HAYATI
Journal of Biosciences

Vol. 30 No. 5, September 957-984
DOI:10.4308/hjb.30.5.957-984
ISSN: 1978-3019
EISSN: 2086-4094

Taxonomic Profiling of Microorganisms Inhabiting Two Solar Salterns that Produce High- and Low-Quality Salts

Ekowati Chasanah[1*], Asri Pratitis[2], Rini Susilowati[2], Agus Heri Purnomo[3], Hedi I. Januar[4], Agustinus R. Uria[5]

[1]*Research Center for Marine and Inland Bioproducts, Research Organization (RO) of Earth and Maritime, National Research and Innovation Agency (BRIN), North Lombok Regency, Province of West Nusa Tenggara 83352, Indonesia*
[2]*Deputy STI Human Resources, BRIN, BJ Habibie Building, Central Jakarta 10340, Indonesia*
[3]*Research Center for Society and Culture, RO of Social Sciences and Humanities, BRIN, Jakarta 12710, Indonesia*
[4]*Research Center for Ecology and Ethnobiology, RO of Life Science and Environment, BRIN, KST Soekarno, Cibinong 16911, Indonesia*
[5]*Faculty of Pharmaceutical Sciences, Hokkaido University. Kita 12, Nishi 6, Kita-ku, Sapporo 060-0812, Japan*

ARTICLE INFO

Article history:
Received February 19, 2022
Received in revised form January 10, 2023
Accepted May 10, 2023

KEYWORDS:
metagenomic taxonomic profiling,
solar salterns,
phytoplankton,
Sampang and Tuban Indonesia,
halophiles

ABSTRACT

The contribution of halophilic microorganisms to the quality of salts produced in solar salterns has recently been recognized but not clearly understood. Using metagenomic 16S rRNA gene sequencing approach, we showed the microbial composition difference between the Tuban crystallization pond (CP-Tuban) that produces low-quality salt and CP-Sampang as a representative solar saltern that yields high-quality salt. Dominant classes in both traditional salterns were represented by γ-proteobacteria and halobacteria that occurred at higher prevalence in CP-Sampang. Microbial taxa, including beneficial genera, in CP-Sampang were more diverse and abundant compared to CP-Tuban. Among 180 genus-level OTUs identified in CP-Sampang, 127 of them were considered unique due to their absence in CP-Tuban. Higher levels of dissolved oxygen (DO) and nutrient (phosphate, nitrate, and ammonia) in the seawater reservoir (SR) of Sampang may contribute to more diverse phytoplankton genera, which could support the growth of beneficial heterotrophic microbes that positively affect the salt quality of the CP-Sampang. Low number of *Dunaleilla* sp. in both CPs do not seem to influence the quality of salts produced. The outcome of these comparative studies provides new insights into the contribution of diverse microbial taxa in correlation with physico-chemical parameters and phytoplankton communities to the high quality of salts produced in traditional solar salterns. The presence of beneficial genera in the enriched microbial cultures could provide an important basis for further applications, such as improving the quality of salt produced and producing unique compounds and enzymes.

1. Introduction

Halophilic microorganisms have particularly been explored to produce unique bioproducts such as biopolymers, hydrolytic enzymes, biosurfactants, biofuels, and silver and selenium nanoparticles, which are useful in biotechnological processes and bioremediation (Abdollahnia *et al.* 2020). Their unique ecological roles in hypersaline environments have been a hot topic in recent years, and studies on microbial community in high-salinity environments including solar salterns have been conducted extensively worldwide (Yang *et al.* 2007; Hedi *et al.* 2009; Najjari *et al.* 2015; Naghoni *et al.* 2017; Barreteau *et al.* 2019; Chasanah *et al.* 2020; Mani *et al.* 2020).

The dominant population of halophilic archaea in hypersaline environments can easily be recognized by the red color that they produce (Oren and Rodriguez-Velera 2011). The red color of crystalization ponds in solar salterns has been associated with the presence of massive archaea within the class Halobacteria (family Halobacteriaceae) that produce C_{50}- carotenoids (Oren 2014a, 2020). Other halophilic microorganisms involved in the red color formation are *Salinibacter ruber* harboring a unique C_{40}-carotenoid acyl glycoside and the unicellular

* Corresponding Author
E-mail Address: ekowati.chasanah@brin.go.id

green microalga *Dunaliella salina* known to contain β-carotene (Oren 2020). It is generally considered that these pigmented microorganisms play an important role in increasing water evaporation in crystalisation ponds, thereby contributing to the salt production process (Oren 2010). The equilibrium of microbiological communities contributes to the production of high-quality salt (NaCl purity of 99.7%) (Javor 2002). However the comparative study of halophilic microbial diversity between solar salterns that produce different quality of salts has not been reported so far.

Solar salterns play important roles for salt production worldwide, since approximately one third of total salt produced for human consumption is derived from this solar evaporation method (Davis 2000). The rest is produced by mining rock salt deposits (Hyler 1935). Solar saltern is mostly built as a multi-pond system, in which seawater initially flows or be pumped to the first set of ponds called stabilization ponds or seawater reservoirs (SR) with 3-5°Baume (Bé). Subsequently seawater that flows through successive evaporation ponds vaporizes due to sunlight and wind, which leads to the increased salinity in precipitation ponds, and finally deposites as sodium salt (halite) in crystallization ponds (CP) (Fernández *et al.* 2014). The crystalization pond, where salt starts to crystalize, is characterized by the saltwater concentration of 25°-28°Be, and in some traditional crystallization ponds were started with 23°Be salt water. Upon salt crystal formation, the waste called bittern is immediately separated from the salt crystals (Hyler 1935).

While studying microbial diversity in solar saltern systems has mostly focused on halophilic microorganisms such as archaea and bacteria (Anton *et al.* 2000; Oh *et al.* 2010; Plominsky *et al.* 2018 Mani *et al.* 2020), the communities of microscopic algae, also called phytoplankton, inhabiting solar salt ponds remain poorly investigated. It has been reported that halophilic phytoplankton such as *Dunaleilla salina* are able to adapt to high salinity and light intensities (Oren 2014b), and their accumulations in the crystallization ponds has been associated with low-quality salt (Davis and Giordano 1995; Giardano *et al.* 2014). However, the negative effect of *D. salina* to the low-quality salt produced in solar salterns remains unclear (Oren 2014b).

The objective of this study was to perform comparative profiling of the microbial communities between two solar salterns that produce salts at different qualities in correlation with physico-chemical parameters and phytoplankton communities. Both solar salterns in our present study are located in East Java where one third of the total salt production in Indonesia come from this region. Sampang solar saltern in Madura Island, a well-known salt production center since Dutch colonialism, was selected in our study as a representative of solar saltern ponds producing high-quality salt. Salt produced in the Sampang solar saltern contained 94.10-97.88% NaCl, 0.1% calcium, and 0.56% magnesium with the moisture content of 5.72% (https://matamaduranews.com/kualitas-garam-madura-, 2019). A saltern pond in Tuban chosen in this study represents salterns that produce low-quality salt. This Tuban salt was reported to contain 86.13% NaCl, 0.028% calcium, 0.0029% magnesium with the moisture content of 12.92% (Suwasono *et al.* 2013).

2. Materials and Methods

2.1. Seawater Sampling and Physico-Chemical Parameter Measurement

Seawater samples were collected from three (3) reservoirs/stabilization ponds (SR) and crystallization ponds (CP), which were randomly chosen at Tuban and Sampang solar salterns. The physical parameters were measured in situ by HACH multi-parameter colorimeter with a single probe. The probe was dipped into 10 cm under water surface. The parameters measured included water temperature, salinity, pH and oxygen demand (DO). The chemical properties were measured *ex situ* using colorimetry method. The collected samples were stored in plastic bottles and kept at about 4°C using a cool-box filled with full crushed ice to avoid biological decompotition. The chemical parameters analyzed were ammonia (NH_4), nitrate (NO_3), and phosphate (PO_4) concentrations, which were measured in three replicates.

2.2. Phytoplankton Sampling and Identification

Phytoplankton samples were obtained by filtering 50 L of pond's water using plankton nets (three replications) of low salinity ponds or called reservoir (SR), intermediate salinity pond and high salinity pond or crystalization pond (CP). The filtered water was transferred to a dark-coated bottle. Preservation was conducted by adding 4% of lugol into filtered

water (Edler 1979). Identification and calculation of phytoplankton cells were carried out in the laboratory. Water sample of 1 ml was initially placed on Sedgewick Rafter-counting Cell (SRC) and covered by a glass-cover. The phytoplankton cells were identified and counted under a light microscope in 10 × 40 magnification. The number and abundance of each phytoplankton genus were determined for each sample. The phytoplankton community was studied by calculating the diversity index (H'), richness (d) and dominance (d). Correlation analysis among environmental factors were performed using SPSS. Multivariate analysis of the environmental conditions and phytoplankton abundance were performed using SPLUS software package (Harrell 2001).

2.3. Microbial Cell Collection and Enrichment

Seawater (1 L) at each sampling site of crystalization ponds was filtered through sterile 0.22-µm polycarbonate membranes (Millipore). A filter membrane disc with the attached microbial cells representing a brine sample was divided into two parts using a sterile scissor. One part was immersed in 70% ethanol in a 250-ml Durham bottle for metagenomic 16S rRNA gene analysis. Another part was immersed in a growth medium for microbial cell enrichment. Both uncultured and enriched microbial samples were placed in a coolbox filled with full crushed ice having temperature of about 4°C and brought to the laboratory. Microbial cells attached on the membrane disc of a brine sample was enriched using a medium containing 0.1% tryptone, 0.05% yeast extract in sterile seawater with different salinities (25%, 30%, 35%) in a shaker waterbath at 30 oC for 24-48 hours with the agitation of 250 rpm. The enriched cell suspension (100 µL) was transferred to the Luria broth plates modified by the addition of seawater from the sampling site. After 24-hour incubation at 30°C, the DNA of the enriched cultures were extracted.

2.4. DNA Isolation and PCR-Amplification

DNA from uncultivated and enriched samples were individually isolated using ZymoBIOMICS DNA Miniprep Kit. Uncultivated samples from Tuban and Sampang are designated here as TU and SU, respectively. TE and SE symbols refer to enriched samples from Tuban and Sampang by mixed cultivation, respectively. The extracted DNA from each sample was visualized on 1% agarose gel. DNA concentration and quality were measured with Qubit 2.0 (Invitrogen) using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific). All DNA samples were individually diluted to the final concentration of 5 ng/µl in 10 mM Tris pH 8.5. The PCR-amplification of the V3 and V4 regions of 16S rRNA genes were carried out using the ready-mix PCR Kit 2x KAPA HiFi HotStart Ready Mix (KAPA Biosystems) based on the primer pair containing overhang adapter sequences at 5'-termini (5`-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3`) and (5`-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3`) (Klindworth *et al.* 2013). The PCR program was set up at 25 cycles, consisting of pre-denaturation at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 30 seconds, and final elongation at 72°C for 5 minutes. The target PCR product (~460 bp) of each sample was cleaned up using AMPure XP beads (BeckmanCoulter, Inc) to remove free primers and primer dimer species.

2.5. Amplicon Library Preparation and Real-Time Sequence Analysis

The purified PCR product of each sample was used to prepare a metagenomic 16S MiSeq paired-end library (2 × 300 bp with at least ~50 bp of overlapping sequence in the middle of paired-end reads) based on the instructions described in the Illumina® Nextera XT Library preparation kit (Illumina). Briefly, the index primer 2 adjacent to the S5 adapter sequence (S502-CTCTCTAT) in combination with the index primer 1 adjacent to the P7 sequence (N701-TAAGGCGA for TU sample, N704-TCCTGAGC for SU sample, N702-CGTACTAG for TE, and N703-AGGCAGAA for SE) were attached to the amplicons using the Nextera XT Index Kit. The generated libraries were quantified using Qubit and Q-PCR, normalized or diluted to 4 nM, and pooled. The pooled amplicon libraries were denatured with NaOH, diluted with hybridization buffer, and re-denatured by heating at 96°C. The resulting denatured and diluted amplicon library was subjected to MiSeq sequencing run. Real-time Analysis (RTA) during the MiSeq sequencing run was conducted using MiSeq Reporter software (illumina) with the Greengenes database (http://greengenes.lbl.gov/) as the reference sequences. The resulting sequence data in RMA6 format created in

the Alignment folder was subsequently analyzed using MEGAN Community Edition (Huson *et al.* 2016) to obtain general comparative insights into the taxonomic profiles at the phylum and class levels among samples from Tuban and Sampang salterns.

2.6. Creating 16S Sequence Classifier Database

Short paired sequence reads generated using the Illumina MiSeq system were converted into fastQ files. All raw sequence reads were end-paired and quality-filtered for length selection and chimera removal according to the amplicon metagenomic procedure described by Geneious (https://www.geneious.com). Briefly, the sequence data in fastQ format was initially imported to Geneious Prime sequence analysis software (ver. 10.2.3, free trial). The separate forward and reverse of sequence reads were paired to generate a single paired read list. The BBDuk plugin (ver. 37.64 by Brian Brusnell) was subsequently used to trim the remaining Illumina adaptors from both ends with the minimal overlap of 24 bp. Short reads of less than 100 bp were removed. The BBMerge tool (ver. 37.64 by Brian Brusnell) was used to merge the paired reads. The paired reads with the expected size of 400-460 bp were extracted. Chimeric reads were subsequently removed from the dataset using UCHIME v4.2.40 (Edgar *et al.* 2011) using RDP-Gold database (5,181 16S rRNA sequences) as the reference sequences. The RDP-Gold database was obtained from the Microbiome Utilities provided by the Broad Institute (http://microbiomeutil.sourceforge.net/). Similar reads were clustered using de novo assembly with the minimum overlap identity of 98%, generating OTU consensus sequences (contigs) as representatives of different reads. Contigs and un-clustered unique sequences were subjected to the Mega BLAST mode of NCBI against the preformatted 16S microbial database downloaded from the updated BLAST databases (https://ftp.ncbi.nlm.nih.gov/blast/db/). The BLAST hits were subsequently processed by removing duplicates and extracting the BLAST hit regions. A 16S sequence classifier database was created from the BLAST hits.

