International Journal of Design & Nature and Ecodynamics
Vol. 20, No. 12, December, 2025, pp. 2773-2781
Journal homepage: http://iieta.org/journals/ijdne

Amplicon-Based 16S rRNA Inventory of a Laboratory-Cultivated Hypersaline Halophilic Biofilm from Indonesian Salt Ponds: A Descriptive Pilot of Bacterial–Archaeal Composition

Ilham Misbakudin Al Zamzami[1], Defri Yona[2], Abd Rahem Faqih[3], Andi Kurniawan[3*]

[1] Doctoral Program of Fisheries Science and Marine, Faculty of Marine Science and Fisheries, University of Brawijaya, Malang 65145, Indonesia
[2] Department of Fishery and Marine Resources Utilization, Faculty of Marine Science and Fisheries, University of Brawijaya, Malang 65145, Indonesia
[3] Department of Fisheries and Marine Resources Management, Faculty of Marine Science and Fisheries, University of Brawijaya, Malang 65145, Indonesia

Corresponding Author Email: andi_k@ub.ac.id

https://doi.org/10.18280/ijdne.201204

Received: 10 November 2025
Revised: 17 December 2025
Accepted: 25 December 2025
Available online: 31 December 2025

Keywords:
extremophile ecology, halophilic microorganisms, hypersaline biofilm, microbial diversity

ABSTRACT

Hypersaline environments support microbial communities with remarkable adaptations to osmotic stress, yet the ecological organization of biofilm-associated microbiomes in these habitats remains poorly understood. This study presents the first amplicon-based 16S rRNA characterization of a laboratory-grown halophilic biofilm derived from Indonesian salt ponds. Biofilm biomass from salinity stages of approximately 2%, 20%, and 40% NaCl was pooled prior to DNA extraction to ensure sufficient yield; therefore, the present study provides a descriptive community inventory rather than comparisons among salinities or biofilm baseline inventory. High-throughput sequencing identified 196 OTUs (Good's coverage = 0.9997) with moderate-to-high richness across alpha-diversity indices. The community was dominated by bacterial genera including *Oceanicaulis*, *Bacillus*, *Halomonas*, and *Limimaricola*, alongside archaeal taxa such as *Haloferax* and *Halohasta*. These taxa are discussed in relation to ecological functions reported in prior hypersaline biofilm studies; however, spatial stratification cannot be inferred from a composite sample. Overall, this work provides baseline taxonomic data for Indonesian salt-pond-derived halophilic biofilms and motivates future spatially resolved and functional validation.

1. INTRODUCTION

Hypersaline environments such as salt lakes, solar salterns, and saline soils represent some of the most extreme ecosystems on Earth, where salinity levels exceed the tolerance limits of most organisms. Despite their harshness, these habitats sustain diverse halophilic and halotolerant microorganisms that have evolved remarkable physiological and molecular strategies to cope with osmotic stress, high ionic strength, and desiccation. Their activities contribute to global biogeochemical cycles through transformations of carbon, nitrogen, and sulfur, thereby linking hypersaline ecosystems to planetary processes [1, 2]. Because of these unique attributes, hypersaline habitats are often regarded as natural laboratories for investigating the limits of life and the resilience of extremophiles. Beyond ecology, halophiles are increasingly valued for their biotechnological potential, producing salt-tolerant enzymes, Osmoprotectants, and secondary metabolites with applications in biofuels, pharmaceuticals, and food industries [3].

Within these habitats, biofilms represent a particularly critical microbial lifestyle. Unlike planktonic populations, biofilm-forming microorganisms are embedded in an extracellular polymeric substance (EPS) matrix that provides structural integrity, mediates adhesion, and buffers against extreme ionic conditions. The biofilm matrix also creates steep microscale gradients in oxygen, nutrients, and salinity, generating distinct niches that allow diverse taxa to coexist [4, 5]. In hypersaline systems, biofilms often consist of both bacteria and archaea, whose interactions enhance community stability under fluctuating conditions. Consequently, hypersaline biofilms are promising model systems for exploring how fine-scale spatial organization influences microbial resilience and ecosystem functioning.

Despite their importance, biofilms in hypersaline environments remain far less studied than planktonic communities or large microbial mats. Previous research has mainly documented dominant taxa and overall diversity patterns, while the stratified architecture of biofilms, particularly the way bacterial and archaeal members partition ecological roles across baseline inventory, remains poorly characterized. The distribution of niches within the EPS matrix, the influence of baseline inventory on functional complementarity, and the role of interdomain interactions in sustaining survival under extreme salinity are still largely unresolved. Clarifying these aspects is essential, as microscale organization is likely fundamental to the ecological persistence and evolutionary strategies of extremophile communities [1, 6, 7].

Recent advances in molecular ecology provide tools to close this gap. High-throughput 16S rRNA gene sequencing, coupled with bioinformatic approaches, has revealed unexpected microbial diversity in hypersaline environments, including novel lineages with unique strategies for osmotic adaptation, phototroph, and polymer degradation [8, 9]. However, most studies stop at taxonomic inventories. Few have integrated these data into ecological models that explicitly consider spatial structure and interdomain interactions within biofilms. As a result, our understanding of hypersaline biofilms as stratified and cooperative assemblages remains limited.

The present study addresses this knowledge gap by investigating the microbial community of a halophilic biofilm from Indonesian salt ponds. Using high-throughput 16S rRNA sequencing and operational taxonomic unit (OTU) based analysis, characterized taxonomic composition, diversity patterns, and potential ecological roles of bacterial and archaeal members. Specifically, the present a descriptive 16S rRNA amplicon inventory of a halophilic biofilm cultivated in a laboratory microcosm using waters collected along an Indonesian salt-pond salinity gradient. Due to low biomass obtained at each salinity stage, biofilm material was pooled prior to DNA extraction. Therefore, this study does not test spatial stratification or salinity-specific differences. Instead, they provide baseline community composition data from an understudied region and outline priorities for future spatially resolved sampling and functional validation.

