EVALUATION OF UNDERGROUND SALINE WATER AS A STABLE WATER SOURCE FOR MARINE MICROALGAE CULTIVATION

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Abstract

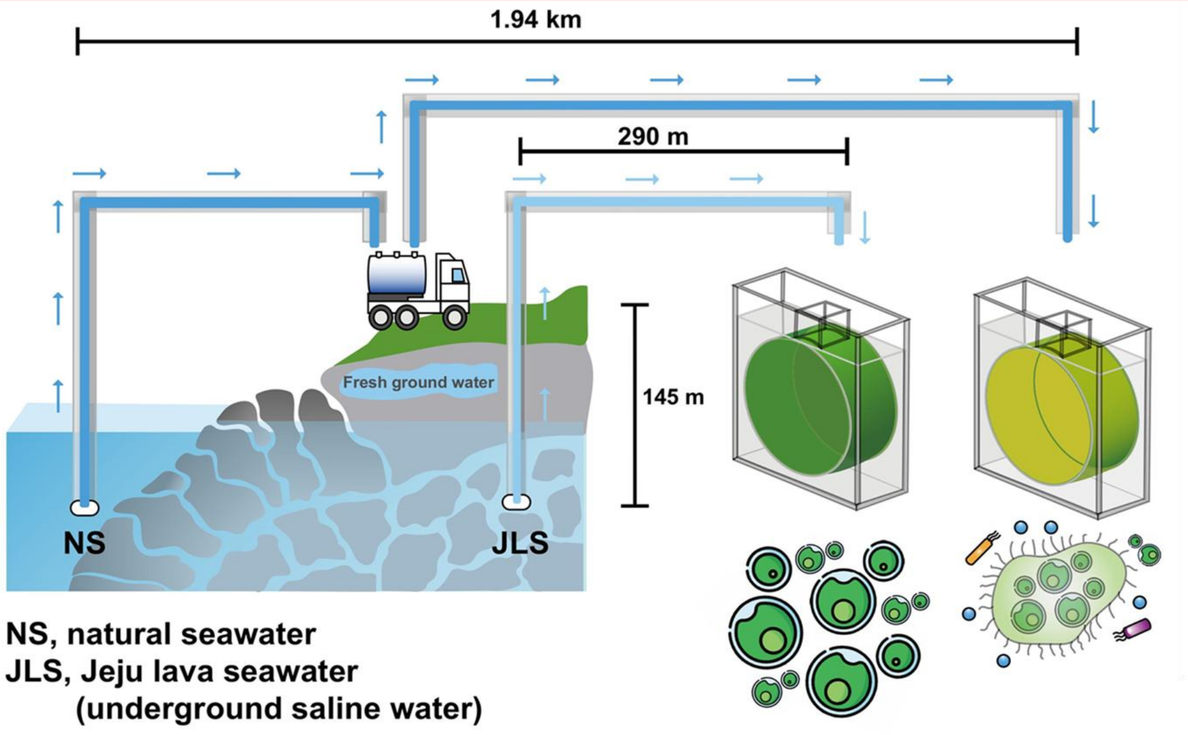
Industrial-scale microalgae cultivation is frequently challenged by biological contamination from grazers, competing algae, bacteria, and viruses, often resulting in culture collapse and productivity loss. Because pretreatment or sterilization of large volumes of water is economically and logistically demanding, securing naturally clean water sources is a promising alternative strategy. In this study, we evaluated the suitability of underground saline water from Jeju Island as a cultivation medium for the marine microalga *Tetraselmis* sp. Algal growth was monitored by flow cytometry, and microbial community structures were comparatively analyzed using metagenomic analysis. Two independent batch cultivation experiments were conducted for each water source. In both batch cultures prepared with untreated coastal seawater, grazer outbreaks occurred during the mid-to-late cultivation phase, resulting in a rapid decline in cell density of more than 60% and premature termination of cultivation. In contrast, cultures grown in underground saline water remained stable without observable contamination in both experimental runs and achieved more than twofold higher biomass productivity. Microbial community analysis revealed that untreated seawater harbored diverse eukaryotic microorganisms, viruses, and numerous bacterial taxa, whereas the underground saline water contained a substantially simplified microbial community, indicating reduced contamination risk. In addition, cultures grown in untreated seawater exhibited altered biomass characteristics and reduced photosynthetic pigment levels, while pigment contents were several-fold higher in the underground saline water cultures. These findings suggest that underground saline water represents a biologically stable and productive water resource for large-scale marine microalgae cultivation, particularly in inland or controlled production systems.