2.7. Classifying Reads Up to Genus Level

All of the 16S rRNA gene sequences (400-460 bp) were classified using the 16S Biodiversity tool RDP Classifier v.2.12 (Wang *et al.* 2007) and subsequently visualized as interactive graph of microbial diversity using Krona (Ondov *et al.* 2011). For high-resolution taxonomic analysis, all amplicon sequences (400-460 bp) of each sample were assigned up to genus level using the Geneious Sequence Classifier (Aaron Kennedy, USDA-APHIS-PPQ and Biomatters) by comparing them to the resulting 16S sequence classifier database created in this work as described above. For classifiying reads at higher taxonomic ranks, the following identity thresholds were used: 97% for Genus, 95% for Family, 90% for Order, 85% for Class, and 80% for Phylum (Taxonomic classification pipeline-BaseClear www.baseclear.com). The classification results in tables were exported and saved as .csv files for further analyses in Excel. The identified taxonomic levels were checked manually from the OTU table and analyzed to determine their composition in each of the environmental samples. The intersections of genera numbers between different samples were visualized in Venn Diagram developed by University of Gent, Bioinformatics and Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/).

2.8. Stastistical Analyses of Genus-Level Alpha-Diversity

Alpha-diversity at the genus level was measured by taking account the number of taxa in the community and the number of read sequences at different taxa (Chernov *et al.* 2015) to get insights into richness and evenness/dominance within metagenomic samples (Chernov *et al.* 2015; Wang *et al.* 2022). The diversity indices calculated here included Margalef index (*K*) (Margalef 1958), and Menhinick index (*M*) (Menhinick 1964), Shannon Entropy index (*H`*) (Shannon 1948), and Simpson diversity index (Simpson 1949). The Simpson index used was in the form 1 – *D*, as *D* value decreases when the taxa evenness increases (Jost 2006). Other diversity indices included Chao1 (Chao 1984; Chao and Yang 1993), iChao1 as the improved version of Chao1 estimator (Chiu *et al.* 2014), and the abundance coverage estimator (ACE) used to estimate the actual number of taxa (Good 1953; Good and Toulmin 1956; Chao and Lee 1992; Chao and Yang 1993). The evenness measure of the Shannon index referring to Pielou evenness (*J`*) (Pielou 1966) was determined to get insight dominant genus-level taxa.

3. Results

3.1. Physico-Chemical Properties and Phytoplankton Biomass

DO values in Sampang ponds were higher than those in Tuban's ponds (Table 1), showing that there was a fairly good oxygenation rate in Sampang ponds compared with that in Tuban ones. No significant difference of pH was observed between both solar saltern ponds. It was found that both Sampang and Tuban locations were rich of nutrients indicated by high concentration of phosphate, nitrate, and ammonia. Although levels of phosphate, nitrate, and ammonia at SR-Sampang were higher compared to those at SR-Tuban, N/P ratio in SR-Tuban was higher than that in SR-Sampang (Table 1). The measurement of phytoplanton biomass in the reservoirs (SR) ponds (Figure 1 and 2) indicated that *Nitszchenia* sp. dominated SR-Tuban, while *Peridinium* sp. dominated SR-Sampang. Species richness of phytoplankton in SR-Tuban was higher than SR-Sampang. Among 19 phytoplankton genera found in Tuban solar salt ponds, 10 of them were significantly abundant. Meanwhile, 13 genera of them were identified in Sampang solar salt ponds. Among the identified genera, *Dunailella* sp. biomass was generally higher at Tuban and Sampang ponds with high salinity compared to low-salinity ponds such as seawater reservoirs (Figure 1). The high biomass of *Dunailella* sp. was particularly found in the ponds with intermediate salt level, followed with the crystallization ponds (CP) with high salinity (Figure 2). Multivariate analysis in Figure 3 shows a correspondence between phytoplankton abundance and water quality.

3.2. Taxonomic Profiling of Saltern Microorganisms

The profiling results of uncultured microorganisms without enrichment as shown in Figure 4A indicated that there were 18 phyla shared between CP-Tuban and CP-Sampang, in which two of them belong to the

Table 1. Chemical and physical characteristics of Tuban and Sampang solar salterns

	Tuban			Sampang		
	Low	Intermediate	High	Low	Intermediate	High
Salinity	30.28±6.06	142.50±9.14	237.50±10.12	33.34±7.57	121.80±0.32	217.25±0.28
DO (mg L-1)	5.52±0.27	5.125±0.29	5.275±0.29	6.23±0.05	6.80±0.00	6.23±0.02
pH	8.41±0.11	8.05±0.11	7.59±0.05	8.65±0.14	8.03±0.09	7.64±0.04
Phosphate (µM)	2.64±0.00	5.26±0.00	5.92±0.40	10.18±4.36	2.63±0.00	2.63±0
Nitrate (µM)	0.90±0.12	2.02±0.25	2.82±0.25	1.08±0.16	3.23±0.81	4.03±0.40
Ammonia (µM)	8.22±3.34	11.01±2.78	26.42±8.34	27.40±9.25	11.74±2.94	7.34±2.20

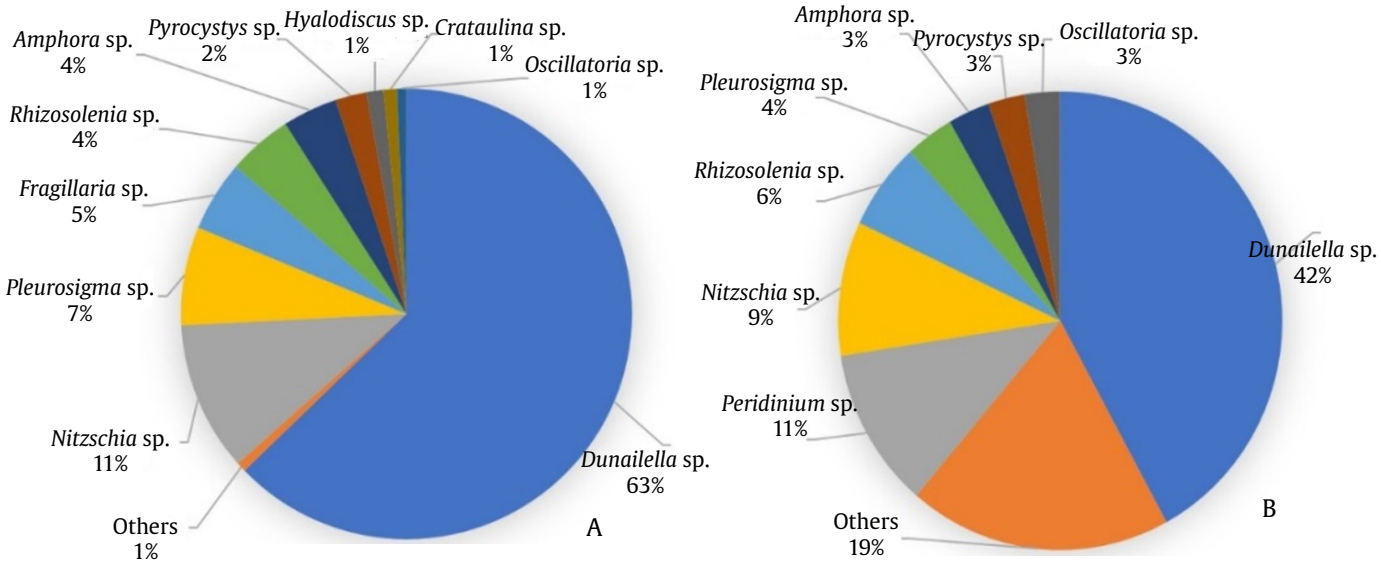


Figure 1. General percentage of phytoplankton genera found in (A) Tuban and (B) Sampang solar salterns

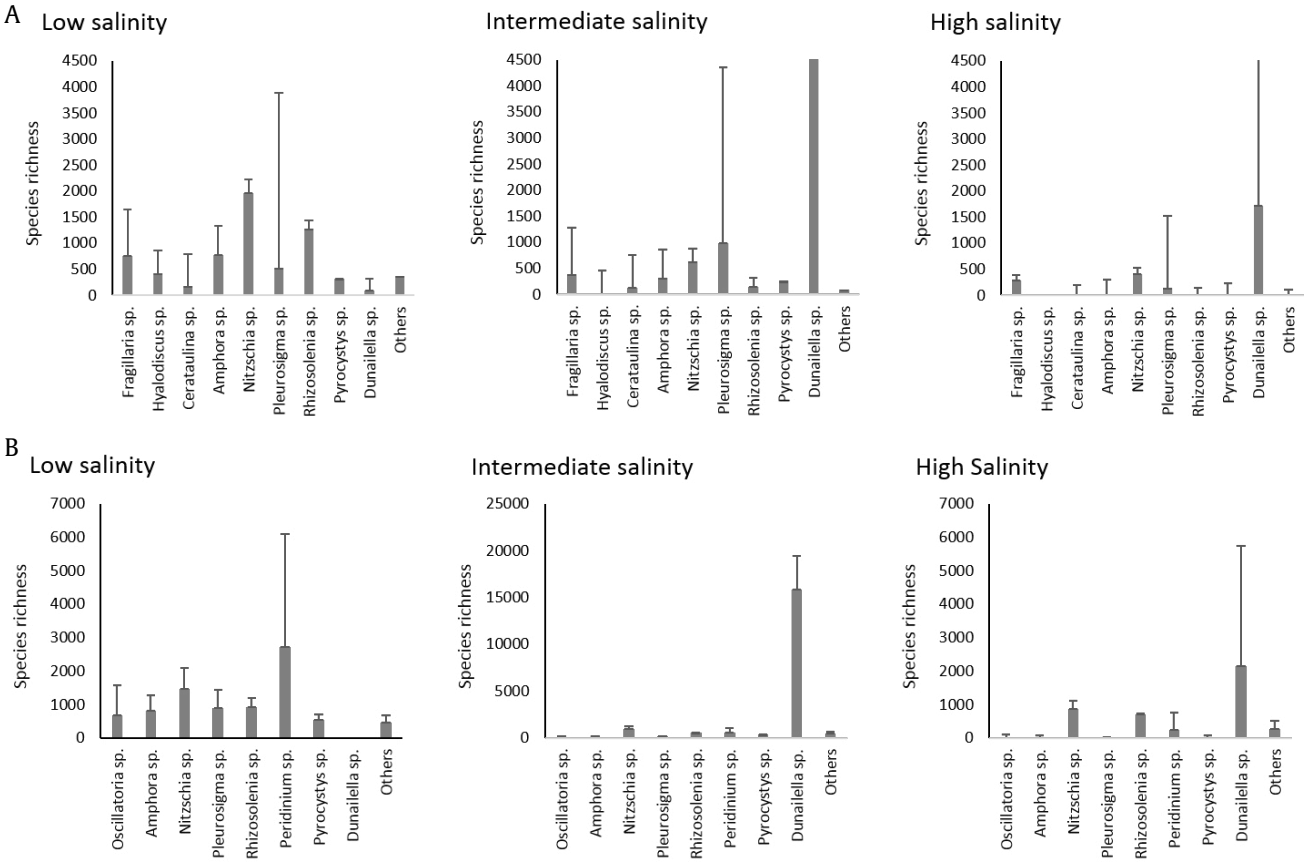


Figure 2. Phytoplankton abundance in Tuban (A) and Sampang (B) solar salt ponds

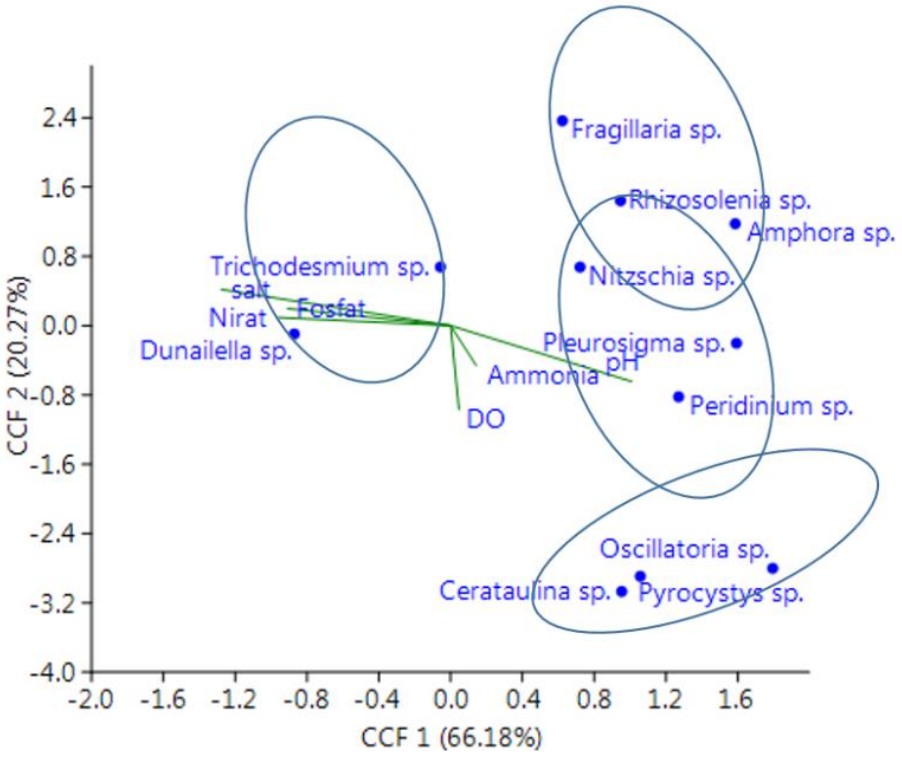


Figure 3. The correspondence of environmental conditions and phytoplankton community in solar salt ponds

