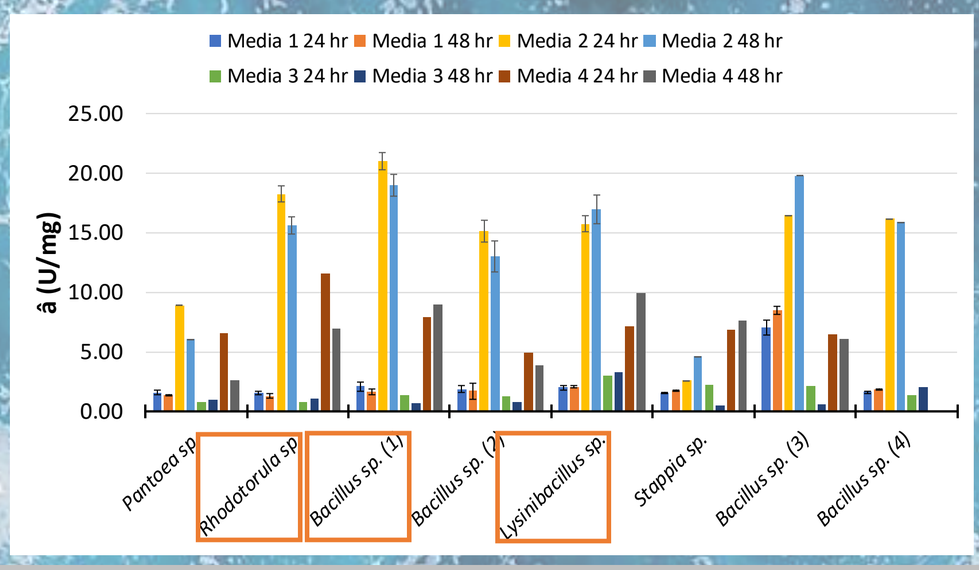

Figure 3: Specific alginate lyase activity of isolates after 24 and 48h of incubation in 4 different media

- Media 2 best for AL production – all isolates

✓ Selected 3 isolates

Comparison of 3 isolates

- Peptone as sole nitrogen source increased AL activity
- Decreased biomass
- *Rhodotorula* sp. showed highest AL activity
- Complex nitrogen sources preferred by isolates

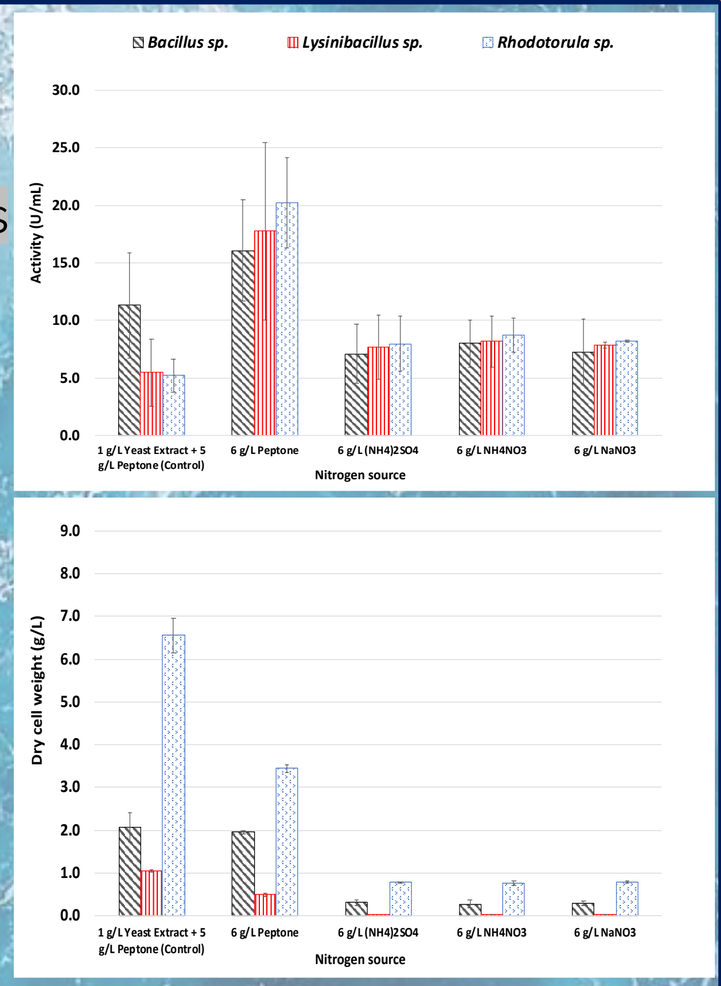

Figure 4: Dry cell weight and AL activity with different nitrogen sources used in fermentation media after 24 h incubation at 30°C,150 rpm agitation (250 mL baffled shake flasks)

Oxygen transfer in stirred reactors

- Impact of added ions on bubble properties
  - ↓ coalescence and ↓ bubble size
  - ↑ gas hold up
- Above 10.5 g/L - the viscosity ↑ results in marked ↓ in liquid mass transfer
  - Repression in K_la

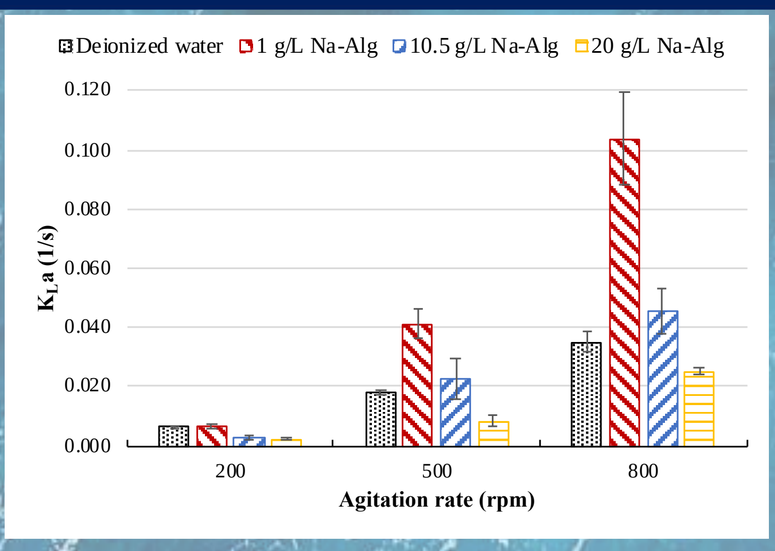

Figure 5: Comparison of K_la measured in different systems in a 1.3L stirred bioreactor. Note, alginate-based solutions were supplemented with 30 g/L NaCl