A key finding is the unusual dominance of *Limimaricola* and *Oceanicaulis*, lineages rarely reported as major components in global hypersaline environments. Their prominence in the Indonesian system suggests biogeographic specialization driven by local environmental gradients. By documenting this baseline inventory and unusual taxonomic composition, the study contributes to broader models of extremophile ecology while highlighting region-specific features that enrich global perspectives. Beyond fundamental insights, these results also have applied relevance, as stratified halophilic biofilms may serve as reservoirs of salt-tolerant enzymes, biosurfactants, and bioremediation agents for high-salinity effluents. This research provides a novel ecological model of hypersaline biofilms as stratified, cooperative consortia where bacterial and archaeal taxa integrate complementary adaptation strategies. It not only fills a critical knowledge gap on fine-scale biofilm architecture but also positions Indonesian salt-pond biofilms as valuable systems for advancing both ecological theory and biotechnological innovation.

2. MATERIAL AND METHODS

2.1 Sampling and experimental design

Biofilm development was conducted in a laboratory microcosm designed to simulate hypersaline conditions typical of Indonesian salt ponds (8°25′26.0″ S, 112°38′18.3″ E). Seawater was collected from three salinity stages representing approximately 2%, 20%, and 40% NaCl, corresponding to early, intermediate, and crystallization ponds, respectively. Each salinity level was maintained in a separate 10-L glass aquarium containing high-density polyethylene (HDPE) panels (15 × 10 cm) as solid substrates for biofilm attachment. The aquaria were incubated for 21 days at 30 ± 1°C under continuous aeration to promote uniform biofilm growth [10]. Information on illumination intensity and exact aeration flow rate was not recorded during this pilot microcosm; future experiments will standardize and report these parameters, given their relevance to phototrophic taxa and biofilm development. At the end of incubation, biofilm growth on individual panels was visibly thin, yielding insufficient biomass for independent DNA extraction. Therefore, biofilm material from all salinity treatments (2%, 20%, and 40%) was pooled to obtain sufficient DNA for sequencing. The resulting composite sample represents the total halophilic consortium developed across the salinity gradient. Accordingly, this study provides a descriptive inventory of community composition rather than a statistical comparison among salinities.

Three independent HDPE panels were deployed per salinity treatment to account for biological variability. The pooled biomass was homogenized under sterile conditions before DNA extraction. Physicochemical parameters (pH, dissolved oxygen, temperature, and salinity) were monitored daily to ensure environmental stability; summarized values are presented in Table 1.

Table 1. Physicochemical parameters during the experiment

Parameters	2%	20%	40%
pH	8.01 ± 0.11	6.83 ± 0.02	7.28 ± 0.06
DO (mg L^{-1})	4.08 ± 0.14	1.35 ± 0.50	1.86 ± 0.53
Temperature (°C)	25.58 ± 0.32	25.68 ± 0.25	25.69 ± 0.24
Salinity (%)	2.00 ± 0.13	20.00 ± 0.24	40.00 ± 0.40

2.2 DNA extraction, controls, and library preparation

Total genomic DNA was extracted from the pooled biofilm using the ZymoBIOMICS™ DNA Kit (Zymo Research, USA), optimized for high-salinity and complex environmental matrices to minimize extraction bias and ensure representative microbial recovery. Extraction blanks (no-template controls) were included in each batch to monitor potential contamination; no detectable DNA or amplification was observed in the blanks. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA), and integrity was verified by 1% agarose gel electrophoresis. Only high-quality DNA (A260/280 ratio between 1.8 and 2.0) was used for subsequent amplification and sequencing. The V3–V4 region of the 16S rRNA gene was amplified with primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 806R (5′-GACTACHVGGGTATCTAATCC-3′) carrying Illumina adapter sequences and unique barcodes. PCR was performed in triplicate (25 µL each) with the following cycling conditions: 95°C for 3 min; 30 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s; final extension 72°C for 5 min. Amplicons were visualized on 2% agarose, purified, equimolarly pooled, and used for library construction following the standard Illumina paired-end (2 × 250 bp) protocol. Library quality and insert size were verified using Qubit fluorometry and Bioanalyzer before sequencing on an Illumina MiSeq platform.

2.3 Bioinformatic processing and taxonomic annotation

Raw paired-end reads were merged with FLASH v1.2.7, quality-filtered in QIIME v1.7.0 (Phred ≥ Q30; length 400–450 bp; no ambiguous bases), and screened for chimeras using UCHIME in USEARCH/UPARSE v7.0.1090 against the SILVA 138 reference database. Non-chimeric reads ("Effective Tags") were clustered into operational taxonomic units (OTUs) at 97% sequence identity using the UPARSE de novo algorithm, ensuring comparability with previous hypersaline biofilm studies. Representative sequences were taxonomically classified using the RDP Classifier (Mothur method) in QIIME v1.7.0 with a confidence threshold of 0.8–1.0 against the SILVA 138 SSU rRNA database. Because the V3–V4 region lacks sufficient resolution for species-level identification, all analyses were restricted to the genus level; species names, where mentioned, are putative and used for descriptive context only. The OTU table was normalized to the smallest sequencing depth to minimize bias in diversity metrics. Alpha-diversity indices (Chao1, ACE, Shannon, Simpson, Good's coverage) were calculated in QIIME. Community composition was summarized as relative abundance plots at the phylum and genus levels, and interactive profiles were generated using KRONA.

3. RESULT AND DISCUSSION

3.1 Sequencing output, OTU generation, and annotation

High-throughput sequencing of hypersaline biofilm samples produced over 1.6 million effective reads, with nearly all sequences successfully assigned to known taxa. Clustering at 97% similarity generated 196 OTUs, reflecting substantial microbial diversity. Although dominant taxa shaped most of the community, the presence of singletons contributed to a long-tail distribution, indicating coexistence of abundant and rare members within the biofilm (Figure 1). This pattern is typical of microbial ecosystems where a few lineages dominate, yet low-abundance taxa maintain additional diversity.

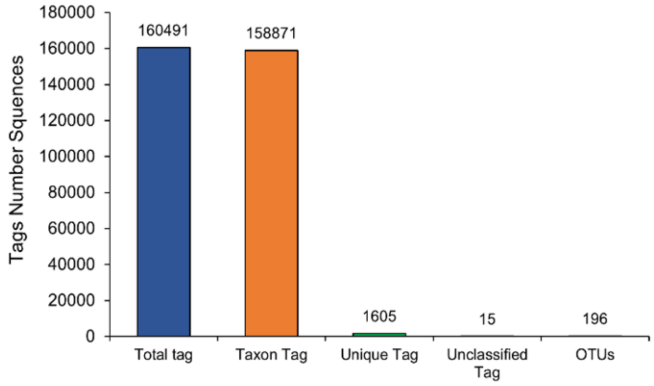

Figure 1. Sequencing tags and OTUs from hypersaline biofilm

3.2 Taxonomic composition of the microbial community

Taxonomic classification at the phylum level showed that the biofilm community was strongly structured by *Proteobacteria* and *Halobacterota*, which together comprised over two-thirds of the total relative abundance (Figure 2). This dominance reflects the dual bacterial–archaeal foundation of hypersaline biofilms, where *Proteobacteria* contribute to heterotrophic metabolism and EPS production, while *Halobacterota* represent the major archaeal lineage adapted to salt-saturated conditions through "salt-in" osmotic strategies.