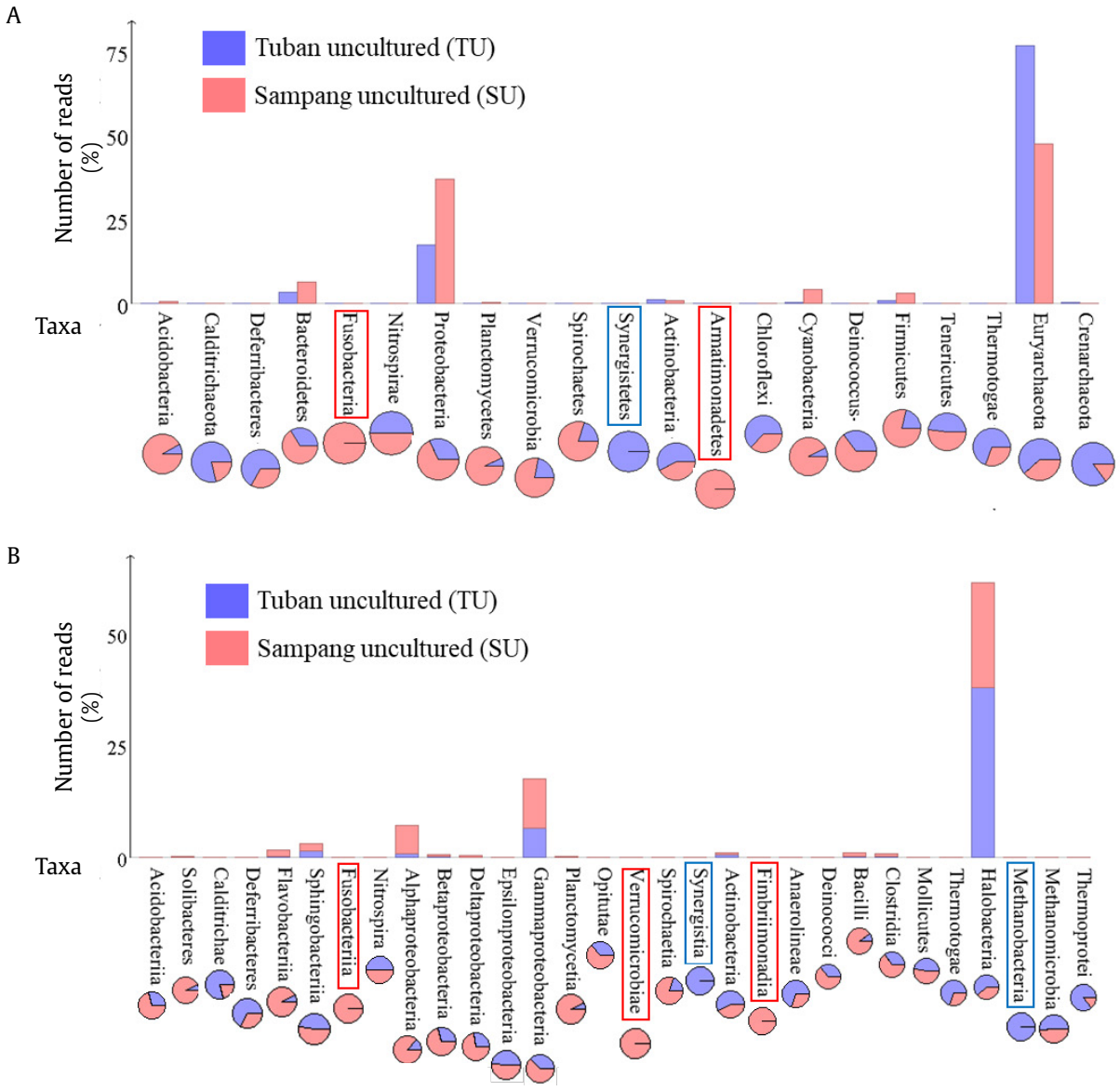


Figure 4. Comparative analysis of the diversity and relative abundance of uncultured microbial communities at the phylum (A) and class (B) levels between CP-Tuban and CP-Sampang. TU, uncultured microbial sample from CP-Tuban; and SU, uncultivated microbial sample from CP-Sampang

archaeal group (Euryarchaeota, Crenarchaeota). The remaining 16 phyla belong to the bacterial group, namely Acidobacteria, Calditrichaeota, Defferribacteres, Bacteroidetes, Nitrospirae, Proteobacteria, Planctomycetes, Verrucomicrobia, Spirochaetes, Actinobacteria, Chloroflexi, Cyanobacteria, Deinococcus, Firmicutes, Tenericutes, and Thermotogae. Interestingly, the phyla Fusobacteria and Armatimonadetes were only detected in Sampang sample, while the phylum Synegistetes was found only in Tuban sample.

As shown in Figure 4B, CP-Tuban with high salt levels (23°Be) was predominantly inhabited by Euryarchaeota (71.03%), followed with Proteobacteria (13.66%), Bacteroidetes (3.03%), Actinobacteria (1.16%), Firmicutes (0.76 %), Cyanobacteria (0.32%), and Crenarchaeota (0.14%). Meanwhile, CP-Sampang was dominated by Euryarchaeota (45.66%), followed with proteobacteria (32.83%). Genus-level 16S sequences from CP-Tuban were dominated by members of Halobacteria, such as *Halohasta* (238 reads), *Halorubrum* (143 reads), *Halobellus* (138 reads), *Halobaculum* (107 reads), *Haloplanus* (93 reads), and *Halomicroarcula* (82 reads). Two members of Proteobacteria *Enterobacter* (85 reads) and *Spiribacter* (501 reads) were prevalent in CP-Tuban, which accounted for 33.7% of the total reads

(Supplementary Table 1). While Genus-level OTUs in CP-Sampang were dominated by members of Halobacteria, such as *Halobaculum*, *Halomicroarcula, Halohasta, Haloarcula, Haloplanus*, and *Halorubrum* (Supplementary Table 2, Figure 5).

We enriched microbial samples derived from both CP-Tuban and CP-Sampang through mix cultivation using a medium added with seawater taken from the sampling sites. It was found that the enriched microbial cultures from CP-Tuban (TE) harbored 12 of the 16 phyla detected in the corresponding uncultured microbial consortium (TU). Phyla detected in TE included calditrichaeota, defferribacteres, bacteroidetes, nitrospirae, proteobacteria, synegistetes, actinobacteria, chloroflexi, cyanobacteria, firmicutes, and tenericutes. Three of them (calditrichaeota, nitrospirae, tenericutes) were not detected in the cultivation-enriched microbes from CP-Sampang (SE). Four of 17 classes identified in TE (calditrichae, flavobacteriia, nitrospira, mollicutes) were not found in SE. Both TE and SE were predominated by δ-proteobacteria and clostridia (Figure 6).

We performed intersection analysis of genera among uncultured microorganisms (TU for CP-Tuban, SU for CP-Sampang) and enriched cultures (TE for CP-Tuban, and SU for CP-Sampang) to indicate shared genus-level OTUs (Figure 5). Among 180 Genus OTUs identified in SU, 54 of them were shared with TU, while 127 OTUs were particularly unique in SU, which were undetected in TU (Figure 5). Further statistical analyses indicated that Margalef index (*K*) and Menhinick index (*M*) calculated for SU were 19.9815 and 2.0418, respectively, which were higher compared to TU (*K* = 7.7725 and *M* = 1.4140) (Figure 7B, Supplementary Table 5). This suggests that genus-level diversity in SU was higher than that in TU, which were supported by the Shannon index (*H`*) and Gini-Simpson (1-*Ds*), Chao, iChao, and ACE indices (Figure 7B, Supplementary Table 5). The Pielou evenness index (*J`*) for TU and SU were similar, namely 0.6396 and 0.6021, respectively. These relatively low *J'* values

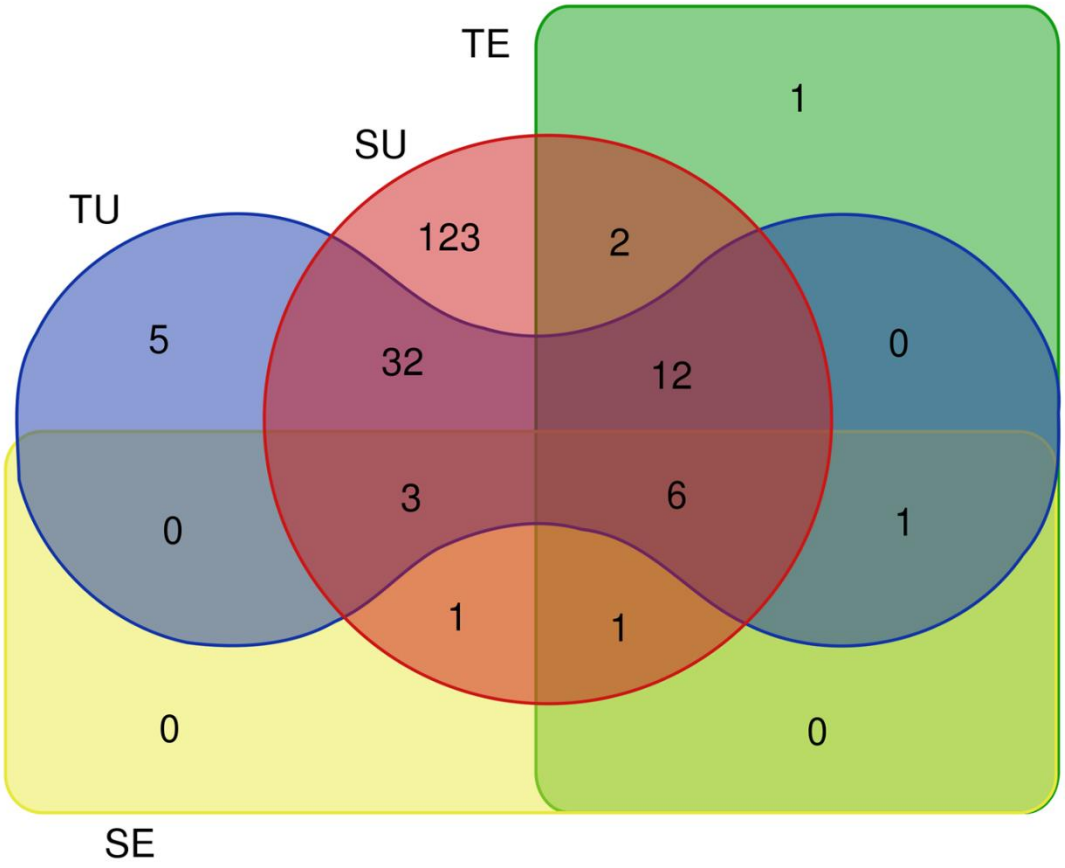


Figure 5. The shared numbers of operational taxonomic units (OTUs) at the genus-level among uncultured microorganisms and enriched microbial cultures in CP-Tuban and CP-Sampang. Note: TU, uncultured microorganisms in CP-Tuban; SU, uncultured microorganisms in CP-Sampang; TE, enriched microbial cultures in CP-Tuban; and SE, enriched microbial cultures in CP-Sampang

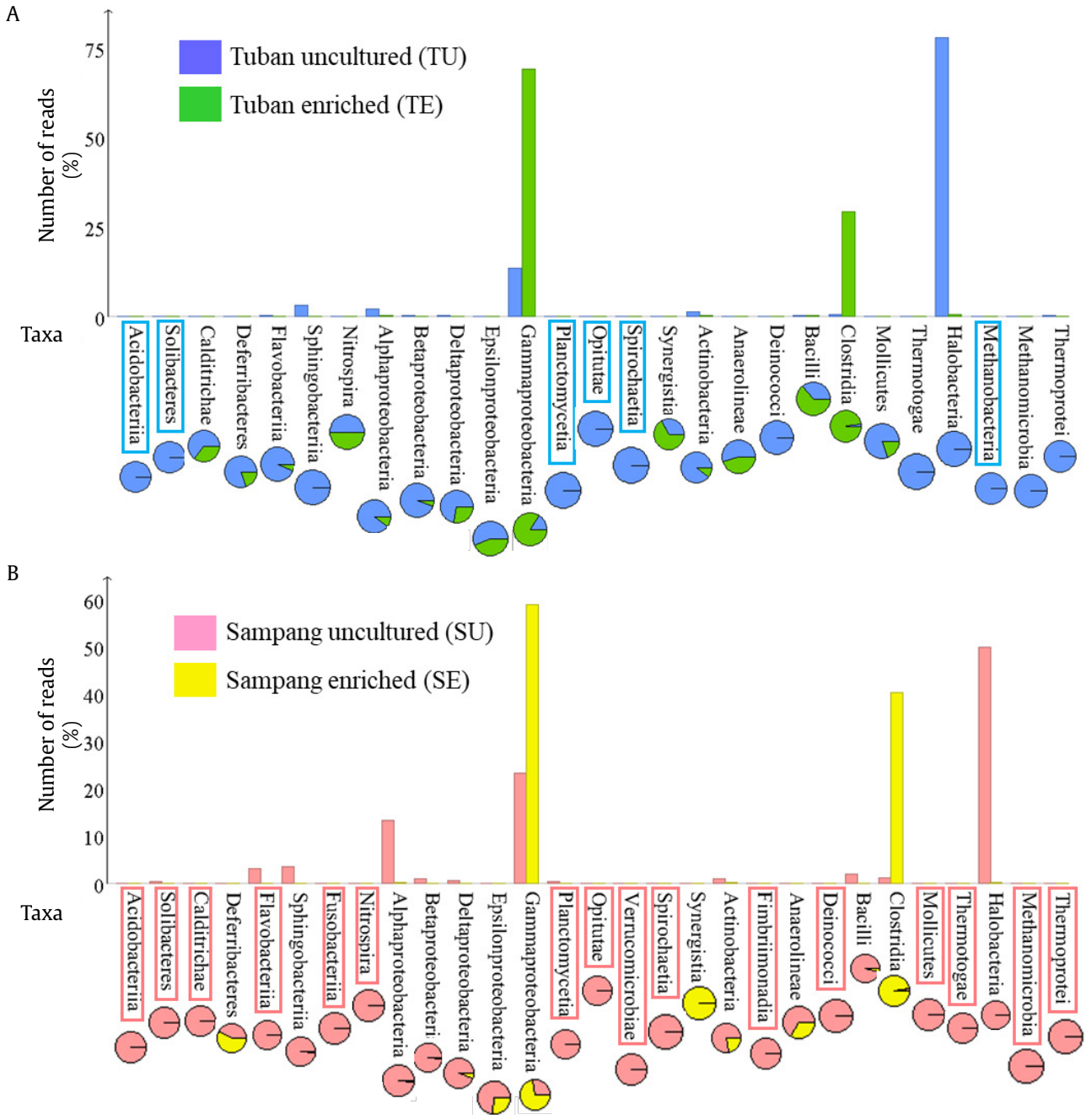


Figure 6. Comparative diversity and abundance at Class level between uncultured microorganisms and cultivation-enriched microorganisms. Microbial samples from CP-Tuban (A) and CP-Sampang (B). Open blue boxes indicate Classes in TU, which were not detected in TE. Open red boxes are Classes in SU, which were not found in SE

may indicate that certain phyla may dominate both TU and SU. Genera generally considered beneficial were shared among SU and TU (Figure 8 and Supplementary Table 6). However, beneficial genera were dominant in SU with 2260 reads compared to TU (603 reads). Dominant beneficial genera in SU were represented by *Haloarcula, Halobaculum, Haloplanus, Halorientalis, Psychroflexus*, and *Rhodosalinus* (Figure 8 and Supplementary Table 6). In addition, there were 26 relatively abundant genera (each OTU >10 reads) in SU, which were not detected in TU (Figure 9 and Supplementary Table 7).

