Pilot-scale *Dunaliella salina* cultivation in Jeju lava seawater: effects on biomass and nutritional composition

Yong-Kyun Ryu*, Woon-Yong Choi, Junseong Kim, Won-Kyu Lee
Jeju Bio Research Center, Korea Institute of Ocean Science and Technology (KIOST), Jeju 63349, Republic of Korea
E-mail: ykyou0111@kiost.ac.kr

KIOST
KOREA INSTITUTE OF OCEAN SCIENCE & TECHNOLOGY

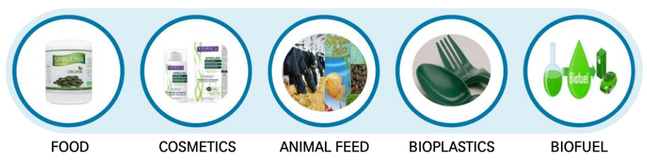
Abstract

Alternative low-pretreatment saline resources could improve the economics and scalability of marine microalgal biomass production. Jeju lava seawater (JLS) was evaluated as a saline water source for *Dunaliella salina* cultivation using a laboratory-scale major-cation supplementation assay followed by 5,000 L pilot-scale open raceway pond cultivation. JLS and natural seawater (NS) had comparable salinity but differed in major cation composition, particularly Mg, K, and Ca. At the laboratory scale, JLS produced the highest endpoint optical density at 750 nm (OD750) after 10 days, whereas Ca and Mg+K supplementation of NS partially reproduced the JLS-associated growth response. NaCl supplementation alone did not improve endpoint growth, indicating that NaCl addition alone was insufficient to account for the JLS-associated response. Under non-sterile, outdoor, pilot-scale cultivation, JLS yielded a higher final biomass concentration than NS (0.475 ± 0.03 vs. 0.387 ± 0.038 g L^{-1} on day 21). JLS-grown biomass also had higher protein and carbohydrate contents, lower ash content, and reduced accumulation of Pb, Cd, and As compared to NS-grown biomass. JLS is a potential, low-pretreatment saline resource for pilot-scale *D. salina* cultivation, with performance benefits associated partly with major cation balance and source water properties. Further validation across seasons and cultivation sites is needed to confirm process robustness.

Background

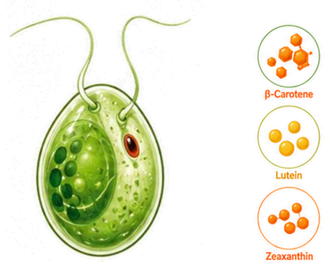
Microalgae as sustainable biomass platforms

- Resource efficient biomass production in increasingly needed
- Microalgae capture CO_2 and produce valuable metabolites
- Major products include amino acids, PUFAs, carotenoids, and phycocyanin
- Applications include food, feed, cosmetics, and biofuel
- Cultivation is commonly performed in raceway ponds or PBRs



Dunaliella salina

- Halotolerant unicellular green microalga
- Important natural source of beta-carotene
- Also produces lutein and zeaxanthin
- Suitable for outdoor biomass and pigment production



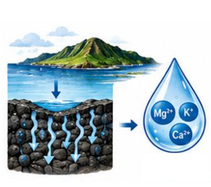
Limitations of natural seawater

- High pumping and pretreatment costs
- Seasonal variations in water composition
- Environmental and regulatory concerns
- Need for alternative saline resources



Jeju lava seawater

- Underground seawater
- Stable temperature and salinity
- Mineral rich and requires less pretreatment
- Promising source for algal cultivation



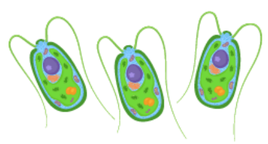
Study objective



Jeju lava seawater (JLS) percolates through volcanic rock below Jeju Island



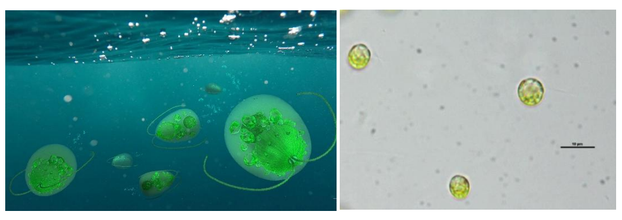
JLS has higher levels of Mg, K, and Ca than surrounding natural seawater (NS), but its salinity is not significantly higher