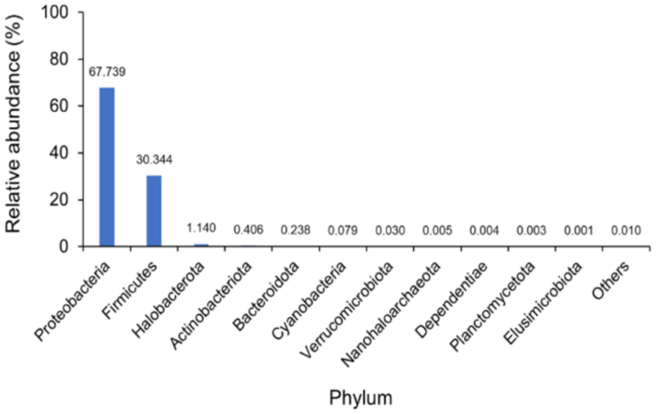

Figure 2. Relative abundance of dominant microbial phyla in the biofilm

At finer resolution, the cladogram (Figure 3) revealed clear clustering of archaeal taxa from the class *Halobacteria* (notably *Haloferax*, *Halorubrum*, and *Haloplanus*) alongside bacterial groups such as *Halomonas* (*Proteobacteria*) and *Salinibacter* (*Bacteroidota*). These lineages are consistent with complementary ecological strategies previously reported for hypersaline biofilms: haloarchaea are typically associated with phototrophy and osmotic balance, while bacterial members often contribute to organic matter degradation and EPS reinforcement. The observed enrichment of these taxa suggests potential metabolic complementarity, although this inference is based solely on taxonomic composition.

Table 2. Top 15 species identified in the hypersaline biofilm

No.	Species	Relative Abundance (%)
1	*Oceanicaulis* sp.	28.638
2	*Bacillus horikoshii*	24.748
3	*Bacillus beringensis*	19.734
4	*Bacillus selenatarsenatis*	10.247
5	*Pseudomonas stutzeri*	6.095
6	*Paraburkholderia kururiensis*	2.523
7	*Roseivivax halotolerans*	2.342
8	*Bifidobacterium pseudolongum*	1.140
9	*Pseudomonas sihuiensis*	0.857
10	*Lactobacillus johnsonii*	0.739
11	*Haloarcula* sp.	0.389
12	*Roseovarius* sp.	0.371
13	*Fictibacillus phosphorivorans*	0.371
14	*Loktanella* sp.	0.211
15	*Lactobacillus reuteri*	0.171

Note: V3–V4 16S rRNA amplicons generally do not provide robust species-level resolution; species names should be interpreted as putative.

The relatively high abundance of *Oceanicaulis* and *Bacillus* (Table 2) distinguishes this community from most previously described hypersaline systems, where these genera are usually minor components. Their prominence may reflect local environmental characteristics of Indonesian salt ponds, indicating a possible biogeographic trend that merits further comparative investigation.