Introduction

- Microalgae are sustainable resources for biofuels and high-value bioproducts, but large-scale cultivation is highly vulnerable to biological contamination.
- Contaminants such as grazers and competing algae can reduce productivity or collapse cultures.
- Conventional sterilization methods are costly and inefficient at industrial scales.
- Jeju lava seawater (JLS) is naturally filtered, clean, and accessible inland.
- **This study evaluated JLS as a cultivation medium for *Tetraselmis* sp. for safer large-scale biomass production.**

Methodology

Schematic of experimental procedure



- Metagenomic analysis
- Flow cytometric analysis
- Biochemical assays
- Microscopic analysis

Fig. 1. Schematic of NS and JLS sampling and experimental procedures

Pilot-scale cultivation using saline groundwater

Fig. 2. Operation of a 200-L scale *Tetraselmis suecica* cultivation system and saline groundwater well drilling methods

Results

Metagenomic analysis of water resources

	JLS	NS
• Biological contamination risk	Low	High
• Bacterial taxa	9 taxa	43 taxa
• Eukaryotic taxa	N.D.	6 taxa
• Viral taxa	N.D.	5 taxa

Cell growth & Flow cytometry analysis

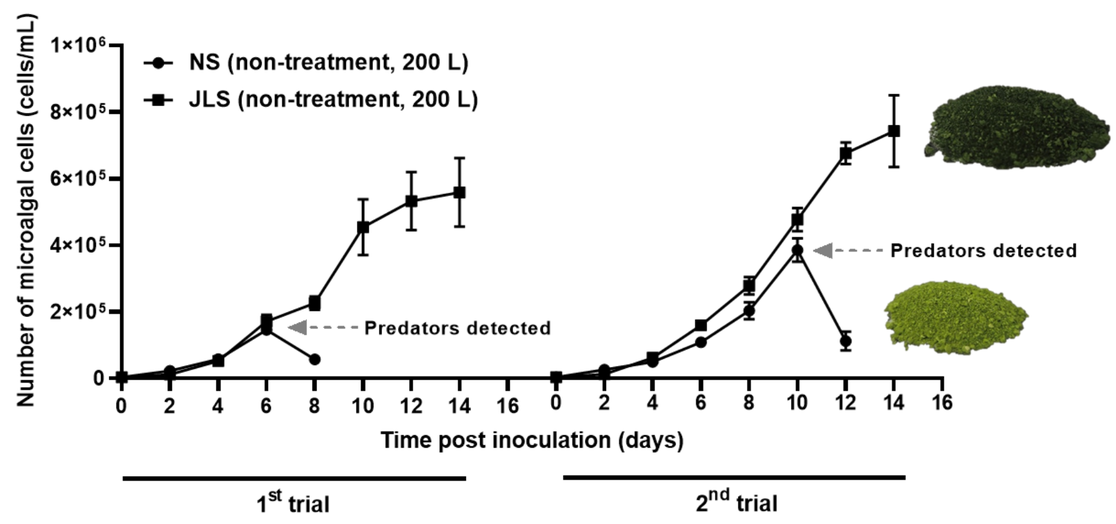


Fig. 1. Growth of *Tetraselmis suecica* during pilot-scale (200 L) cultivation

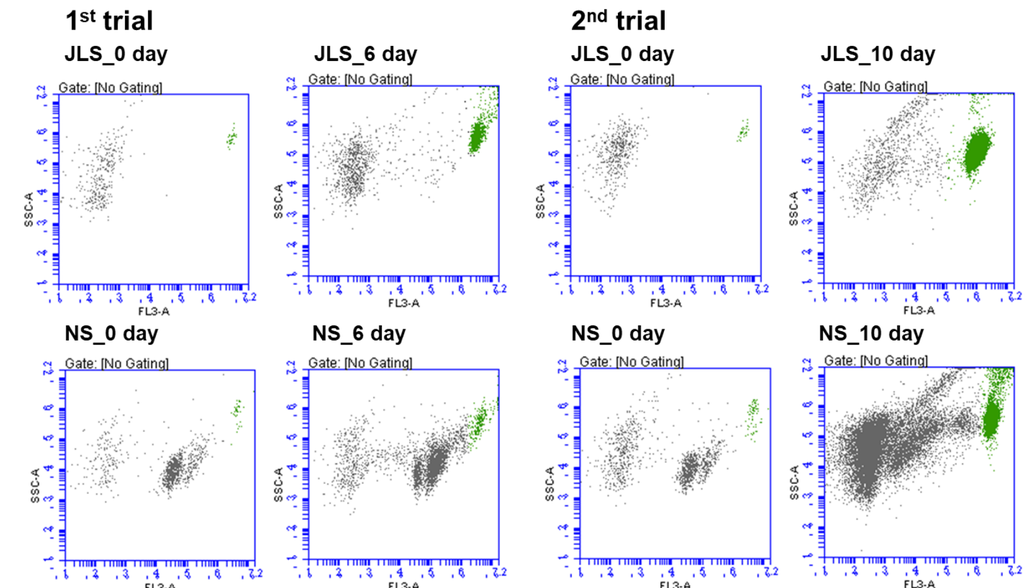


Fig. 2. Flow cytometry analysis of *Tetraselmis suecica* cell populations

Observation of biological contamination

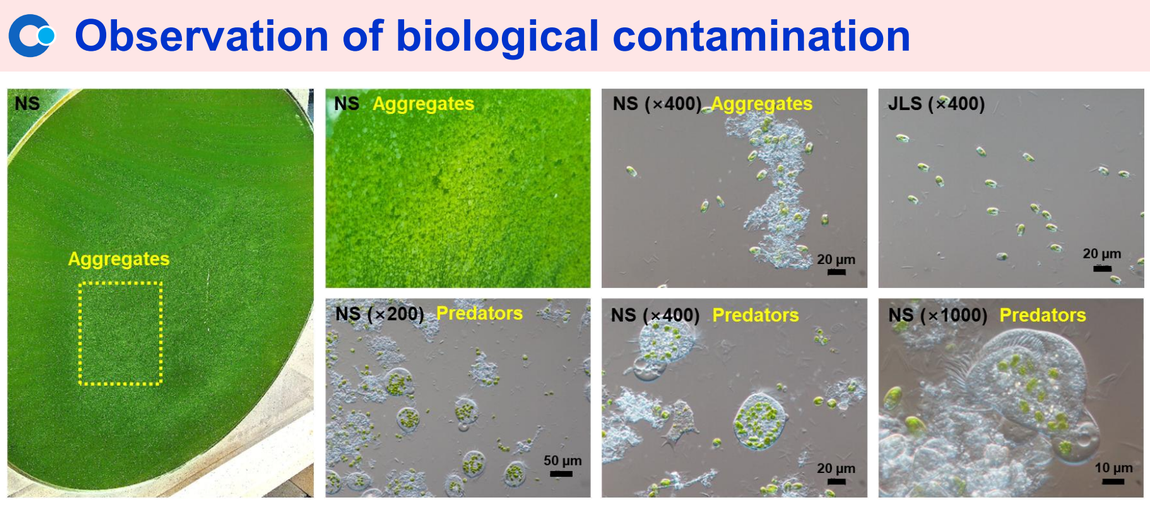


Fig. 3. Images of grazer-induced predation and aggregate formation

Photosynthetic pigments content

Pigments (mg/g)	JLS	NS	
• Chlorophyll a	268.91 ± 13.45	104.41 ± 5.22	*2.6-fold*
• Chlorophyll b	403.45 ± 20.17	138.51 ± 6.93	*2.9-fold*
• Violaxanthin	23.31 ± 1.83	8.06 ± 0.64	*2.9-fold*
• Lutein	61.07 ± 4.89	22.26 ± 1.78	*2.7-fold*

Conclusion

- **Jeju lava seawater (JLS, underground saline water) supported higher biomass production of *T. suecica* than natural seawater (NS).**
- **JLS reduced biological contamination, with no predator occurrence observed during cultivation.**
- **Photosynthetic pigment production of *T. suecica* in JLS cultures was 2.6–2.9 times higher than in NS cultures.**
- **JLS shows strong potential as an inland-accessible water source for contamination-resilient microalgae production.**