Comparison of *Dunaliella salina* cultivation in JLS vs NS

Materials & Methods

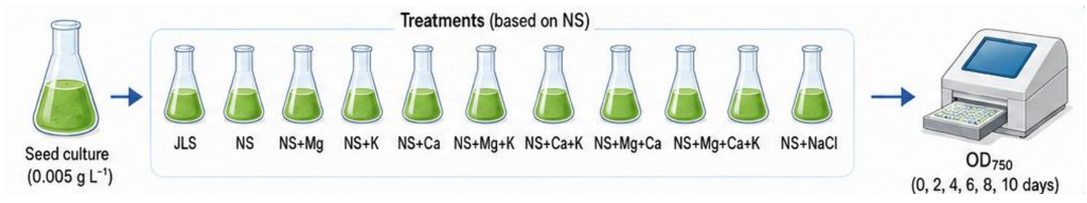
Dunaliella salina



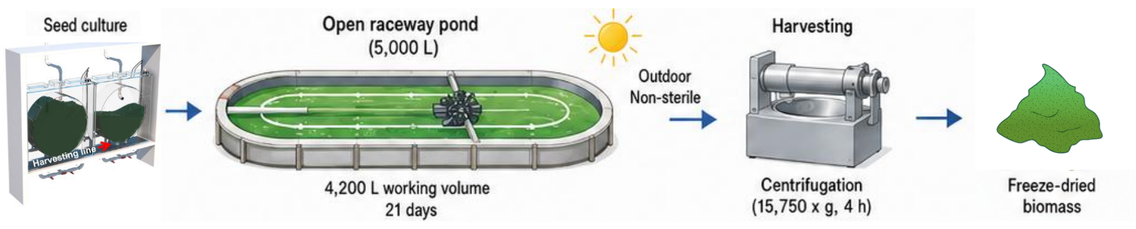
Phylum	Chlorophyta
Class	Chlorophyceae
Order	Chlamydomonadales
Family	Dunaliellaceae
Genus	*Dunaliella*
Species	*D.salina*

- Protein
- Minerals
- Fats
- Carbohydrates
- Fiber
- Pigments
- Vitamines

Laboratory supplementation assay



Pilot scale outdoor cultivation



Biomass composition analysis

Statistics analysis

- All analyses were performed in triplicate and data are presented as mean ± standard deviation (n = 3)
- For laboratory-scale supplementation assay, final OD_{750} values on day 10 were compared among treatments using one-way ANOVA followed by Tukey's multiple-comparison test
- For pairwise comparisons between JLS and NS in pilot-scale endpoint measurements, unpaired two-tailed Student's *t*-tests were used

Results

Table 1. Chemical composition of JLS and NS used in this study.

Parameter (mg L^{-1})	JLS	NS	Difference in JLS relative to NS
Na	8360.0 ± 68.1	8220.0 ± 66.4	+1.7%
Mg	1640.0 ± 15.6	1240.0 ± 32.4	+32.3%
K	391.5 ± 11.0	264.0 ± 26.3	+48.3%
Ca	75.2 ± 4.1	57.4 ± 2.5	+31.0%
Sr	7.1 ± 0.8	5.6 ± 0.6	+26.8%
B	4.2 ± 0.1	3.2 ± 0.2	+31.3%
Cu	<0.005	<0.005	
Fe	<0.01	<0.01	
Ge	<0.0001	<0.0001	
Mn	<0.001	<0.001	
Se	<0.001	<0.001	
V	<0.0001	<0.0001	
Zn	<0.005	<0.005	
Salinity (‰)	33.1 ± 0.7	32.7 ± 0.5	

