

Introduction and Background

- Alginate lyases are enzymes of the *polysaccharide lyase family* that catalyse the breakdown of alginate into unsaturated alginate oligosaccharides.
 - Alginate is a linear copolymer found within cell wall of brown macroalgae/seaweed.
- Alginate lyases are promising enzymes for application in the food, agricultural, and pharmaceutical industries^[1].
 - The produced products (alginate oligosaccharides) have shown bioactivities with application in medicines and plant stimulants/biofertilizers^[2].
 - The enzyme's ability to modify alginate viscosity is useful to food and pharmaceutical uses of alginate.
 - Useful for extraction of other valuable compounds found in brown seaweeds.
- Alginate lyases have been found in organisms that consume brown seaweed.
 - For example, the gut microflora of marine and terrestrial molluscs that consume kelp.

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^[3].
- ✓ Identified using 16S and ITS rRNA.

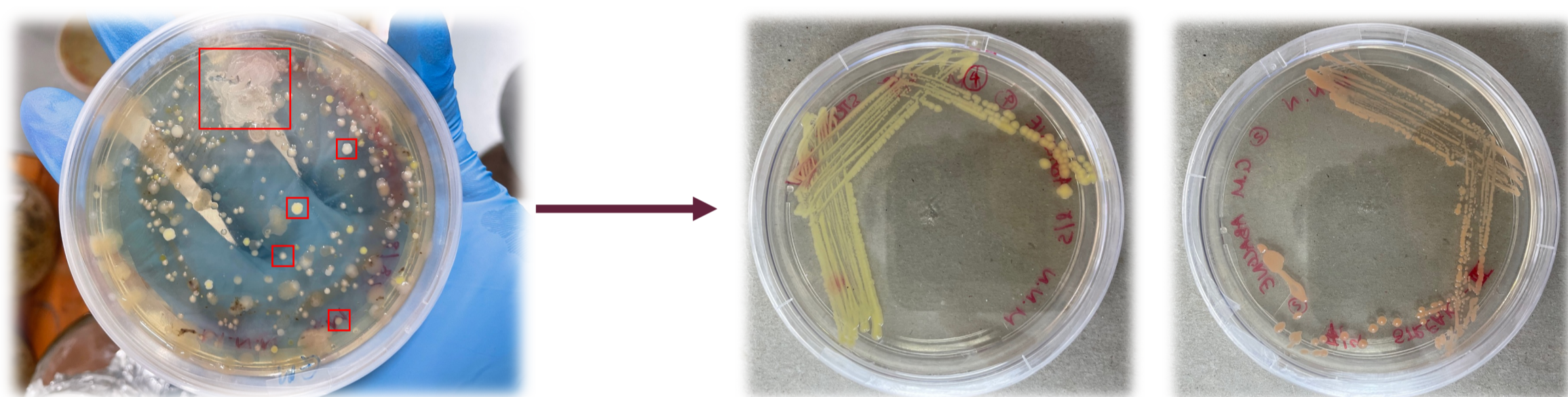


Figure 1: Different colonies streaked to purity.