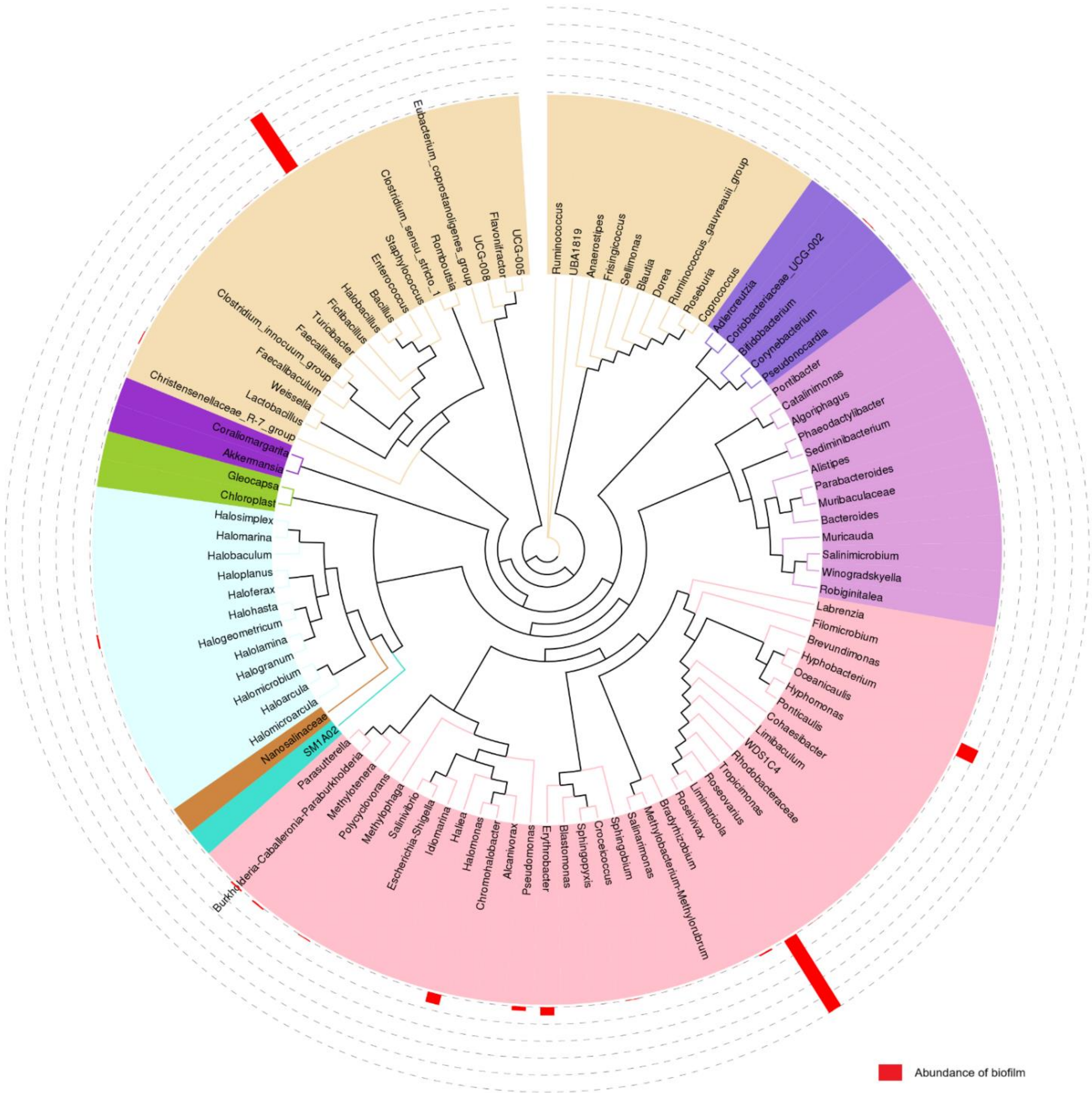

Figure 3. Cladogram of bacterial and archaeal genera in the biofilm

3.3 Alpha diversity and richness patterns

Alpha diversity analysis revealed a moderately rich and balanced community, with 196 observed OTUs and diversity indices indicating even distribution among dominant and minor taxa. Richness estimators (Chao1 and ACE) closely matched observed values, suggesting that most taxa were successfully captured. Importantly, Good's coverage of 0.9997 confirmed near-complete sequencing depth, providing confidence in the representativeness of the dataset (Table 3). In contrast, the Indonesian biofilm demonstrated both substantial taxonomic richness and balanced representation of bacterial and archaeal lineages, underscoring the ecological uniqueness of its stratified structure.

Rarefaction analysis (Figure 4) further confirmed that sequencing depth was sufficient, with the curve plateauing near 196 OTUs, while the rank abundance distribution (Figure 5) reflected a community shaped by a few abundant lineages supported by a diverse rare biosphere. Such a pattern mirrors other hypersaline environments where cooperative interactions between dominant taxa and low-abundance specialists are thought to enhance ecological resilience under fluctuating salinity. Thus, the Indonesian biofilm not only fits within global patterns of halophilic diversity but also expands them by demonstrating a more balanced bacterial–archaeal coexistence than many previously studied systems.

Table 3. Alpha diversity of halophilic biofilm

Parameters	Value
Observed OTUs	196
Shannon	3.482
Simpson	0.821
Chao1	199.6
ACE	198.948
Goods coverage	0.9997
PD whole tree	21.353

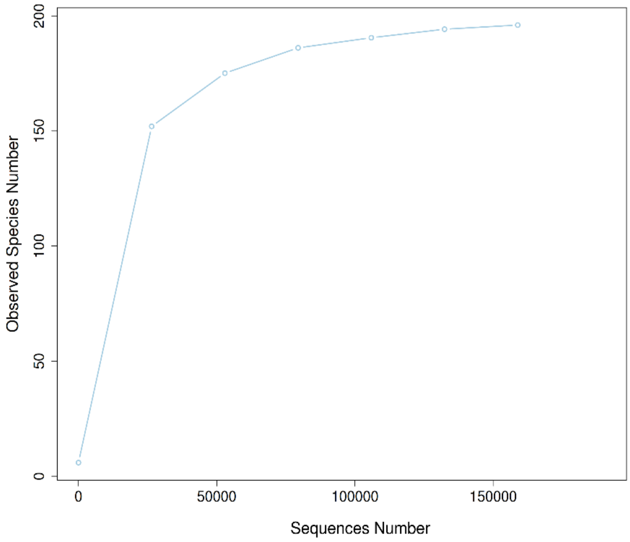

Figure 4. Rarefaction curves of hypersaline biofilm sequences

3.4 Comparative insights with other hypersaline

Comparative analysis of halophilic microbial communities across hypersaline environments revealed consistent dominance of both Bacteria and Archaea, with taxonomic variation depending on location and physicochemical gradients (Table 4). In the current study, biofilm samples from Indonesian salt ponds were dominated by bacterial genera such as *Limimaricola*, *Bacillus*, *Oceanicaulis*, and *Halomonas*, alongside archaeal taxa including *Halohasta* and *Haloferax*.

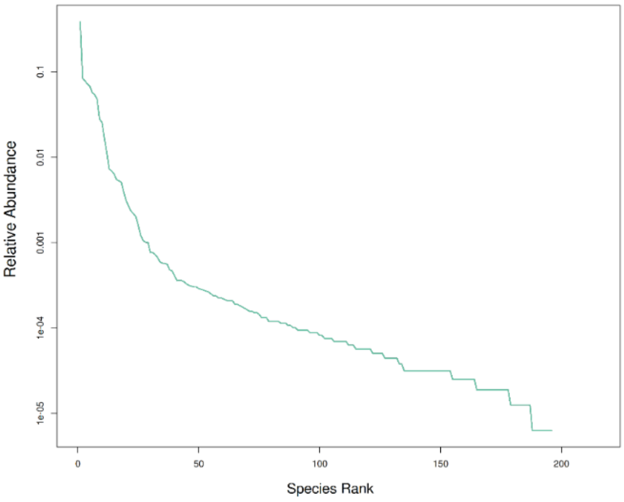

Figure 5. Rank-abundance distribution of biofilm taxa

3.5 Discussion

3.5.1 Ecological structure and comparison to global systems

The ecological structure of halophilic biofilms revealed in this study demonstrates a highly stratified and functionally diverse microbial consortium. The bacterial domain by *Oceanicaulis* sp. (28.6%) and *Bacillus horikoshii* (24.7%), while archaeal representatives such as *Haloferax* and *Halohasta* were also consistently detected. This taxonomic pattern indicates that both bacterial and archaeal lineages contribute to the biofilm structure, with dominance shared rather than restricted to a single group. The presence of a long-tail rare biosphere further demonstrated coexistence between abundant and low-abundance taxa within the EPS matrix.