JLS, Jeju lava seawater; NS, natural seawater. Data are expressed as mean ± standard deviation (n = 3)

Figure 1. Laboratory-scale growth response of *Dunaliella salina* to major-cation supplementation in natural seawater (a) Time-course OD750 profiles during 10 d of cultivation. (b) Final OD750 on day 10. Data are expressed as the mean ± SD (n = 3). Different letters in the same row indicate significant differences (Tukey's test, $p < 0.05$)
JLS, Jeju lava seawater; NS, natural seawater; OD, optical density; SD, standard deviation

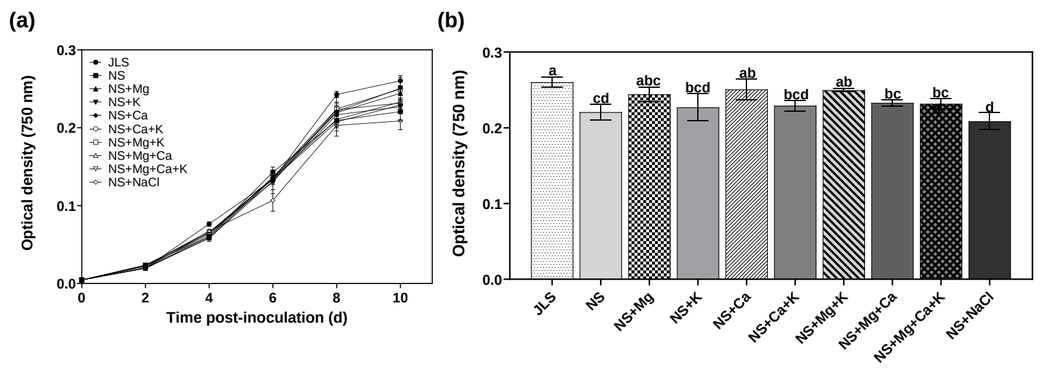


Figure 2. Pilot-scale microalgae cultivation of *Dunaliella salina* (a) 200 L seed culture for inoculating a 5,000 L open raceway pond, (b) media prepared using various saline water sources, (c) cultures at 5 and 20 d using underground seawater, and (d) harvested biomass of *D. salina* grown in underground seawater