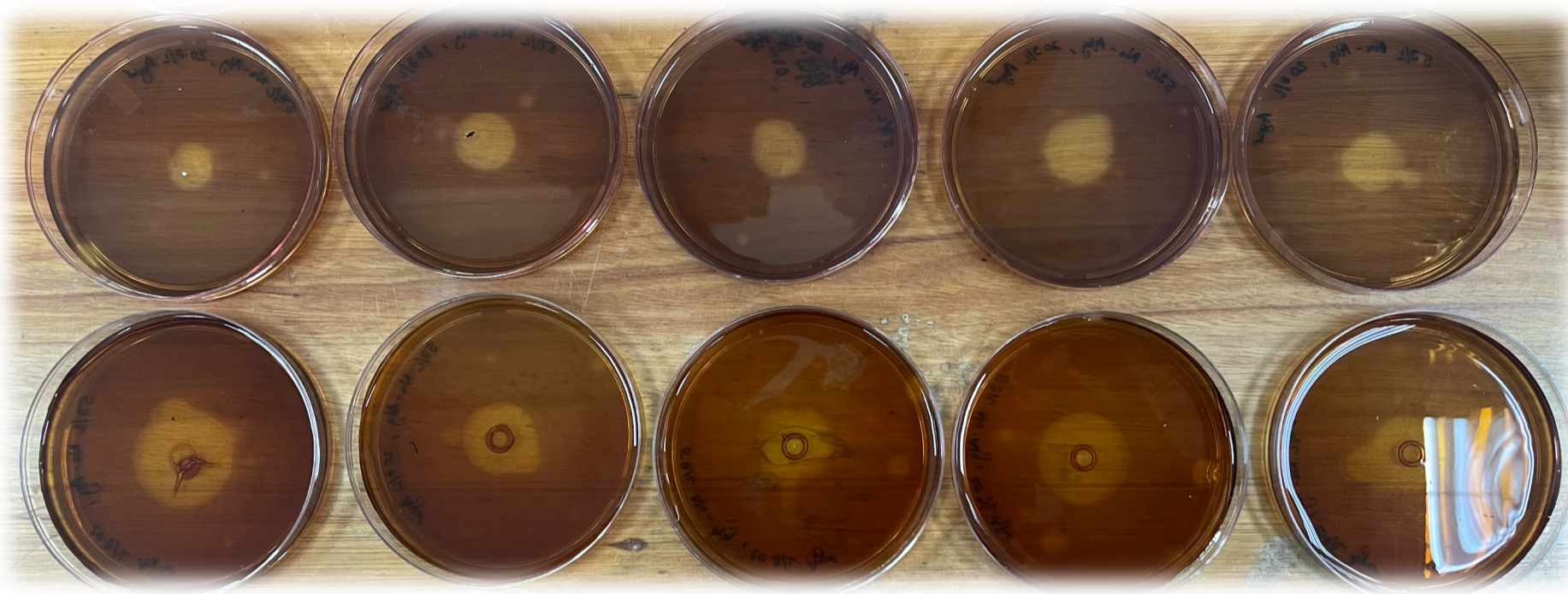


Figure 2: Plate assay indicating alginate lyase activity.

Quantifying Alginate Lyase Activity

- ✓ Each isolate was tested in 4 different media.
 - Alginate used as sole carbon source.
 - ✓ 250 mL baffled shake flasks, 1 mL seed culture, 30°C and 150 rpm, sampled 24 and 48 hours.
 - ✓ Alginate lyase activity (a) determined by DNS reducing sugars (RS) assay^[4].
 - Alginate digested by crude enzyme.
- $$a \left(\frac{U}{mL} \right) = \frac{C_{RS,60min} - C_{RS,initial}}{60 \text{ min}}$$
- ✓ 1 unit (U) of alginate lyase activity def. as the amount of enzyme required to release 1 µg of RS per minute.
 - ✓ Specific activity(â)-standardized by protein content.