Among 180 genus-level OTUs in SU, 12 of them were found in the enriched cultures (SE) (Figure 5, Supplementary Table 4, and Supplementary Figure 1), which were identified as *Salinivibrio, Halomonas,*

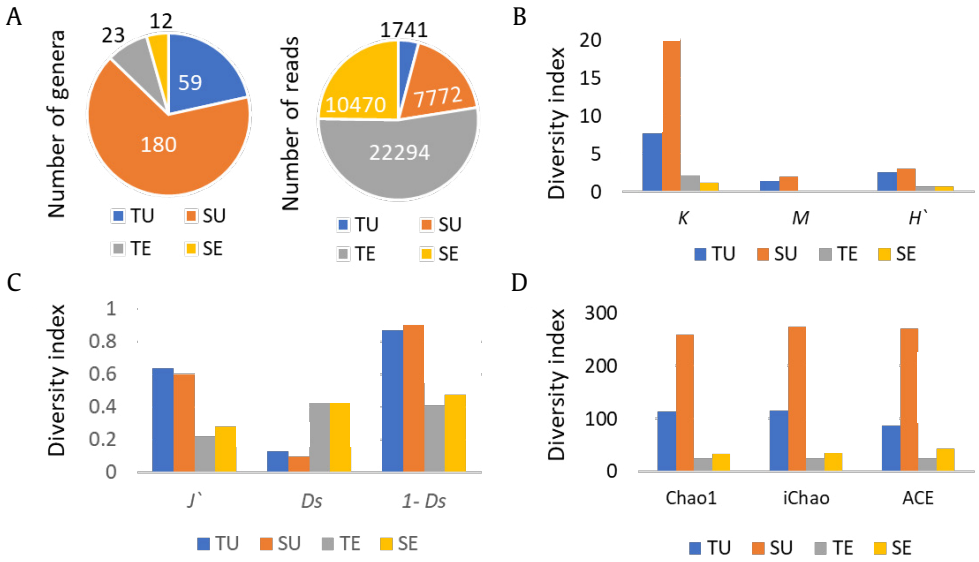


Figure 7. Richness and alpha-diversity indices of genera identified in CP-Tuban and CP-Sampang samples. (A) Numbers of genus-level OTUs and reads, (B) comparison between the samples based on Margalef richness index (*K*), Menhinick index (*M*), and Shannon Entropy index (*H`*), (C) diversity comparison based on Pielou evenness (*J`*), Simpson diversity index (D_s), and Gini-Simpson index (1-*Ds*), (D) sample comparison based on Chao1, iChao, and ACE indices. Note: CP = crystallization ponds, TU = CP-Tuban uncultured, SU = CP-Sampang uncultured, TE = CP-Tuban enriched, SE = CP-Sampang enriched

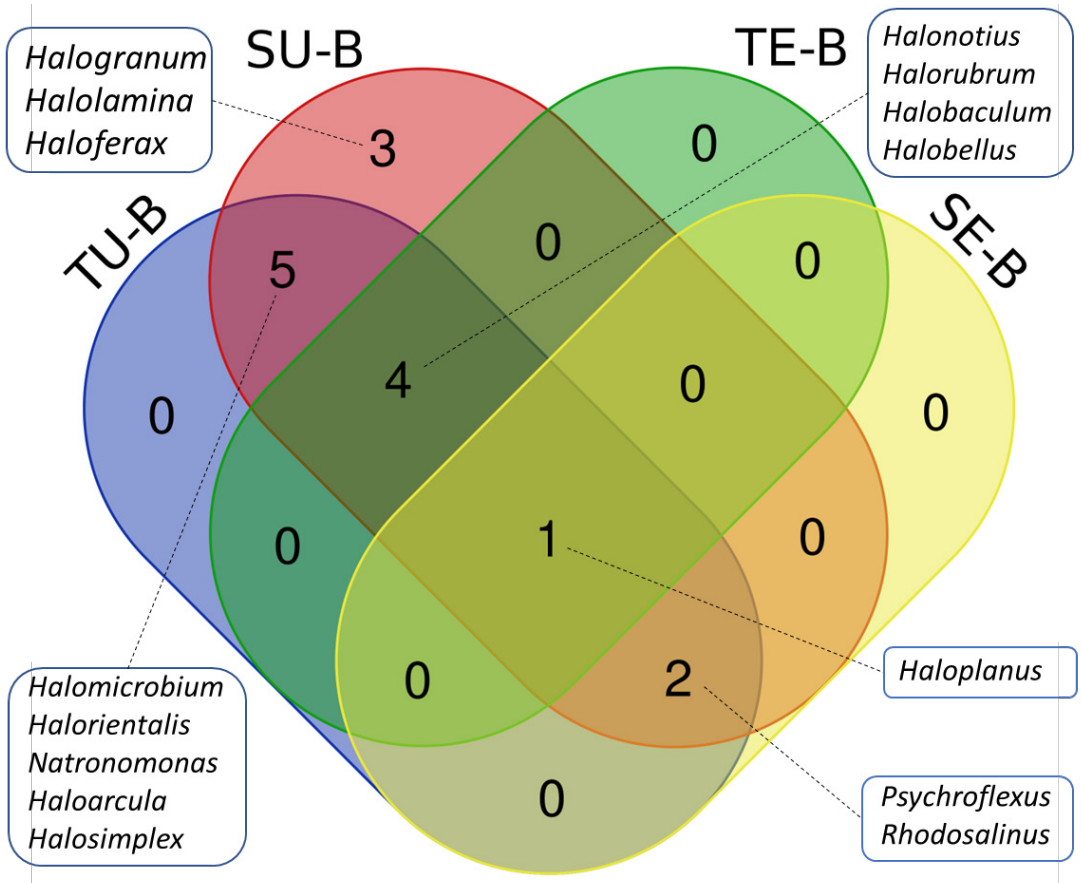


Figure 8. The number of shared genera within Halobacteriaceae, Flavobacteriaceae, and Rhodobacteraceae families detected in Tuban and Sampang samples, which are considered beneficial to the salt quality. Note: CP = crystallization ponds, TU-B = CP-Tuban uncultured beneficial, SU-B = CP-Sampang uncultured beneficial, TE-B = CP-Tuban enriched beneficial, SE-B = CP-Sampang enriched beneficial

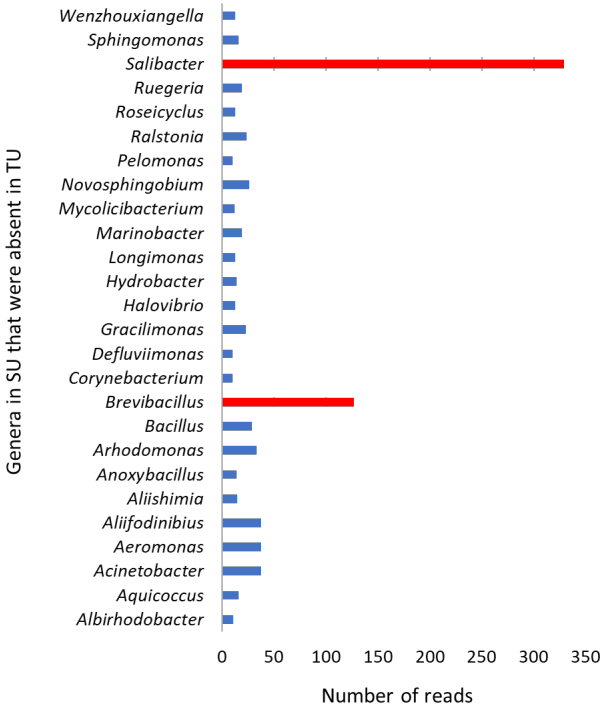


Figure 9. Genera of uncultured microorganisms relatively dominant in CP-Sampang (SU), which were undetected in CP-Tuban (TU)

Haloplanus, *Halobacillus*, *Salinigranum*, *Rhodosalinus*, *Streptomyces*, *Nocardioides*, *Vibrio*, *Enterobacter*, and *Psychroflexus* (Supplementary Table 2 and 4). Among 59 genus-level OTUs in TU, 19 of them were represented in the enriched cultures (TE) (Figure 5), which included *Salinigranum*, *Salinivibrio*, *Halonotius*, *Streptomyces*, *Oceanospirillum*, *Marinomonas*, *Halanaerobium*, *Pseudomonas*, *Pseudoalteromonas*, *Chromohalobacter*, *Halomonas*, *Enterobacter*, *Haloplanus*, *Halorubrum*, *Halohasta*, *Oceanococcus*, *Halobaculum*, *Halomicroarcula*, and *Halobellus* (Supplementary Table 1 dan 3). This indicates that beneficial genera were present in the enriched cultures of both CP-Tuban and CP-Sampang samples (SE and TE). Among 8 genus-level OTUs shared between TE and SE (Figure 5), three of them (*Halomonas*, *Halobacillus*, *Haloplanus*) are known as beneficial genera.

3.3. Availability of DNA Sequence Datasets and Accession Numbers

The sequences obtained in the present study were deposited in the Sequence Read Archive (SRA) via the National Center for Biotechnology Information (NCBI) under the BioProject accession number PRJNA680192. It contains sequence files in FastQ format with the following accession numbers: SRX9591214 for uncultured CP-Tuban (T4U), SRX9591215for enriched CP-Tuban (T4C), SRX9591217 for enriched CP-Sampang (S3C or SE), and SRX9591216 for uncultured CP-Sampang (S3U).

4. Discussion

In this work, we report the composition of phytoplankton and microbial populations in two solar salterns that produce different salt quality. Solar salt ponds in Tuban were mostly located in settlement areas far from the sea, while Sampang solar ponds were in the seashores far from the people settlement areas. This contributed to the difference between two solar salterns in term of nutrient level, phytoplankton density, and microbial diversity. DO levels of Sampang ponds were higher compared to those of Tuban ponds, suggesting a fairly good oxygenation rate in Sampang ponds compared with that in Tuban. However, there is no significant difference of pH observed between both solar salterns. The levels of nutrients (nitrate, phosphate, and ammonia) varied with the pond salinity levels, which contributed to the dynamics of phytoplankton population in each level of the salt ponds.

Multivariate analysis showed 4 phytoplankton groups at different levels of environmental conditions (nitrate, phosphate, ammonia, and DO) in the salt ponds. The direction of positive x-axis appears to have three groups of phytoplankton community characterized by high pH and DO. On the contrary, *Dunailella* sp. profile is located on the negative x-axis, showing that its presence corresponded with high salinity, phosphate and nitrate levels. It means that *Dunaleilla* favors high concentrations of phosphate and nitrate as well as high salinity in the ponds. It has been suggested that the presence of *Dunaleilla* in solar salt ponds is associated with salt quality (Giordano *et al.* 2014), as it photosynthetically produces glycerol that can be utilized by halophilic archaea as carbon and energy source in hypersaline environments such as in the crystalization pond (Oren 2010). In the other hand, a high quantity of polysaccharides excreted by *Dunaleilla* can negatively affect the quality of the salt produced (Oren 2014b). Therefore, controlling

phosphate and nitrate levels that regulate the growth of *Dunaleilla* population can be considered as an effective strategy to maintain good salt quality in the CP. In this study, low number of *Dunaleilla* spp. has been detected in the seawater reservoirs of both Tuban and Sampang. A higher number of *Dunaleilla* spp. was found at the crystalization ponds of both solar salterns, amounting to 1,500–2,000 cells/ml. However, this number was lower than that of *D. salina* usually present in saltern crystallizer ponds as previously reported by Oren 2020, which was in the range of 10^3 and 10^4 cells/ml. This suggests that the presence of *Dunaleilla* in both Tuban and Sampang salterns gave no significant effect to the quality of salts produced.

Our data suggested the difference in microbial diversity and abundance between the crystalization ponds (CP) of Tuban and CP-Sampang. This difference was correlated with their different environmental physico-chemical properties, which affected microbial composition. For example, at the phylum level, it was found the presence of Fusobacteria and Armatimonadetes in CP-Sampang, which were not detected in CP-Tuban. Furthermore, three classes (Fusobacteria, Verrucomicrobiae, Fimbriimonadia) identified in CP-Sampang were absent in CP-Tuban. Verrucomicrobiae was reported as the sixth most abundant bacterial phylum in ocean water after Proteobacteria, Bacteriodetes, Deferribacteres, Actinobacteria and Cyanobacteria (Freitas *et al.* 2012). Only one class in CP-Tuban (Synergistia) was not observed in CP-Sampang. These results provide a general insight that microbial communities in CP-Sampang were more diverse compared to those in CP-Tuban.