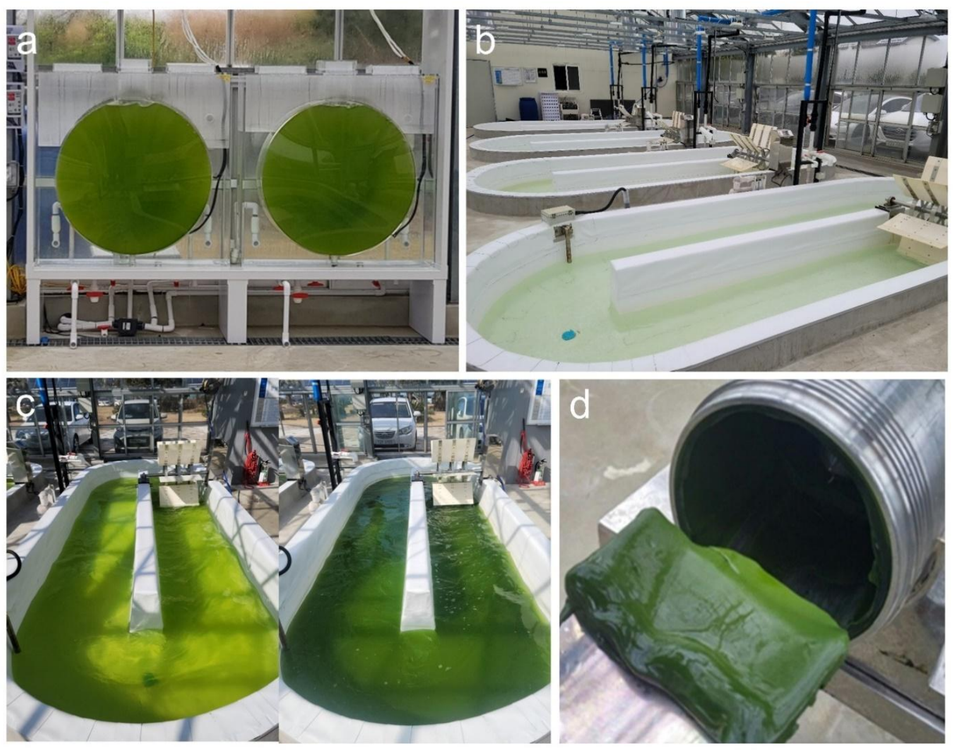


Figure 3. Comparison of (a) biomass concentration, with representative cytograms (FL3-H vs. SSC-A) on day 21 shown as an inset, and (b) pigment concentration of *Dunaliella salina* biomass cultured using JLS or NS. Data are presented as mean ± standard deviation.
$*p < 0.05$, $**p < 0.01$, $***p < 0.001$. JLS, Jeju lava seawater; NS, natural seawater

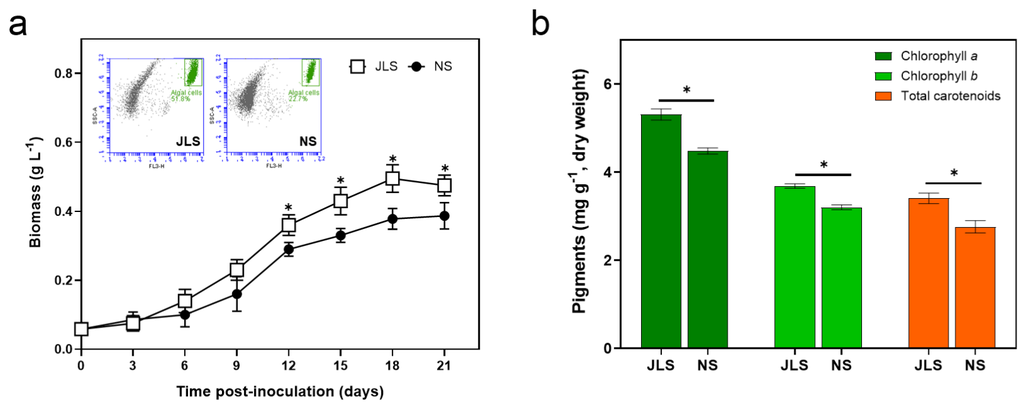


Table 2. Heavy metal contents of *Dunaliella salina* biomass cultured using JLS or NS

Constituent	JLS	NS	*p*-value	Significance
Carbohydrates (%)	38.69 ± 1.07	36.37 ± 0.79	<0.05	*
Protein (%)	22.27 ± 0.87	18.53 ± 0.52	<0.01	**
Lipid (%)	11.39 ± 0.16	11.25 ± 0.19	-	ns
Moisture (%)	2.05 ± 0.04	4.38 ± 0.05	<0.0001	****
Ash (%)	25.60 ± 1.12	29.46 ± 1.60	<0.05	*
Pb (mg Kg^{-1})	2.93 ± 0.03	5.73 ± 0.06	<0.0001	****
Cd (mg Kg^{-1})	0.47 ± 0.12	1.37 ± 0.25	<0.01	**
As (mg Kg^{-1})	N.D.	2.87 ± 0.32	-	-
Sn (mg Kg^{-1})	N.D.	N.D.	-	-
Hg (mg Kg^{-1})	0.00 ± 0.00	0.00 ± 0.00	-	ns

JLS, Jeju lava seawater; NS, natural seawater; N.D., not detectable; ns, not significant. Data are presented as mean ± standard deviation

Conclusion

1. Improved biomass production
JLS enhanced pilot-scale *D. salina* biomass accumulation compared with natural seawater.

2. Importance of ionic balance
Ca and Mg+K supplementation partially reproduced the JLS growth response, whereas NaCl alone did not.

3. Better biomass composition
JLS-grown biomass showed higher protein and carbohydrate contents, lower ash, and reduced Pb, Cd, and As accumulation