Table 4. Comparative dominant taxa in global hypersaline environments

No.	Site Project	Bacteria Dominant	Archaea Dominant	Reference
1	Salt pond, Indonesia (current research)	*Limimaricola, Bacillus, Oceanicaulis, Halomonas*	*Halohasta, Haloferax*	-
2	Great Salt Lake, USA	*Proteobacteria, Cyanobacteria*	*Halobacteria (Haloferax/Halorubrum)*	[11]
3	Salton Sea, USA	*Proteobacteria*	*Halobacteria*	[12]
4	Isla Cristina Saltern, Spain	*Proteobacteria, Bacteroidetes*	*Halobacteria*	[13]
5	Eilat Solar Saltern, Israel	*Cyanobacteria*	*Halobacteria*	[14]
6	Shark Bay, Australia	*Cyanobacteria, Proteobacteria*	Archaea diverse (lower)	[9]
7	Guerrero Negro, Mexico	*Cyanobacteria, Proteobacteria*	*Halobacteria*	[15]
8	Dead Sea, Jordan/Israel	*Proteobacteria, Bacteroidetes*	*Haloferax/Halorubrum*	[16]
9	Magadi Lake, Kenya	*Haloalkaliphilic*	*Haloarchaea*	[17]
10	Sambhar Salt Lake, India	*Firmicutes, Proteobacteria, Bacteroidetes*	*Euryarchaeota (Halobacteria)*	[18]
11	Kutch Saline Desert, India	*Proteobacteria*	*Haloarchaea*	[19]
12	Chott El Jerid, Tunisia	*Firmicutes, Proteobacteria*	*Halobacteria*	[20]
13	Lake Urmia, Iran	*Proteobacteria*	*Halobacteria*	[21]
14	Salar de Atacama, Chile	*Proteobacteria*	*Halobacteria*	[11]
15	Namib Desert Salt Pans	*Proteobacteria, Actinobacteria*	*Halobacteria*	[22]

These findings extend previous work by demonstrating that hypersaline biofilms are not homogeneous but organized into a baseline inventory shaped by gradients of oxygen, nutrients, and salinity [2, 23, 24]. The high abundance of *Firmicutes* such as *Bacillus horikoshii* may indicate a potential role in EPS production and osmoprotectant synthesis, functions that have been frequently reported for related taxa in hypersaline systems [25], while *Alphaproteobacteria* such as *Oceanicaulis* are often reported to degrade complex organic substrates, and their presence here may suggest similar ecological roles [26]. These complementary activities collectively stabilize the biofilm matrix and support resilience under osmotic stress. The co-occurrence of *Haloferax* and *Halohasta* illustrates the salt-in strategy of osmotic adaptation [27, 28], contrasting with the compatible solute approach of their bacterial partners. Similar bacterial–archaeal partitioning has been documented in hypersaline lakes and soils [11, 24], but the simultaneous prominence of *Oceanicaulis* in this system highlights a biogeographic distinction from other global sites.

The EPS matrix, composed of polysaccharides, proteins, lipids, and extracellular DNA (eDNA), formed the structural foundation of the biofilm. EPS promotes adhesion to substrates such as the HDPE panels used in this study, while also buffering cells against desiccation and ion toxicity [29-31]. In line with previous studies, eDNA appears to act both as a scaffold and a nutrient reservoir [23]. The presence of rare taxa within this matrix may reflect specialization within micro-niches; however, their roles remain speculative without direct functional validation. This emphasizes the need for cautious interpretation of rare taxa in taxonomic datasets.

The ecological structure observed here also illustrates how environmental filters and microbial interactions jointly shape hypersaline communities. Laboratory simulations across salinity gradients (2–40%) demonstrated shifts in community composition consistent with prior reports: Firmicutes and Proteobacteria are favored at intermediate salinities, whereas archaeal lineages such as *Euryarchaeota* dominate under extreme brine conditions [13, 24]. Comparative studies from Guerrero Negro and Israeli solar salterns [4, 5, 22] support the view that hypersaline biofilms are governed by a balance of deterministic factors (e.g., salinity, pH, substrate availability) and stochastic processes (e.g., immigration of rare taxa). The Indonesian biofilm is characterized by the dominance of *Oceanicaulis* and *Bacillus* within the bacterial domain, together with *Haloferax* and *Halohasta* among Archaea. This combination is consistent with baseline inventory functional models proposed in previous studies, although the present data derived from pooled biofilm samples do not directly demonstrate spatial stratification. This structure extends our understanding of how microbial communities persist in extreme niches and highlights regional variations that enrich global perspectives on hypersaline biofilm ecology.

3.5.2 Functional implications and metabolic potential

The taxonomic composition of the hypersaline biofilm suggests important functional roles in carbon turnover, nutrient cycling, and ecological stability under extreme osmotic stress. Dominant bacterial lineages such as *Oceanicaulis* and *Bacillus* are taxa commonly associated with the degradation of complex organics and EPS production in other hypersaline systems. While such functions cannot be confirmed from 16S rRNA data alone, the observed taxa imply potential ecological roles contributing to matrix stability and nutrient cycling within the biofilm [21, 32].

Archaeal taxa, particularly *Haloferax* and *Halohasta*, add another dimension to metabolic potential. These haloarchaea employ the salt-in strategy of osmotic balance and are frequently associated with rhodopsins and retinal-based proton pumps that support phototrophic energy capture [33]. Light-driven proton transport has been shown to enhance resilience in hypersaline lakes when organic substrates are scarce [2]. In addition, haloarchaea often encode nitrogen cycling pathways, including denitrification, which further contributes to nutrient turnover in extreme saline ecosystems [24]. The coexistence of heterotrophic bacteria and phototrophic/denitrifying archaea thus exemplifies metabolic complementarity, enhancing overall ecological stability.

Rare but persistent taxa such as *Roseivivax*, *Loktanella*, and *Bifidobacterium pseudolongum* were detected at low abundance. Their ecological roles in this system remain unclear; however, their presence highlights possible niche specialization and should be considered as observations for future research rather than confirmed functions. Because 16S rRNA sequencing provides only taxonomic inference, the specific metabolic contributions of these rare members cannot be directly established. Their presence, however, hints at possible niche specialization and redundancy that may reinforce community resilience [11, 22].

Taken together, the inferred functional structure of the halophilic biofilm reflects a consortium where bacteria dominate EPS production and organic matter degradation, archaea contribute to phototrophy and osmotic balance, and minor taxa potentially expand metabolic flexibility. This integrated network may allow the biofilm to act both as a biogeochemical reactor and as a stability reservoir in extreme environments. Beyond ecological significance, these traits could be harnessed for biotechnological applications such as bioremediation of saline effluents, enzymatic production under high ionic strength, and bioenergy generation via light-driven proton pumps.