Class Synergistia (member of phylum Synergestes) is a part of normal microbiota of animals and human, which has been isolated from a variety of sites in humans including oral cavity (Vartoukian *et al.* 2009). The presence of this class in CP-Tuban might be as the consequences of the saltern location that was near the human settlement, which could affect the quality of salt produced in CP-Tuban. Meanwhile, phyla Proteobacteria was present in higher amount in CP-Sampang compared to CP-Tuban. Further analysis of reads that belong to Proteobacteria indicated the dominant presence of *Salinivibrio* and *Enterobacter* in both salterns (Supplementary Table 1 and 2). Members of *Salinivibrio* belong to halophilic bacteria commonly found in brines, salted foods, and hypersaline environments (Gorriti *et al.* 2014). Genome sequencing indicated the presence of genes related to arsenic, NaCl, and UV radiation resistance as well as genes for DNA repair mechanism and xanthorhodopsin, enabling them to thrive in extreme environments with high salinity and temperature (Gorriti *et al.* 2014). Due to the common presence for *Salinivibrio* in marine environments, it may not represent a health concern, as reported for *S. costicola* (Flores *et al.* 2021). The prevalent 16S rRNA reads of *Enterobacter* identified in CP-Tuban and CP-Sampang mostly showed high homology (>98% identity) with those of *E. cloacae* and *E. mori* (Supplementary Table 1 and 2). *E. cloacae* is distributed widely in various environments, and it is present as commensal microflora in the human and animal intestinal tracts and as pathogens in plants and insects (Davin-Regli and Pagès 2015). We propose that controling the occurance of *Enterobacter* to low levels is very important to increase the quality of salts produced in traditional solar salterns.

Archaeal abundance in CP-Tuban accounted for 71.03% compared 45.66% for CP-Sampang. This indicates a significant difference in the ratio of archaea and bacteria abundance between both solar salterns. Genera within the family Halobacteriaceae (currently Haloferacaceae family) are generally considered beneficial to salt production process (Oren 2010, 2014a; Oren and Rodriguez-Valera 2011). Among 40 genera known in family Halobacteriaceae (Oren 2014a), beneficial genera within this family were detected in both CP-Tuban and CP-Sampang (Figure 8 and Supplementary Table 6). Although within phylum Euryarchaeota, Halobacteria dominated CP-Tuban by 70.96% and CP-Sampang by 45.60%, CP-Sampang harbored beneficial genera at higher number and abundance (2260 reads) compared to CP-Tuban (603 reads). It was found that three beneficial genera *Halobaculum* (596 reads), *Haloplanus* 903 (reads), and *Haloarcula* (95 reads) (Koecher *et al.* 2009; Oren 2010, 2014a; So *et al.* 2022) were present in higher abundance in CP-Sampang compared to those in CP-Tuban (Figure 8 and Supplementary Table 6). These 3 archaeal genera were also identified in the Indramayu Indonesia solar saltern (Chasanah *et al.* 2020). Their abundant presence in CP-Sampang can be regarded as good indication of salt quality, since these archaea require high $MgCl_2$ (Hallsworth *et al.* 2007; Shimoshige *et al.* 2013), thereby lowering Mg^{2+} contamination as impurties in the salt produced in the salterns.

Psychroflexus and *Rhodosalinus* from Flavobacteriaceae and Rhodobacteraceae families were detected in both CP-Tuban and CP-Sampang. They may represent beneficial bacteria due to the presence of carotenoid pigments, as reported for *Psychroflexus* (Zhong *et al.* 2016). Although so far there is no report about carotenoid content in *Rhodosalinus*, its red-colored colonies (Guo *et al.* 2017) may indicate its ability to produce certain pigments. The prevalence of *Psychroflexus* and *Rhodosalinus* in CP-Sampang (533 reads) was higher compared to that in CP-Tuban (40 reads) (Supplementary Table 6), suggesting the potential contribution of these genera to the high quality of Sampang salt. Another pigment-producing genus dominating CP-Sampang was *Pseudomonas* (347 reads). Around 72% of the identified *Pseudomonas* reads showed high homology (≥98.65% identity) with the 16S rRNA gene sequence of *P. stutzeri*. In contrast, only 8 *Pseudomonas* sequence reads were detected in CP-Tuban. Marine *P. stutzeri* was reported as the producer of an extracellular black-colored polymeric pigment called melanin (Kumar *et al.* 2013). This raises a question whether the dominant presence of *Pseudomonas*, especially *P. stutzeri*, could contribute to the higher salt quality.

The cultivation-based enrichment method described in this work was based on sterile seawater from both CP-Tuban and CP-Sampang with different salinities (25, 30, 35°Be). It was found that 19 out of 59 genera identified in uncultivated CP-Tuban (TU) were represented in the enriched cultures (TE), while 11 of 180 genera in SU were present in SE (Figure 5 and Supplementary Figure 1). Interestingly, the enriched cultures from both CP-Tuban and CP-Sampang (TE and SE) samples contained beneficial genera that belong to family Halobacteriaceae. TE particularly harbored *Haloplanus, Halorubrum, Halobaculum, Halonotius, Halomicroarcula*, and *Halobellus*. SE contained *Haloplanus, Psychroflexus*, and *Rhodosalinus*. The presence of beneficial genera in the enriched samples suggests the potential application of these combined enriched cultures for improving the quality of salt produced in solar salterns.

In conclusion, our taxonomic profiling studies showed the difference in microbial composition between the crystallization pond (CP) of Tuban and CP-Sampang with the salinity level of 23° Baume (Be). Beneficial microbes from the families Halobacteriaceae, Flavobacteriaceae, and Rhodobacteraceaein were detected in both traditional solar salterns. However, CP-Sampang harbored more diverse and abundant beneficial microorganisms compared to CP-Tuban. This may contribute to the higher quality of salts produced in Sampang solar saltern. Levels of DO and nutrient (phosphate, nitrate, and ammonia) in the seawater reservoir (SR) of Sampang were higher than those at SR-Tuban, most likely due to the location of Sampang saltern near the open sea. These higher physico-chemical parameters may contribute to more diverse phytoplankton genera at SR-Sampang (13 genera) compared to 10 genera at SR-Tuban. Furthermore, the presence of diverse phytoplankton in SR may give advantage to salt quality, as the organic matters that they produce may flow into higher salinity-level ponds, which support the growth of beneficial heterotrophic microbes in CP. However, controlling the density of phytoplankton population in SR is important to prevent the overgrowth of exopolysaccharide-producers, such as *Dunaleilla* and *Nitszchenia*, which may enter CP and decrease salt quality. Relatively low number of *Dunaleilla* sp. cells in the CP of both solar salterns do not seem to affect the quality of salts produced. The cultivation-based enrichment of some beneficial genera derived from CP-Tuban and CP-Sampang provide an important basis for various application of enriched cultures, especially in salt quality improvement and production of unique enzymes and natural products.

Conflict of Interest

No financial conflict of interest exists in relation to this work. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Acknowledgments

This work was supported by the Ministry of Marine Science and Fisheries and National Research and Innovation Agency (BRIN), RIIM 3 B-2458/III.4/HK/5/2023.

All authors equally contributed based on their expertise from designing of the study, lab works, and writing manuscript. All authors have agreed a final version of the manuscript.

References

Abdollahnia, M., Id, A.M., Mashreghi, M., Id, H.E., 2020. Exploring the potentials of halophilic prokaryotes from a solar saltern for synthesizing nanoparticles : The case of silver and selenium. *PLOS ONE.* 15, 1–18. https://doi.org/10.1371/journal.pone.0229886

Anton, J., Rosella-Mora, R., Rodriguez-Valera, F., Amann, R., 2000. Extremely halophilic bacteria in Crystallizer Ponds from Solar Salterns. *Appl. Environ. Microbiol.* 66, 3052–3057.

Barreteau, H., Vandervennet, M., Guedon, L., Point, V., Canaan, S., Rebuffat, S., Peduzzi, J., Carre-Mlouka, A., 2019. *Haloarcula sebkhae* sp . nov ., an extremely halophilic archaeon from *Algerian hypersaline* environment. *Int J Syst Evol Microbiol*. 69, 732–738. https://doi.org/10.1099/ijsem.0.003211

Chasanah, E., Marraskuranto, E., Sugiyono, Pratitis, A., Nursid, M., Yogiara., 2020. Comparative diversity analysis of halophiles at two polar saltern systems in Indramayu, West Java, Indonesia. *Letters in Applied Microbiology*. 72, 1–10. https://doi.org/10.1111/lam.13401

Chao, A., 1984. Nonparametric estimation of the number of classes in a population. *Scand. J. Stat.* 11, 265–270.

Chao, A., Lee, S.M., 1992. Estimating the number of classes via sample coverage. *J. Am. Stat. Assoc.* 87, 210–217. https://doi.org/10.1080/01621459.1992.10475194

Chao, A., Yang, M.C.K., 1993. Stopping rules and estimation for recapture debugging with unequal failure rates. *Biometrika.* 80, 193–201. https://doi.org/10.2307/2336768

Chernov, T.I., Tkhakakhova, A.K., Kutovaya, O.V., 2015. Assessment of diversity indices for the characterization of the soil prokaryotic community by metagenomic analysis. *Eurasian Soil Sci.* 48, 410–415. https://doi.org/10.1134/S1064229315040031

Chiu, C.H., Wang, Y.T., Walther, B.A., Chao, A., 2014. An improved nonparametric lower bound of species richness via a modified good–turing frequency formula. *Biometrics.* 70, 671–682. https://doi.org/10.1111/biom.12200

Davin-Regli, A., Pagès, J.M., 2015. Enterobacter aerogenes and *Enterobacter cloacae*; versatile bacterial pathogens confronting antibiotic treatment. *Front. Microbiol.* 6, 392. https://doi.org/10.3389/fmicb.2015.00392

Davis, J.S., Giordano, M., 1995. Biological and physical events involved in the origin , effects , and control of organic matter in solar saltworks Biological and physical events involved in the origin , effects , and control of organic matter in solar saltworks. *International Journal of Salt Lake Research*. 4, 335–347. https://doi.org/10.1007/BF01999117

Davis, J.S., 2000. Structure, function and management of the biological system for seasonal solar saltworks. *Global NEST: The Int Journal*. 3, 217–226. https://doi.org/10.30955/gnj.000175

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics*. 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Edler, L., 1979. Recommendations on methods for marine biological studies in the Baltic Sea. Phytoplankton and chlorophyll. *The Baltic Marine Biologists.* 5, 1-38.

Fernández, A.B., Vera-Gargallo, B., Sánchez-Porro, C., Ghai, R., Papke, R.T., Rodriguez-Valera, F., Ventosa, A., 2014. Comparison of prokaryotic community structure from Mediterranean and Atlantic saltern concentrator ponds by a metagenomic approach. *Frontiers in Microbiology*. 5, 1–12. https://doi.org/10.3389/fmicb.2014.00196

Flores, B., González, B., Bravo, A., Mora-Sánchez, B., Torres, D., Jirón, W., Sheleby-Elías, J., Balcázar, J.L., 2021. Identification of pathogenic bacteria in fishes caught in the Pacific off Nicaragua. *Ciencias Marinas.* 47, 175–184. https://doi.org/10.7773/cm.v47i3.3212

Freitas, S., Hatosy, S., Fuhrman, J.A., Huse, S.M., Welch, D.B.M., Sogin, M.L., Martiny, A.C., 2012. Global distribution and diversity of marine *Verrucomicrobia. The ISME Journal*. 6, 1499–1505. https://doi.org/10.1038/ismej.2012.3

Giordano, M., Bargnesi, F., Mariani, P., Cryptogamie, S., 2014. *Dunaliella salina* (Chlorophyceae) affects the quality of NaCl crystals *Dunaliella salina* (Chlorophyceae) affects the quality of NaCl crystals. *Cryptogamie, Algologie*. 35, 285–302. https://doi.org/10.7872/crya.v35.iss3.2014.285

Good, I.J., 1953. The population frequencies of species and the estimation of population parameters. *Biometrika.* 40, 237–264. https://doi.org/10.2307/2333344

Good, I.J., Toulmin, G.H., 1956. The number of new species, and the increase in population coverage, when a sample is increased. *Biometrika.* 43, 45–63. https://doi.org/10.2307/2333577

Gorriti, M.F., Dias, G.M., Chimetto, L.A., Trindade-Silva, A.E., Silva, B.S., Mesquita, M.M., Gregoracci, G.B., Farias, M.E., Thompson, C.C., Thompson, F.L., 2014. Genomic and phenotypic attributes of novel salinivibrios from stromatolites, sediment and water from a high altitude lake. *BMC Genom.* 15, 473.

Guo, L., Ling, S., Li, C., Chen, G., Du, Z., 2017. *Rhodosalinus sediminis* gen . nov ., sp . nov ., isolated from marine saltern. *Int J Sys Evol Microbiol*. 67, 5108–5113. https://doi.org/10.1099/ijsem.0.002424

Hallsworth, J.E., Yakimov, M.M., Golyshin, P.N., Gillion, J.L., D'Auria, G., de Lima Alves, F., La Cono, V., Genovese, M., McKew, B.A., Hayes, S.L., Harris, G., Giuliano, L., Timmis, K.N., McGenity, T.J., 2007. Limits of life in MgCl2-containing environments: chaotropicity defines the window. *Environ Microbiol.* 9, 801-13. https://doi.org/10.1111/j.1462-2920.2006.01212.x. PMID: 17298378

Harrell, F.E., 2001. *Regression Modelling Strategies With Applications to Linear Models Logistic Regression and Survival Analysis*. Springer-Verlag, New York.