Results - Selection and Enzyme Production

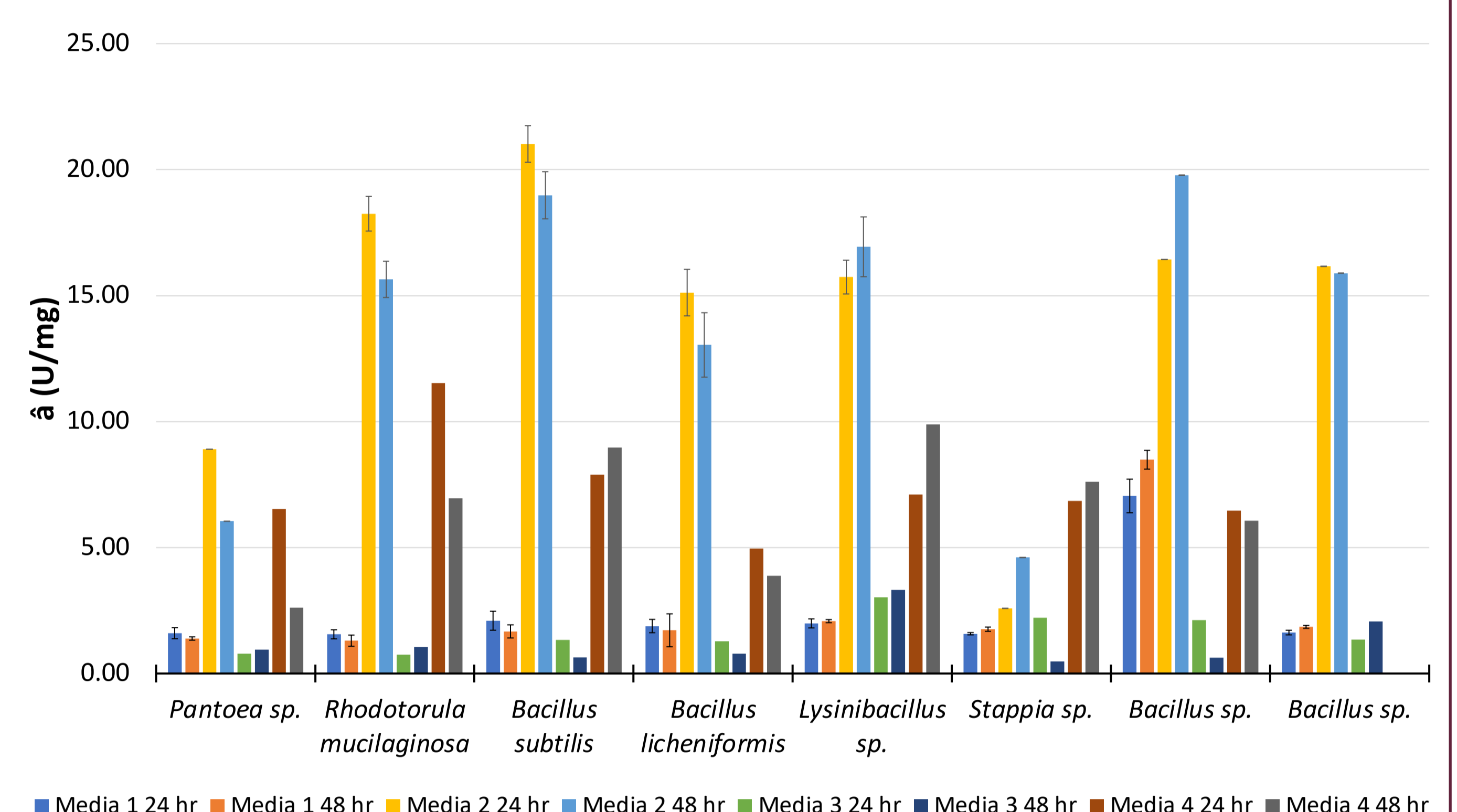


Figure 3: Crude specific alginate lyase activity.

- Selected isolates based on activity, source of isolation, and the type of microorganism.
 - ✓ *Bacillus subtilis*, *Lysinibacillus sp.*, and *Rhodotorula mucilaginosa*
- Selected isolates were further evaluated for growth and enzyme production in 500 mL shake flasks

Table 1: Summary of growth and enzyme production results from initial studies.

	<i>Bacillus subtilis</i>	<i>Lysinibacillus sp.</i>
Average specific productivity (U/g h)	0.94	0.66
Alginate Media Maximum Biomass (mg/mL)	3.06	2.73
Glucose Media Maximum Biomass (mg/mL)	2.46	0.774
Maximum Crude Volumetric Activity (U/mL)	20.54	25.44
Crude Protein Content (mg/mL)	1.19	1.86
Maximum Crude Specific Activity (U/mg)	17.27	13.78
Alginate Media Specific Growth Rate (1/h)	0.309	0.107
Glucose Modified Media Specific Growth Rate (1/h)	0.283	0.206

References

- Cheng, D., Jiang, C., Xu, J., Liu, Z. & Mao, X. 2020. Characteristics and applications of alginate lyases: A review. *International Journal of Biological Macromolecules*. 164:1304-1320. DOI: 10.1016/j.ijbiomac.2020.07.199.
- Liu, J., Yang, S., Li, X., Yan, Q., Reaney, M.J.T. & Jiang, Z. 2019. Alginate Oligosaccharides: Production, Biological Activities, and Potential Applications. *Comprehensive Reviews in Food Science and Food Safety*. 18(6):1859-1881. DOI: 10.1111/1541-4337.12494.
- Sawant, S.S., Salunke, B.K. & Kim, B.S. 2015. A rapid, sensitive, simple plate assay for detection of microbial alginate lyase activity. *Enzyme and Microbial Technology*. 77:8-13. DOI: 10.1016/j.enzmictec.2015.05.003.
- Miller, G.L. 2002. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*. 31(3):426-428. DOI: 10.1021/AC60147A030.