Nevertheless, these functional interpretations are necessarily putative, as they are based solely on 16S rRNA taxonomic profiles rather than direct genomic or transcriptomic evidence. While such data provide valuable insight into community structure and potential metabolic strategies, they cannot confirm gene content or expression. Future studies employing shotgun metagenomics and metatranscriptomics are therefore essential to validate these predictions, uncover the genetic basis of observed adaptations, and resolve the active metabolic processes underpinning hypersaline biofilm stability.

3.5.3 Extremophile adaptation strategies

Halophilic biofilms persist in hypersaline environments by combining complementary strategies that mitigate osmotic, oxidative, and nutritional stresses. In our dataset, bacterial members such as *Bacillus* spp. likely adopt the compatible solute strategy, synthesizing or accumulating osmolytes including ectoine, glycine betaine, and trehalose, which stabilize proteins and maintain turgor under high salinity [21, 34]. In contrast, archaeal taxa such as *Haloferax* and *Halohasta* employ the salt-in strategy, characterized by high intracellular KCl and acidic proteomes enriched in negatively charged amino acids that preserve protein solubility in saturated brines [2]. The coexistence of these divergent mechanisms within a single biofilm highlights functional redundancy and community-level resilience under fluctuating salinity regimes.

Structural adaptations further reinforce this resilience. EPS production, prominent in *Bacillus*, provides hydration, ion buffering, and adhesion, while EPS enriched with eDNA may facilitate horizontal gene transfer and the rapid spread of adaptive traits [7]. Previous studies have shown that hypersaline biofilms often exhibit micro-gradients of oxygen and light that support niche specialization. In our pooled community, the coexistence of *Oceanicaulis*, *Bacillus*, and *Haloferax* genera suggests a similar ecological partitioning, though no direct baseline inventory or physicochemical profiling was performed [11, 22].

Additional strategies extend the biofilm's adaptive capacity. Haloarchaeal rhodopsins (bacteriorhodopsins and halorhodopsins) enable light-driven proton pumping and ATP synthesis under nutrient limitation [2]. Stress-tolerant enzymes such as halophilic amylases, lipases, and proteases, previously reported in *Firmicutes* and *Haloarchaea*, retain catalytic activity in high ionic strength, enhancing both ecological persistence and biotechnological potential [24]. The persistence of rare taxa, including *Roseivivax* and *Loktanella*, likely reflects niche-specific adaptations that further enhance community resilience [22]. Collectively, these findings show that adaptation in halophilic biofilms is not uniform but stratified and taxon-specific, integrating bacterial compatible solutes, archaeal salt-in mechanisms, and EPS-mediated structural defenses. This dynamic configuration extends previous models of hypersaline adaptation by demonstrating how baseline inventory and taxonomic complementarity jointly sustain biofilm function in one of Earth's most hostile environments.

3.5.4 Biotechnological potential of biofilm halophiles from Indonesia

The taxonomic composition, particularly the dominance of Bacillus and haloarchaea, indicates possible sources of salt-tolerant enzymes and metabolites. These prospective applications are discussed further in the conclusion as avenues for future research. These enzymes have been explored for industrial processes requiring high ionic strength, including food fermentation, detergent formulation, and biofuel production [21, 35]. The Indonesian salt pond context, with its fluctuating salinity gradients (2–40%), further suggests that local isolates may harbor unique enzymatic adaptations, though experimental validation is still required.

Archaea, such as *Haloferax* and *Halohasta,* may also provide resources of interest. Their rhodopsin-based proton pumps and stress-tolerant pigments have been proposed for bioenergy applications and optogenetics. For instance, haloarchaeal rhodopsins have been considered as sustainable bionanomaterials for solar energy harvesting and photonic devices, while carotenoids from halophiles are valued for their antioxidant properties and potential nutraceutical uses [2]. Although these applications remain largely conceptual in the context of this biofilm, they illustrate the biotechnological avenues that could be tested in future studies.

The detection of low-abundance but metabolically versatile taxa, including *Roseivivax* and *Loktanella*, further points to potential sources of novel biomolecules. Related lineages have been reported to produce rhodopsin-like proteins and secondary metabolites with antimicrobial activity [11]. In addition, the EPS matrix enriched with extracellular DNA (eDNA) highlights the potential of halophilic biofilms as reservoirs for biopolymer extraction. Halophilic EPS have been studied for their stability under extreme conditions and possible applications as bioflocculants, emulsifiers, or bioplastic precursors [36, 37]. Their metal-binding capacity also suggests avenues for saline wastewater remediation, though direct evidence from this study remains pending.

Positioning these findings within the Indonesian context underscores both ecological and socio-economic relevance. Salt ponds and aquaculture systems in the region face growing challenges from saline effluents and organic waste. Indigenous halophilic biofilms could, in principle, be developed as biofilm-based bioreactors or as sources of extremozymes, offering locally adapted strategies for waste management and value-added production. However, such applications remain potential and require future validation through experimental assays, metagenomic screening, and pilot-scale trials. Thus, the biofilm described here represents not only a valuable ecological model of extremophilic adaptation but also a prospective biotechnological asset for Indonesia, bridging microbial diversity exploration with industrial and environmental innovation.

4. CONCLUSIONS

This study provides the first amplicon-based 16S rRNA characterization of a halophilic biofilm from Indonesian salt ponds, describing community composition consistent with previously reported baseline inventory in hypersaline environments. The notably high relative abundance of *Limimaricola* and *Oceanicaulis* highlights possible regional influences on community structure and contributes new descriptive data to the broader understanding of halophilic diversity. Beyond ecological relevance, the biofilm's taxonomic and functional repertoire positions it as a potential reservoir of salt-tolerant enzymes, biosurfactants, and bioremediation agents. However, these applications remain prospective. Future research should integrate shotgun metagenomics and metatranscriptomics to verify functional potential, conduct experimental assays to validate enzymatic activities, and compare biofilms across diverse Indonesian salt ponds to assess environmental drivers of unique taxa dominance. Such efforts will strengthen the link between taxonomy, function, and application, and advance both ecological understanding and biotechnological exploitation of hypersaline biofilms.

ACKNOWLEDGMENT

This research was funded by the Doctoral Dissertation Research Program (PDD), Directorate of Research and Community Service, Directorate General of Research and Development, Ministry of Higher Education, Science and Technology of the Republic of Indonesia, with contract number: 064/C3/DT.05.00/PL/2025 and 00567/UN10.A0501/B/PT.01. 03.2/2025. The author would like to express the deepest gratitude to the Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, and the Coastal and Marine Research Centre of Brawijaya University for the facilities and equipment that greatly assisted this research. The authors gratefully acknowledge the technical and analytical support provided by the Safety of Fishery Products Laboratory, Brawijaya University, for assistance in preparing samples.