Hedi, A., Sadfi, N., Fardeau, M., Rebib, H., Cayol, J., Ollivier, B., Boudabous, A., 2009. Studies on the biodiversity of halophilic microorganisms isolated from El-Djerid Salt Lake (*Tunisia*) under aerobic conditions. *Int J of Microbiology*. 2009, 731786. https://doi.org/10.1155/2009/731786

Huson, D.H., Beier, S., Flade, I., Górska, A., ElHadidi, M., Mitra, S., Ruscheweyh H.J., Tappu R., 2016. MEGAN community edition- interactive exploration and analysis of large-scale microbiome sequencing data. *PLoS. Comput. Biol.* 12, e1004957. https://doi.org/10.1371/journal.pcbi.1004957

Hyler, J.E., 1935. The production of salt. *J. Chem. Educ*. 12, 203–207. https://doi.org/10.1021/ed012p203

Javor, B.J., 2002. Industrial microbiology of solar salt production. *J of Industrial Microbiology and Biotechnology*. 28, 42–47. https://doi.org/10.1038/sj/jim/7000173

Jost, L., 2006. Entropy and diversity. *Oikos.* 113, 363–375. https://doi.org/10.1111/j.2006.0030-1299.14714.x

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glo¨ckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, 41, 1-11. https://doi.org/10.1093/nar/gks808

Koecher, S., Breitenbach, J., Müller, V., Sandmann, G., 2009. Structure, function and biosynthesis of carotenoids in the moderately halophilic bacterium *Halobacillus halophilus. Arch Microbiol.* 191, 95-104. https://doi.org/10.1007/s00203-008-0431-1

Kumar, C.G., Sahu, N., Reddy, G.N., Prasad, R.B.N., Nagesh, N., Kamal, A., 2013. Production of melanin pigment from *Pseudomonas stutzeri* isolated from red seaweed *Hypnea musciformis. Letters in Applied Microbiology*. 57, 295–302. https://doi.org/10.1111/lam.12111

Margalef, R., 1958. Information theory in ecology. *General Systems*. 3, 36-71.

Mani, K., Taib, N., Hugoni, M., Bronner, G., Bragança, J. M., Debroas, D., 2020. Transient dynamics of Archaea and Bacteria in Sediments and Brine Across a Salinity Gradient in a Solar Saltern of Goa , India. *Frontiers in Microbiology*. 11, 1–19. https://doi.org/10.3389/fmicb.2020.01891

Menhinick, E.F., 1964. A comparison of some species-individuals diversity indices applied to samples of field insects. *Ecology*. 45, 859–861. https://doi.org/10.2307/1934933

Naghoni, A., Emtiazi, G., Amoozegar, M. A., Cretoiu, M. S., Lucas, J., Etemadifar, Z., Abolhassan, S., Fazeli, S., Bolhuis, H., 2017. Microbial diversity in the hypersaline Lake Meyghan, Iran. *Sci Rep*. 7, 1–13. https://doi.org/10.1038/s41598-017-11585-3

Najjari, A., Elshahed, M.S., Cherif, A., Youssef, H., 2015. Patterns and determinants of halophilic archaea (Class Halobacteria) diversity in Tunisian Endorheic Salt Lakes and Sebkhet Systems. *Applied and Environmental Microbiology*. 81, 4432–4441. https://doi.org/10.1128/AEM.01097-15

Oh, D., Porter, K., Russ, B., Dyall-smith, D.B.M., 2010. Diversity of Haloquadratum and other haloarchaea in three , geographically distant, Australian saltern crystallizer ponds. *Extremophiles*. 14, 161–169. https://doi.org/10.1007/s00792-009-0295-6

Ondov, B.D., Bergman, N.H., Phillippy, A.M., 2011. Interactive metagenomic visualization in a web browser. *BMC Bioinformatics*. 12, 1–9. https://doi.org/10.1186/1471-2105-12-385

Oren, A., 2010. Industrial and environmental applications of halophilic microorganisms. *Environmental Technology*. 31, 825–834. https://doi.org/10.1080/09593330903370026

Oren, A., Rodríguez-Valera, F., 2011. The contribution of halophilic Bacteria to the red coloration of saltern crystallizer ponds (1). *FEMS Microbiol Ecol.* 36, 123-130. https://doi.org/10.1111/j.1574-6941.2001.tb00832.x

Oren, A., 2014a. The family Halobacteriaceae, in: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea.* Springer, Berlin Heidelberg, pp. 41–121. https://doi.org/10.1007/978-3-642-38954-2_313

Oren A., 2014b. The ecology of Dunaliella in high-salt environments. *J Biol Res (Thessalon).* 18, 1-8. https://doi.org/10.1186/s40709-014-0023-y

Oren, A., 2020. The microbiology of red brines. *Advances in Applied Microbiology*. 113, 58–99. https://doi.org/10.1016/bs.aambs.2020.07.003

Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. *J. Theor. Biol.* 13, 131–144. https://doi.org/10.1016/0022-5193(66)90013-0

Plominsky, A.M., Carlos, H.C., Delherbe, N., Podell, S., Salvador, R.F., Ugalde, J.A., Engh, G. van den, Hanselmann, K., Ulloa, O., Iglesia, R.D.la, Allen, E.E., Trefault, N., 2018. Distinctive archaeal composition of an artisanal crystallizer pond and functional insights into salt-saturated hypersaline environment adaptation. *Frontiers in Microbiology*. 9, 1–13. https://doi.org/10.3389/fmicb.2018.01800

Shannon, C.E., 1948. A mathematical theory of communication. *Bell Syst. Tech. J.* 27, 623–656. https://doi.org/10.1002/j.1538-7305.1948.tb00917.x

Shimoshige, H., Yamada, T., Minegishi, H., Echigo, A., Shimane, Y., Kamekura, M., Itoh, T., Usami, R., 2013. *Halobaculum magnesiiphilum* sp. nov., a magnesium-dependent haloarchaeon isolated from commercial salt. *Int J Syst Evol Microbiol.* 63, 861-866. https://doi.org/10.1099/ijs.0.037432-0

Simpson, E.H., 1949. Measurement of diversity. *Nature.* 163, 688–688. https://doi.org/10.1038/163688a0

So, Y., Chhetri G., Kim , I., Kang, M., Kim, J., Lee, B., Jang, W., Seo, T., 2022. *Halomonas antri* sp. nov., a carotenoid-producing bacterium isolated from surface seawater. *Int J Syst Evol Microbiol.* 72. https://doi.org/10.1099/ijsem.0.005272. PMID: 35238736

Suwasono, B., Munazid, A., Widodo, A., 2013. Keragaman kualitas air laut, garam rakyat, dan garam evaporasi bertingkat di wilayah pesisir Jawa Timur. *J. Segara.* 9, 145-155.

Vartoukian, S.R., Palmer, R.M., Wade, W.G., 2009. Diversity and morphology of members of the phylum “Synergistetes” in periodontal health and disease. *Applied and Environmental Microbiology*. 75, 3777–3786. https://doi.org/10.1128/AEM.02763-08

Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naı¨ve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*. 73, 5261–5267. https://doi.org/10.1128/AEM.00062-07

Wang, Q., Wang, C., Xiang, X., Xu, H., Han, G., 2022. Analysis of microbial diversity and succession during Xiaoqu Baijiu fermentation using high-throughput sequencing technology. *Eng. Life Sci.* 22, 495–504. https://doi.org/10.1002/elsc.202200015

Yang, Y., Cui, H., Zhou, P., Liu, S., 2007. *Haloarcula amylolytica* sp . nov ., an extremely halophilic archaeon isolated from Aibi salt lake in Xin-Jiang, China. *Int J of Systematic and Evolutionary Microbiology*, 57, 103–106. https://doi.org/10.1099/ijs.0.64647-0

Zhong, Z.P., Liu, Y., Wang, F., Zhou, Y., Liu, H., Liu, Z., 2016. *Psychroflexus salis* sp. nov. and *Psychroflexus planctonicus* sp. nov., isolated from a salt lake. *International Journal of Systematic and Evolutionary Microbiology*. 66, 125-131. https://doi.org/10.1099/ijsem.0.000687

Supplementary Table 1. Genus-level classification of uncultivated microorganisms detected in CP-Tuban (TU). Red highlight indicates ≥98.65% sequence identity; and blue highlight indicates the sequence identity of 97.00 to 98.65%

Genus	Closest sequences	Reads	
Alcanivorax	*Alcanivorax venustensis*	1	1
Alteromonas	*Alteromonas mediterranea*	6	7
	Alteromonas mediterranea	1	
Brevibacillus	*Brevibacillus thermoruber*	7	7
Chromohalobacter	*Chromohalobacter salexigens*	1	1
Enterobacter	*Enterobacter cloacae*	46	85
	Enterobacter mori	35	
	Enterobacter cloacae	2	
	Enterobacter mori	2	
Halanaerobacter	*Halanaerobacter jeridensis*	1	1
Halanaerobium	*Halanaerobium praevalens*	6	7
	Halanaerobium praevalens	1	
Halapricum	*Halapricum salinum*	6	6
Haloarcula	*Haloarcula marismortui, Haloarcula quadrata*	1	2
	Haloarcula salaria	1	
Halobaculum	*Halobaculum gomorrense*	2	107
	Halobaculum gomorrense, Halobaculum magnesiiphilum	3	
	Halobaculum gomorrense	24	
	Halobaculum magnesiiphilum	36	
	Halobaculum roseum	42	
Halobellus	*Halobellus litoreus*	1	138
	Halobellus limi	2	
	Halobellus ramosii, Halobellus rarus	1	
	Halobellus inordinatus, Halobellus ramosii	27	
	Halobellus inordinatus	1	
	Halobellus limi	5	
	Halobellus ramosii	101	
Halohasta	*Halohasta litorea*	134	238
	Halohasta litchfieldiae, Halohasta litorea	4	
	Halohasta litchfieldiae	2	
	Halohasta litorea	98	
Halomicroarcula	*Halomicroarcula limicola, Halomicroarcula pellucida*	1	82
	Halomicroarcula limicola	12	
	Halomicroarcula pellucida	69	
Halomicrobium	*Halomicrobium zhouii*	1	1
Halomonas	*Halomonas meridiana*	1	2
	Halomonas smyrnensis	1	
Halonotius	*Halonotius pteroides*	39	39
Haloplanus	*Haloplanus natans*	40	93
	Haloplanus salinus	23	
	Haloplanus natans, Haloplanus salinus	4	
	Haloplanus aerogenes, Haloplanus salinus	1	
	Haloplanus natans, Haloplanus salinus	2	
	Haloplanus natans	20	
	Haloplanus salinus	3	
Halorientalis	*Halorientalis persicus*	1	6
	Halorientalis brevis	5	
Halorubellus	*Halorubellus litoreus*	1	1
Halorubrum	*Halorubrum kocurii*	2	143
	Halorubrum orientale	15	
	Halorubrum aidingense, Halorubrum kocurii	3	
	Halorubrum kocurii, Halorubrum lacusprofundi	2	
	Halorubrum aidingense, H. kocurii H. lacusprofundi	1	
	Halorubrum aidingense, H. kocurii, H. yunnanense	2	
	Halorubrum aidingense	3	
	Halorubrum cibi	1	

Supplementary Table 1. Continued

Genus	Closest sequences	Reads	
	Halorubrum coriense	1	
	Halorubrum ejinorense	1	
	Halorubrum halodurans	2	
	Halorubrum kocurii JCM 14978	3	
	Halorubrum lipolyticum	2	
	Halorubrum orientale	98	
	Halorubrum rutilum	1	
	Halorubrum sodomense	4	
	Halorubrum terrestre	1	
	Halorubrum trueperi	1	
Halosimplex	*Halosimplex pelagicum*	1	4
	Halosimplex pelagicum	3	
Herbiconiux	*Herbiconiux flava*	4	4
Idiomarina	*Idiomarina fontislapidosi*	3	4
	Idiomarina taiwanensis	1	
Ilumatobacter	*Ilumatobacter fluminis*	1	1
Kerstersia	*Kerstersia similis*	1	1
Leisingera	*Leisingera daeponensis*	1	1
Marinomonas	*Marinomonas communis*	1	1
Marivita	*Marivita hallyeonensis*	4	4
Marinobacterium	*Marinobacterium georgiense*	1	1
Massilia	*Massilia agri*	2	3
	Massilia namucuonensis	1	
Methylophilus	*Methylophilus luteus*	1	1
Natronomonas	*Natronomonas moolapensis*	22	31
	Natronomonas moolapensis	9	
Natronotalea	*Natronotalea proteilytica*	1	1
Nevskia	*Nevskia terrae*	1	1
Nioella	*Nioella nitratireducens*	3	3
Nitrincola	*Nitrincola schmidtii*	2	2
Oceanococcus	*Oceanococcus atlanticus*	1	1
Oceanospirillum	*Oceanospirillum linum*	1	24
	Oceanospirillum sanctuarii	23	
Paracoccus	*Paracoccus aminovorans*	1	1
Phaeodactylibacter	*Phaeodactylibacter luteus*	1	1
Phenylobacterium	*Phenylobacterium koreense*	1	1
Ponticoccus	*Ponticoccus marisrubri*	4	4
Pontimonas	*Pontimonas salivibrio*	1	2
	Pontimonas salivibrio	1	
Pseudoalteromonas	*Pseudoalteromonas undina*	4	5
	Pseudoalteromonas ruthenica	1	
Pseudomonas	*Pseudomonas knackmussii*	4	8
	Pseudomonas stutzeri	4	
Psychroflexus	*Psychroflexus aestuariivivens*	1	5
	Psychroflexus salarius	4	
Rhodosalinus	*Rhodosalinus sediminis*	35	35
Rhodovibrio	*Rhodovibrio sodomensis*	4	4
Reyranella	*Reyranella massiliensis*	1	1
Roseicyclus	*Roseicyclus mahoneyensis*	6	6
Roseovarius	*Roseovarius atlanticus*	1	1
Salinigranum	*Salinigranum salinum*	2	25
	Salinigranum rubrum	8	
	Salinigranum salinum	15	
Salinivenus	*Salinivenus lutea*	1	1