REFERENCES

[1] Wong, H.L., MacLeod, F.I., White, III, R.A., Visscher, P.T., Burns, B.P. (2020). Microbial dark matter filling the niche in hypersaline microbial mats. Microbiome, 8(1): 135. https://doi.org/10.1186/s40168-020-00910-0

[2] Oren, A. (2024). Novel insights into the diversity of halophilic microorganisms and their functioning in hypersaline ecosystems. npj Biodiversity, 3(1): 18. https://doi.org/10.1038/s44185-024-00050-w

[3] Gupta, A., Mishra, R., Rai, S., Bano, A., Pathak, N., Fujita, M., Kumar, M., Hasanuzzaman, M. (2022). Mechanistic insights of plant growth promoting bacteria mediated drought and salt stress tolerance in plants for sustainable agriculture. International Journal of Molecular Sciences, 23(7): 3741. https://doi.org/10.3390/ijms23073741

[4] Gao, L., Huang, Y., Liu, Y., Mohamed, O.A.A., Fan, X., Wang, L., Li, L., Ma, J. (2022). Bacterial community structure and potential microbial coexistence mechanism associated with three halophytes adapting to the extremely hypersaline environment. Microorganisms, 10(6): 1124. https://doi.org/10.3390/microorganisms10061124

[5] Parrilli, E., Tutino, M.L., Marino, G. (2022). Biofilm as an adaptation strategy to extreme conditions. Rendiconti Lincei. Scienze Fisiche e Naturali, 33(3): 527-536. https://doi.org/10.1007/s12210-022-01083-8

[6] Campbell, M.A., Grice, K., Visscher, P.T., Morris, T., Wong, H.L., White, III, R.A., Burns, B.P., Coolen, M.J.L. (2020). Functional gene expression in Shark Bay hypersaline microbial mats: Adaptive responses. Frontiers in Microbiology, 11: 560336. https://doi.org/10.3389/fmicb.2020.560336

[7] Kurniawan, A., Aziz Amin, A., Yanuar, A.T., Pramudia, Z., Susanti, Y.A.D., Zamzami, I.M.A., Kurniaty, R., Hakim, L., Ardian, G., Amenan, M. (2024). Exploring viability and innovation requirements for novel salt production: A case study of Kangen Beach, Malang Regency's South Coast, Indonesia.Cogent Social Sciences, 10(1): 2434667. https://doi.org/10.1080/23311886.2024.2434667

[8] Bhattarai, B., Bhattacharjee, A.S., Coutinho, F.H., Goel, R.K. (2021). Viruses and their interactions with bacteria and archaea of hypersaline Great Salt Lake. Frontiers in Microbiology, 12: 701414. https://doi.org/10.3389/fmicb.2021.701414

[9] Skoog, E.J., Fournier, G.P., Bosak, T. (2023). Assessing the influence of HGT on the evolution of stress responses in microbial communities from Shark Bay, Western Australia. Genes, 14(12): 2168. https://doi.org/10.3390/genes14122168

[10] Kurniawan, A., Pramudia, Z., Susanti, Y.A.D., Zamzami, I.M.A., Yamamoto, T. (2024). Comparative biosorption proficiency in intact and autoclaved biofilm matrices. Journal of Ecological Engineering, 25(4): 131-141. https://doi.org/10.12911/22998993/183943

[11] Kurth, D., Elias, D., Rasuk, M.C., Contreras, M., Farías, M.E. (2021). Carbon fixation and rhodopsin systems in microbial mats from hypersaline lakes Brava and Tebenquiche, Salar de Atacama, Chile. Plos One, 16(2): e0246656. https://doi.org/10.1371/journal.pone.0246656

[12] Hawley, E.R., Schackwitz, W., Hess, M. (2014). Metagenomic sequencing of two Salton Sea microbiomes. Genome Announcements, 2(1): 10-1128. https://doi.org/10.1128/genomea.01208-13

[13] Fernández, A.B., León, M.J., Vera, B., Sánchez-Porro, C., Ventosa, A. (2014). Metagenomic sequence of prokaryotic microbiota from an intermediate-salinity pond of a saltern in Isla Cristina, Spain. Genome Announcements, 2(1): 10-1128. https://doi.org/10.1128/genomea.00045-14

[14] Sørensen, K.B., Canfield, D.E., Oren, A. (2004). Salinity responses of benthic microbial communities in a solar saltern (Eilat, Israel). Applied and Environmental Microbiology, 70(3): 1608-1616. https://doi.org/10.1128/aem.70.3.1608-1616.2004

[15] Harris, J.K., Caporaso, J.G., Walker, J.J., Spear, J.R., et al. (2012). Phylogenetic stratigraphy in the Guerrero Negro hypersaline microbial mat. The ISME Journal, 7(1): 50-60. https://doi.org/10.1038/ismej.2012.79

[16] Jacob, J.H., Hussein, E.I., Shakhatreh, M.A.K., Cornelison, C.T. (2017). Microbial community analysis of the hypersaline water of the Dead Sea using high-throughput amplicon sequencing. MicrobiologyOpen, 6(5): e00500. https://doi.org/10.1002/mbo3.500

[17] Kambura, A.K., Mwirichia, R.K., Kasili, R.W., Karanja, E.N., Makonde, H.M., Boga, H.I. (2016). Bacteria and Archaea diversity within the hot springs of Lake Magadi and Little Magadi in Kenya. BMC Microbiology, 16(1): 136. https://doi.org/10.1186/s12866-016-0748-x

[18] Mehta, P., Yadav, M., Ahmed, V., Goyal, K., Pandey, R., Chauhan, N.S. (2021). Culture-independent exploration of the hypersaline ecosystem indicates the environment-specific microbiome evolution. Frontiers in Microbiology, 12: 686549. https://doi.org/10.3389/fmicb.2021.686549

[19] Pandit, A.S., Joshi, M.N., Bhargava, P., Shaikh, I., Ayachit, G.N., Raj, S.R., Saxena, A.K., Bagatharia, S.B. (2015). A snapshot of microbial communities from the Kutch: One of the largest salt deserts in the World. Extremophiles, 19(5): 973-987. https://doi.org/10.1007/s00792-015-0772-z