Supplementary Table 1. Continued

Genus	Closest sequences	Reads	
Salinivibrio	*Salinivibrio costicola subsp. alcaliphilus*	1	34
	Salinivibrio kushneri	8	
	Salinivibrio proteolyticus	8	
	Salinivibrio sharmensis	7	
	Salinivibrio costicola subsp. alcaliphilus, S. kushneri	2	
	Salinivibrio costicola, S. proteolyticus	1	
	Salinivibrio costicola, S. kushneri S. sharmensis	2	
	Salinivibrio costicola, Salinivibrio kushneri	2	
	Salinivibrio costicola, Salinivibrio sharmensis	1	
	Salinivibrio kushneri	1	
	Salinivibrio sharmensis	1	
Spiribacter	*Spiribacter roseus*	9	501
	Spiribacter curvatus, Spiribacter roseus	1	
	Spiribacter roseus	491	
Staphylococcus	*Staphylococcus capitis*	2	2
Streptomyces	*Streptomyces abikoensis*	5	47
	Streptomyces tritici	37	
	Streptomyces abikoensis, Streptomyces tritici	3	
	Streptomyces abikoensis	1	
	Streptomyces tritici	1	
Vibrio	*Vibrio hyugaensis*	1	1
Winogradskyella	*Winogradskyella aquimaris*	1	1
	Total	1741	1741
Unclassified	*Halomicroarcula limicola, Halomicrobium zhouii*	10	12
	Halopeptonella vilamensis, Spiribacter roseus	1	
	Halopeptonella vilamensis, Spiribacter roseus	1	
	Grand total	1753	1753

Supplementary Table 2. Genus-level classification of uncultivated microorganisms in CP-Sampang (SU). Red highlight indicates ≥98.65% identity; and blue highlight indicates the identity of 97.00 to 98.65%. Yellow highlights indicate unique genera in CP-SU that were not detected in TU

Genus	Closest sequences	Reads	
Acidovorax	*Acidovorax radicis*	1	1
Aeromicrobium	*Aeromicrobium ginsengisoli*	2	2
Albirhodobacter	*Albirhodobacter marinus*	11	12
	Albirhodobacter marinus	1	
	Alcanivorax venustensis ISO4	4	4
Alcanivorax	*Aliifodinibius salicampi*	3	77
Aliifodinibius	*Aliifodinibius halophilus, Aliifodinibius salicampi*	18	
	Aliifodinibius salicampi, Aliifodinibius sediminis	3	
	Aliifodinibius halophilus, A. salicampi, A. sediminis	8	
	Aliifodinibius halophilus	19	
	Aliifodinibius salicampi	26	
Aliishimia	*Aliishimia ponticola*	7	22
	Aliishimia ponticola	15	
Alishewanella	*Alishewanella aestuarii* B11	7	7
Alteromonas	*Alteromonas mediterranea*	3	5
	Alteromonas mediterranea	1	
	Alteromonas pelagimontana	1	
Aquabacterium	*Aquabacterium parvum*	1	2
	Aquabacterium fontiphilum	1	
Aquicoccus	*Aquicoccus porphyridii*	16	16
Arcobacter	*Arcobacter aquimarinus*	1	8
	Arcobacter cloacae	1	
	Arcobacter aquimarinus	1	
	Arcobacter butzleri	1	
	Arcobacter pacificus	4	

Supplementary Table 2. Continued

Genus	Closest sequences	Reads	
Acinetobacter	*Acinetobacter baumannii*	11	40
	Acinetobacter johnsonii	7	
	Acinetobacter junii	4	
	Acinetobacter lwoffii	6	
	Acinetobacter modestus	5	
	Acinetobacter schindleri	6	
	Acinetobacter johnsonii	1	
Aeromonas	*Aeromonas caviae*	34	43
	Aeromonas veronii	7	
	Aeromonas caviae	1	
	Aeromonas veronii	1	
Aliiglaciecola	*Aliiglaciecola coringensis*	1	1
Anoxybacillus	*Anoxybacillus flavithermus subsp. yunnanensis* str. E13	9	14
	Anoxybacillus mongoliensis	4	
	Anoxybacillus vitaminiphilus	1	
Arhodomonas	*Arhodomonas recens*	33	33
Bacillus	*Bacillus flexus*	1	29
	Bacillus thioparans	3	
	Bacillus zhanjiangensis	1	
	Bacillus carboniphilus	10	
	Bacillus panaciterrae	1	
	Bacillus solimangrovi	3	
	Bacillus zhanjiangensis	2	
	Bacillus alkalinitrilicus, B. carboniphilus, B, taeanensis	3	
	Bacillus carboniphilus, Bacillus taeanensis	1	
Barrientosiimonas	*Barrientosiimonas marina*	1	1
Bdellovibrio	*Bdellovibrio bacteriovorus*	1	1
Bradyrhizobium	*Bradyrhizobium cytisi*	19	45
	Bradyrhizobium namibiense	22	
	Bradyrhizobium namibiense	4	
Brevundimonas	*Brevundimonas albigilva*	6	15
	Brevundimonas aurantiaca	3	
	Brevundimonas naejangsanensis	3	
	Brevundimonas aurantiaca	1	
	Brevundimonas naejangsanensis	2	
Brucella	*Brucella papionis*	1	1
Brevibacillus	*Brevibacillus invocatus*	3	127
	Brevibacillus thermoruber	121	
	Brevibacillus thermoruber	3	
Brevibacterium	*Brevibacterium sediminis*	1	1
Caenispirillum	*Caenispirillum salinarum* AK4	2	2
Corynebacterium	*Corynebacterium accolens*	2	10
	Corynebacterium amycolatum	5	
	Corynebacterium jeikeium	1	
	Corynebacterium otitidis	1	
	Corynebacterium coyleae	1	
Caulobacter	*Caulobacter hibisci*	4	4
Chromohalobacter	*Chromohalobacter salexigens* DSM 3043	1	5
	Chromohalobacter salexigens DSM 3043	4	
Clostridium	*Clostridium huakuii*	3	3
Comamonas	*Comamonas phosphati*	8	9
	Comamonas phosphati	1	
Cronobacter	*Cronobacter malonaticus*	1	1
Cutibacterium	*Cutibacterium acnes*	1	1
Cribrihabitans	*Cribrihabitans pelagius*	2	2
Dactylococcopsis	*Dactylococcopsis salina* PCC 8305	9	9
Defluviimonas	*Defluviimonas nitratireducens*	10	10
Devosia	*Devosia insulae* DS-56	6	6
Dolosigranulum	*Dolosigranulum pigrum*	1	1
Erythrobacter	*Erythrobacter nanhaisediminis*	2	2

Supplementary Table 2. Continued

Genus	Closest sequences	Reads	
Enterobacter	*Enterobacter cloacae*	932	1938
	Enterobacter mori	967	
	Enterobacter cloacae	14	
	Enterobacter mori	25	
Escherichia	*Escherichia fergusonii* ATCC 35469	8	9
	Escherichia fergusonii ATCC 35469	1	
Exiguobacterium	*Exiguobacterium acetylicum*	1	1
Fabibacter	*Fabibacter misakiensis*	2	2
Flavitalea	*Flavitalea antarctica*	1	1
Francisella	*Francisella philomiragia*	1	1
Fenollaria	*Fenollaria massiliensis*	1	1
Glycocaulis	*Glycocaulis albus*	4	4
Gordonia	*Gordonia hongkongensis*	6	6
Granulicatella	*Granulicatella adiacens*	1	1
Guyparkeria	*Guyparkeria hydrothermalis*	2	2
Gracilimonas	*Gracilimonas tropica*	16	23
	Gracilimonas tropica	4	
	Gracilimonas halophila	3	
Halanaerobacter	*Halanaerobacter jeridensis, H. lacunarum*	1	27
	Halanaerobacter lacunarum	1	
	Halanaerobium praevalens	13	
	Halanaerobacter jeridensis	1	
	Halanaerobacter lacunarum	8	
	Halanaerobacter lacunarum, H. salinarius	3	
Halapricum	*Halapricum salinum*	3	9
	Halapricum salinum	6	
Haliea	*Haliea salexigens*	1	1
Haloarchaeobius	*Haloarchaeobius baliensis*	1	1
Haloarcula	*Haloarcula salaria*	2	95
	Haloarcula marismortui ATCC 43049	1	
	Haloarcula salaria	90	
	Haloarcula tradensis	2	
Halobacillus	*Halobacillus profundi*	1	1
Halobaculum	*Halobaculum roseum*	25	582
	Halobaculum gomorrense	18	
	Halobaculum magnesiiphilum	179	
	Halobaculum roseum	353	
	Halobaculum magnesiiphilum, Halobaculum roseum	7	
Halobellus	*Halobellus limi*	1	32
	Halobellus litoreus	1	
	Halobellus rarus	1	
	Halobellus clavatus	2	
	Halobellus inordinatus, Halobellus ramosii	23	
Halodesulfurarchaeum	*Halodesulfurarchaeum formicicum*	3	3
Haloferax	*Haloferax larsenii*	1	1
Halogranum	*Halogranum gelatinilyticum*	1	1
Halohasta	*Halohasta litchfieldiae*	11	515
	Halohasta litorea	257	
	Halohasta litchfieldiae, Halohasta litorea	2	
	Halohasta litchfieldiae	3	
	Halohasta litorea	239	
	Halohasta litchfieldiae, Halohasta litorea	3	
Halolamina	*Halolamina litorea*	1	2
	Halolamina sediminis	1	422
Halomicroarcula	*Halomicroarcula limicola*	32	
	Halomicroarcula pellucida	1	
	Halomicroarcula limicola, Halomicrobium zhouii	8	
	Halomicroarcula limicola, Halomicroarcula pellucida	16	
	Halomicroarcula limicola	37	
	Halomicroarcula pellucida	319	
	Halomicroarcula limicola, Halomicroarcula pellucida	9	

Supplementary Table 2. Continued

Genus	Closest sequences	Reads	
Halomicrobium	*Halomicrobium zhouii*	3	3
Halomonas	*Halomonas aestuarii*	1	44
	Halomonas denitrificans	1	
	Halomonas halophila	12	
	Halomonas johnsoniae	1	
	Halomonas lutescens	11	
	Halomonas meridiana	10	
	Halomonas axialensis, Halomonas lutescens	1	
	Halomonas axialensis, Halomonas meridiana	3	
	Halomonas aestuarii	3	
Haloplanus	*Haloplanus natans*	700	894
	Haloplanus salinus	127	
	Haloplanus aerogenes, Haloplanus natans	1	
	Haloplanus natans, Haloplanus salinus	5	
	Haloplanus natans, Haloplanus salinus	19	
	Haloplanus aerogenes	2	
	Haloplanus natans	36	
	Haloplanus salinus	4	
Halorientalis	*Halorientalis persicus*	11	54
	Halorientalis brevis, Halorientalis persicus	1	
	Halorientalis brevis	20	
	Halorientalis persicus	22	
Halorubellus	*Halorubellus litoreus*	1	4
	Halorubellus salinus	2	
	Halorubellus salinus	1	
Halonotius	*Halonotius pteroides*	3	3
Halorubrum	*Halorubrum kocurii* JCM 14978	2	57
	Halorubrum orientale	1	
	Halorubrum rutilum	1	
	Halorubrum sodomense	2	
	Halorubrum xinjiangense	1	
	Halorubrum sodomense, Halorubrum tebenquichense	1	
	Halorubrum coriense	4	
	Halorubrum ejinorense	2	
	Halorubrum lipolyticum	15	
	Halorubrum orientale	3	
	Halorubrum rutilum	18	
	Halorubrum tebenquichense	1	
	Halorubrum xinjiangense	1	
	Halorubrum sodomense, Halorubrum tebenquichense	5	
Halosimplex	*Halosimplex pelagicum*	3	3
Halospina	*Halospina denitrificans*	3	3
Halovibrio	*Halovibrio denitrificans*	13	13
Henriciella	*Henriciella algicola*	1	1
Herbiconiux	*Herbiconiux flava*	14	14
Hydrobacter	*Hydrobacter penzbergensis*	14	14
Idiomarina	*Idiomarina aquatica*	2	46
	Idiomarina fontislapidosi	8	
	Idiomarina seosinensis	4	
	Idiomarina taiwanensis	19	
	Idiomarina aquatica	2	
	Idiomarina fontislapidosi	3	
	Idiomarina piscisalsi	1	
	Idiomarina taiwanensis	6	
	Idiomarina piscisalsi, Idiomarina seosinensis	1	
Ilumatobacter	*Ilumatobacter fluminis* YM22-133	8	8
Kerstersia	*Kerstersia similis*	2	2
Kluyvera	*Kluyvera intermedia*	1	1

Supplementary Table 2. Continued

Genus	Closest sequences	Reads	
Kushneria	*Kushneria konosiri*	1	1
Klebsiella	*Klebsiella quasipneumoniae* subsp. *quasipneumoniae*	1	3
	Klebsiella quasipneumoniae subsp. *quasipneumoniae*	2	
Legionella	*Legionella pneumophila* subsp. *pascullei*	2	2
Lewinella	*Lewinella nigricans*	3	3
Limnoluna	*Candidatus Limnoluna rubra*	1	1
Litorisediminivivens	*Litorisediminivivens gilvus*	5	7
	Litorisediminivivens gilvus	2	
Lysobacter	*Lysobacter gummosus*	1	1
Longibacter	*Longibacter salinarum*	3	4
	Longibacter salinarum	1	
Longimonas	*Longimonas halophila*	10	13
	Longimonas halophila	3	
Mameliella	*Mameliella alba*	3	9
	Mameliella alba	6	
Marinomonas	*Marinomonas ostreistagni*	1	1
Maritimibacter	*Maritimibacter alkaliphilus*	2	2
Marinobacter	*Marinobacter hydrocarbonoclasticus* ATCC 49840	2	19
	Marinobacter salsuginis SD-14B	3	
	Marinobacter segnicrescens	3	
	Marinobacter algicola DG893	2	
	Marinobacter persicus	3	
	Marinobacter salsuginis SD-14B	3	
	Marinobacter segnicrescens	3	
Marispirillum	*Marispirillum indicum*	4	4
Marivibrio	*Marivibrio halodurans*	1	1
Massilia	*Massilia putida*	2	2
Methylophaga	*Methylophaga thiooxydans* DMS010	2	2
Methyloversatilis	*Methyloversatilis discipulorum*	1	1
Methylophilus	*Methylophilus leisingeri*	29	64
	Methylophilus luteus	9	
	Methylophilus rhizosphaerae	14	
	Methylophilus leisingeri, Methylophilus luteus	8	
	Methylophilus luteus	2	
	Methylophilus rhizosphaerae	1	
	Methylophilus leisingeri, Methylophilus luteus	1	
Methylorubrum	*Methylorubrum populi* BJ001	2	2
Micrococcus	*Micrococcus luteus*	1	1
Mixta	*Mixta calida*	2	2
Moraxella	*Moraxella osloensis*	1	1
Mycolicibacterium	*Mycolicibacterium aubagnense*	8	15
	Mycolicibacterium chlorophenolicum	3	
	Methylobacterium bullatum	1	
	Mycolicibacterium aubagnense	2	
	Mycolicibacterium chlorophenolicum	1	
Natronomonas	*Natronomonas gomsonensis*	2	3
	Natronomonas pharaonis DSM 2160	1	
Nevskia	*Nevskia aquatilis*	1	109
	Nevskia terrae	5	
	Nevskia soli	87	
	Nevskia terrae	16	
Nioella	*Nioella nitratireducens*	7	9
	Nioella nitratireducens	2	
Nitrincola	*Nitrincola lacisaponensis*	1	6
	Nitrincola schmidtii	5	
Niveispirillum	*Niveispirillum fermenti*	1	1
Nocardioides	*Nocardioides marinus*	1	2
	Nocardioides marinus	1	