[20] Ben Abdallah, M., Karray, F., Kallel, N., Armougom, F., Mhiri, N., Quéméneur, M., Cayol, J.L., Erauso, G., Sayadi, S. (2018). Abundance and diversity of prokaryotes in ephemeral hypersaline lake Chott El Jerid using Illumina Miseq sequencing, DGGE and qPCR assays.Extremophiles, 22(5): 811-823. https://doi.org/10.1007/s00792-018-1040-9

[21] Kheiri, R., Mehrshad, M., Pourbabaee, A.A., Ventosa, A., Amoozegar, M.A. (2023). Hypersaline Lake Urmia: A potential hotspot for microbial genomic variation. Scientific Reports, 13(1): 374. https://doi.org/10.1038/s41598-023-27429-2

[22] Martínez-Alvarez, L., Ramond, J.B., Vikram, S., León-Sobrino, C., Maggs-Kölling, G., Cowan, D.A. (2023). With a pinch of salt: Metagenomic insights into Namib Desert salt pan microbial mats and halites reveal functionally adapted and competitive communities. Applied and Environmental Microbiology, 89(12): e00629-23. https://doi.org/10.1128/aem.00629-23

[23] Fröls, S., Dyall-Smith, M., Pfeifer, F. (2012). Biofilm formation by haloarchaea. Environmental Microbiology, 14(12): 3159-3174. https://doi.org/10.1111/j.1462-2920.2012.02895.x

[24] Ding, Y., Ke, J., Hong, T., Zhang, A., Wu, X., Jiang, X., Shao, S., Gong, M., Zhao, S., Shen, L., Chen, S. (2025). Microbial diversity and ecological roles of halophilic microorganisms in Dingbian (Shaanxi, China) saline-alkali soils and salt lakes. BMC Microbiology, 25(1): 287. https://doi.org/10.1186/s12866-025-03997-3

[25] Upadhyaya, C., Patel, H., Patel, I., Upadhyaya, T. (2025). Extremophilic exopolysaccharides: Bioprocess and novel applications in 21st century. Fermentation, 11(1): 16. https://doi.org/10.3390/fermentation11010016

[26] Liu, J., Zheng, Y., Lin, H., Wang, X., et al. (2019). Proliferation of hydrocarbon-degrading microbes at the bottom of the Mariana Trench. Microbiome, 7(1): 47. https://doi.org/10.1186/s40168-019-0652-3

[27] Amoozegar, M.A., Siroosi, M., Atashgahi, S., Smidt, H., Ventosa, A. (2017). Systematics of haloarchaea and biotechnological potential of their hydrolytic enzymes. Microbiology, 163(5): 623-645. https://doi.org/10.1099/mic.0.000463

[28] Zamzami, I.M.A., Yona, D., Faqih, A.R., Kurniawan, A. (2025). Decoding halophilic biofilm development across salinity gradients in hypersaline environments. Biodiversitas Journal of Biological Diversity, 26(6): 2773-2785. https://doi.org/10.13057/biodiv/d260622

[29] Salamah, L.N., Al-Zamzami, I.M., Pramudia, Z., Susanti, Y.A.D., Dhea, L.A., Kurniawan, A. (2024). Distribution of microplastics in Lusi Island, Sidoarjo, Indonesia. IOP Conference Series: Earth and Environmental Science, 1328(1): 012012. https://doi.org/10.1088/1755-1315/1328/1/012012

[30] Mas'ud, F., Musa, M., Lestariadi, R.A., Al Zamzami, I.M. (2025). Sustainable vannamei shrimp farming in Bonorowo, Indonesia wetlands: Growth performance, land suitability, and ecological challenges. Egyptian Journal of Aquatic Biology and Fisheries, 29(4): 897-919. https://doi.org/10.21608/ejabf.2025.442574

[31] Zamzami, I.M.A., Yona, D., Faqih, A.R., Kurniawan, A. (2025). Halophilic bacteria in biotechnology: A seven-decade scientometric analysis of global research trends, knowledge gaps, and emerging applications (1955-2024). Journal of Ecological Engineering, 26(10): 252-271. https://doi.org/10.12911/22998993/205826

[32] Perwira, I.Y., Ulinuha, D., Al Zamzami, I.M., Ahmad, F.H., Kifly, M.T.H., Wulandari, N. (2020). Environmental factors associated with decomposition of organic materials and nutrients availability in the water and sediment of Setail River, Banyuwangi, Indonesia. IOP Conference Series: Earth and Environmental Science, 493(1): 012025. https://doi.org/10.1088/1755-1315/493/1/012025

[33] Weng, M.M., Klempay, B., Bowman, J.S., Fisher, L., Camplong, C., Doran, P.T., Rundell, S., Glass, J.B., Dutta, A., Pontefract, A., Bartlett, D.H., Schmidt, B., Johnson, S.S. (2025). Light cues drive community-wide transcriptional shifts in the hypersaline South Bay Salt Works. Communications Biology, 8(1): 450. https://doi.org/10.1038/s42003-025-07855-w

[34] Sharma, P., Kapoor, D., Kumar, R., Husen, A. (2025). Rhizosphere and osmolytes coordination in plant systems under adverse situations. In Roles of Osmolytes in Changing Environment, pp. 101-117. https://doi.org/10.1016/b978-0-443-26549-5.00020-5

[35] Ismail, E., Prihanto, A.A., Kartikaningsih, H., Huda, N., Al Zamzami, I.M. (2025). Isolation and characterization of a novel acid protease from striped marlin (Kajikia audax) stomach with potential as a rennet substitute in dairy processing. Egyptian Journal of Aquatic Biology and Fisheries, 29(4): 1593-1613. https://doi.org/10.21608/ejabf.2025.443720

[36] Kanik, M., Munro-Ehrlich, M., Fernandes-Martins, M.C., Payne, D., et al. (2020). Unexpected abundance and diversity of phototrophs in mats from morphologically variable microbialites in Great Salt Lake, Utah. Applied and Environmental Microbiology, 86(10): e00165-20. https://doi.org/10.1128/aem.00165-20

[37] Sepe, F., Costanzo, E., Ionata, E., Marcolongo, L. (2025). Biotechnological potential of extremophiles: Environmental solutions, challenges, and advancements. Biology, 14(7): 847. https://doi.org/10.3390/biology14070847