Supplementary Table 2. Continued

Genus	Closest sequences	Reads	
Nocardia	*Nocardia kroppenstedtii*	1	1
Novosphingobium	*Novosphingobium capsulatum*	16	26
	Novosphingobium subterraneum	2	
	Novosphingobium capsulatum	2	
	Novosphingobium nitrogenifigens DSM 19370	6	
Oceanococcus	*Oceanococcus atlanticus*	54	57
	Oceanococcus atlanticus	3	
Oceanisphaera	*Oceanisphaera donghaensis*	1	7
	Oceanisphaera sediminis	6	
Oceanospirillum	*Oceanospirillum linum*	1	1
Paracoccus	*Paracoccus hibiscisoli*	4	4
Pararhodobacter	*Pararhodobacter aggregans*	1	1
Pelomonas	*Pelomonas saccharophila*	9	10
	Pelomonas saccharophila	1	
Phaeodactylibacter	*Phaeodactylibacter luteus*	1	2
	Phaeodactylibacter xiamenensis	1	
Phenylobacterium	*Phenylobacterium koreense*	12	12
Piscicoccus	*Piscicoccus intestinalis* NBRC 104926	1	1
Polycyclovorans	*Polycyclovorans algicola* TG408	1	8
	Polycyclovorans algicola TG408	7	
Ponticoccus	*Ponticoccus marisrubri*	1	1
Pontimonas	*Pontimonas salivibrio*	5	8
	Pontimonas salivibrio	3	
Prochlorococcus	*Prochlorococcus marinus subsp. pastoris*	2	2
Pseudoalteromonas	*Pseudoalteromonas gelatinilytica*	1	39
	Pseudoalteromonas haloplanktis ATCC 14393	1	
	Pseudoalteromonas undina	18	
	Pseudoalteromonas haloplanktis, *P. undina*	9	
	Pseudoalteromonas undina	7	
	Pseudoalteromonas haloplanktis ATCC 14393, *P. undina*	3	
Pseudomonas	*Pseudomonas geniculata*	5	347
	Pseudomonas hibiscicola	9	
	Pseudomonas oleovorans, *Pseudomonas stutzeri*	1	
	Pseudomonas alcaligenes	6	
	Pseudomonas chengduensis	3	
	Pseudomonas fluvialis	1	
	Pseudomonas gessardii	4	
	Pseudomonas guguanensis	1	
	Pseudomonas guineae	1	
	Pseudomonas knackmussii B13	30	
	Pseudomonas oleovorans	5	
	Pseudomonas oryzihabitans	1	
	Pseudomonas sihuiensis	8	
	Pseudomonas stutzeri	255	
	Pseudomonas trivialis	2	
	Pseudomonas knackmussii B13	3	
	Pseudomonas sihuiensis	1	
	Pseudomonas stutzeri	8	
	Pseudomonas trivialis	1	
	Pseudomonas fluvialis, *Pseudomonas knackmussii* B13	1	
	Pseudomonas hibiscicola	1	
Pseudaeromonas	*Pseudaeromonas sharmana*	3	3
Pseudolysinimonas	*Pseudolysinimonas kribbensis*	1	1
Pseudonocardia	*Pseudonocardia alni*	1	1
Pseudotenacibaculum	*Pseudotenacibaculum haliotis*	2	2
Pseudoxanthomonas	*Pseudoxanthomonas japonensis*	1	2
	Pseudoxanthomonas kaohsiungensis	1	

Supplementary Table 2. Continued

Genus	Closest sequences	Reads	
Psychroflexus	*Psychroflexus salarius*	25	26
	Psychroflexus salarius	1	
Ralstonia	*Ralstonia pickettii*	22	24
	Ralstonia pickettii	2	
Rhizobium	*Rhizobium rosettiformans* W3	1	2
	Rhizobium rosettiformans W3	1	
Rhodosalinus	*Rhodosalinus sediminis*	476	507
	Rhodosalinus sediminis	31	
Roseicyclus	*Roseicyclus mahoneyensis*	13	44
	Roseicyclus mahoneyensis	31	
Rubinisphaera	*Rubinisphaera brasiliensis* DSM 5305	1	1
Rugosibacter	*Rugosibacter aromaticivorans*	1	1
Reyranella	*Reyranella massiliensis* 521	2	29
	Reyranella graminifolii	4	
	Reyranella massiliensis 521	23	
Rheinheimera	*Rheinheimera japonica*	3	8
	Rheinheimera nanhaiensis E407-8	4	
	Rheinheimera japonica	1	
Rhodovibrio	*Rhodovibrio sodomensis*	85	96
	Rhodovibrio sodomensis	11	
Roseicitreum	*Roseicitreum antarcticum*	2	2
Roseivivax	*Roseivivax halodurans*	1	3
	Roseivivax halotolerans	2	
Roseovarius	*Roseovarius aestuariivivens*	1	1
Ruegeria	*Ruegeria intermedia*	19	19
Saccharopolyspora	*Saccharopolyspora halophila*	1	1
Salibacter	*Salibacter halophilus*	327	329
	Salibacter halophilus	2	
Salinicola	*Salinicola salarius*	1	1
Salinigranum	*Salinigranum rubrum*	36	72
	Salinigranum salinum	36	
Salinirepens	*Salinirepens amamiensis*	1	1
Salinivenus	*Salinivenus lutea*	1	1
Salinivibrio	*Salinivibrio kushneri, Salinivibrio sharmensis*	4	121
	Salinivibrio costicola subsp. alcaliphilus	8	
	Salinivibrio kushneri	13	
	Salinivibrio proteolyticus	36	
	Salinivibrio sharmensis	53	
	Salinivibrio kushneri	1	
	Salinivibrio proteolyticus	2	
	Salinivibrio sharmensis	4	
Salipiger	*Salipiger thiooxidans*	5	5
Shewanella	*Shewanella seohaensis*	1	2
	Shewanella seohaensis	1	
Shinella	*Shinella curvata*	3	3
Sinirhodobacter	*Sinirhodobacter ferrireducens*	1	1
Sphingobacterium	*Sphingobacterium siyangense*	2	2
Sphingorhabdus	*Sphingorhabdus buctiana*	1	1
Schlesneria	*Schlesneria paludicola*	6	6
Selenihalanaerobacter	*Selenihalanaerobacter shriftii*	2	2
Sphingomonas	*Sphingomonas jeddahensis, S. kyeonggiensis*	1	16
	Sphingomonas jeddahensis	2	
	Sphingomonas kyeonggiensis	3	
	Sphingomonas kyungheensis	4	
	Sphingomonas olei	1	
	Sphingomonas prati	3	
	Sphingomonas piscinae	1	
	Sphingomonas prati	1	

Supplementary Table 2. Continued

Genus	Closest sequences	Reads	
Sphingopyxis	*Sphingopyxis solisilvae*	1	1
Spiribacter	*Spiribacter roseus*	1	154
	Spiribacter roseus	153	
Staphylococcus	*Staphylococcus warneri*	1	1
Streptomyces	*Streptomyces abikoensis, Streptomyces tritici*	2	12
	Streptomyces abikoensis	1	
	Streptomyces tritici	6	
	Streptomyces tritici	1	
	Streptomyces abikoensis, Streptomyces tritici	2	
Spongiibacter	*Spongiibacter marinus*	1	2
	Spongiibacter taiwanensis	1	
Sulfitobacter	*Sulfitobacter faviae*	3	5
	Sulfitobacter pontiacus	2	
Sulfurimonas	*Sulfurimonas autotrophica*	1	1
Sulfurospirillum	*Sulfurospirillum alkalitolerans*	2	2
Synechococcus	*Synechococcus rubescens*	2	2
Terrimonas	*Terrimonas aquatica*	1	2
	Terrimonas soli	1	
Thalassospira	*Thalassospira australica*	1	1
Tamilnaduibacter	*Tamilnaduibacter salinus*	2	2
Thiohalobacter	*Thiohalobacter thiocyanaticus*	1	1
Vibrio	*Vibrio alginolyticus*	7	8
	Vibrio alginolyticus	1	
Variovorax	*Variovorax humicola*	1	1
Virgibacillus	*Virgibacillus carmonensis*	2	2
Wenzhouxiangella	*Wenzhouxiangella sediminis*	6	13
	Wenzhouxiangella marina	1	
	Wenzhouxiangella sediminis	6	
	Total	7772	7772
Unclassified	*Enterobacter cloacae, Klebsiella quasipneumoniae*	6	27
	Enterobacter cloacae, E. mori, Klebsiella pneumoniae	2	
	Cronobacter malonaticus, Enterobacter mori	1	
	E. cloacae, Klebsiella quasipneumoniae subsp. *quasipneumoniae*	5	
	Halapricum salinum, Halomicrobium zhouii	2	
	Litorisediminivivens gilvus, Roseovarius pacificus	1	
	Aestuariibacter salexigens, Alteromonas oceani	1	
	Altererythrobacter xinjiangensis, Novosphingobium nitrogenifigens	9	
	Grand total	7799	7799

Supplementary Table 3. Enriched cultures at Genus level in CP-Tuban (TE). Red highlights indicate the sequence identity of (≥98.65%); and blue highlights indicate the sequence identity of 97.03-98.64%

Genus	Closest sequences	Reads	
Aliifodinibius	*Aliifodinibius halophilus*	1	1
Chromohalobacter	*Chromohalobacter salexigens*	5	8
	Chromohalobacter salexigens	3	
Enterobacter	*Enterobacter cloacae*	1	2
	Enterobacter mori	1	
Halanaerobium	*Halanaerobium praevalens*	5043	5581
	H. saccharolyticum subsp. senegalense	7	
	H. praevalens, H. saccharolyticum subsp. *senegalense*	291	
	H. praevalens, H. saccharolyticum subsp. *senegalense*	34	
	Halanaerobium praevalens	206	
Halobacillus	*Halobacillus profundi*	30	31
	Halobacillus profundi	1	
Halobaculum	*Halobaculum magnesiiphilum*	1	2
	H. gomorrense, H. magnesiiphilum	1	
Halobellus	*Halobellus ramosii*	3	4
	Halohasta litorea	1	

Supplementary Table 3. Continued

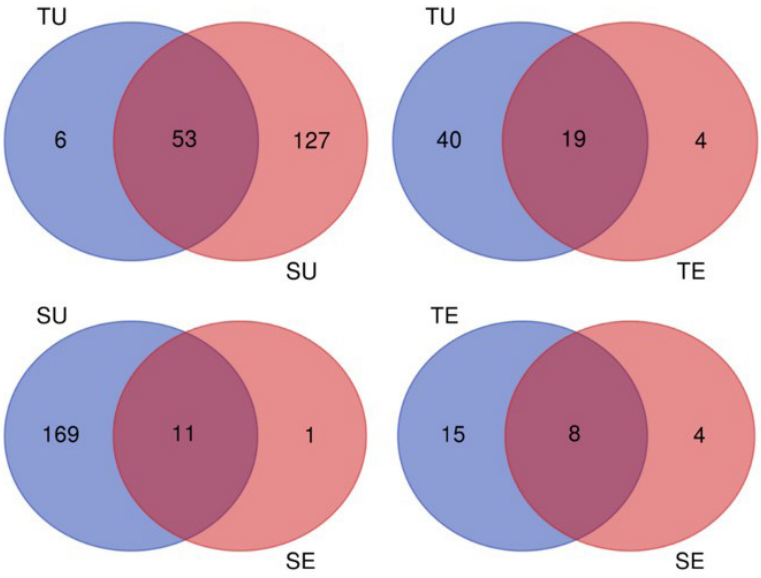
Genus	Closest sequences	Reads	
Halohasta	*Halohasta litorea*	2	2
Halomicroarcula	*Halomicroarcula limicola*	1	1
Halomonas	*Halomonas denitrificans*	1	181
	Halomonas halophila	83	
	Halomonas smyrnensis	28	
	Halomonas halophila	50	
	Halomonas smyrnensis	19	
Halonotius	*Halonotius pteroides*	2	2
Haloplanus	*Haloplanus natans*	4	4
Halorubrum	*Halorubrum orientale*	1	3
	Halorubrum orientale	1	
	Halorubrum sodomense	1	
Marinomonas	*Marinomonas ostreistagni*	3	3
Oceanococcus	*Oceanococcus atlanticus*	1	1
Oceanospirillum	*Oceanospirillum sanctuarii*	1	7
	Oceanospirillum linum	2	
	Oceanospirillum sanctuarii	4	
Orenia	*Orenia chitinitropha*	242	242
Pseudoalteromonas	*Pseudoalteromonas undina*	1	2
	Pseudoalteromonas ruthenica	1	
Pseudomonas	*Pseudomonas stutzeri*	1	1
Salinigranum	*Salinigranum rubrum*	2	3
	Salinigranum salinum	1	
Salinivibrio	*Salinivibrio costicola* subsp. *alcaliphilus*	528	16202
	Salinivibrio kushneri	4775	
	Salinivibrio proteolyticus	2262	
	Salinivibrio sharmensis	4787	
	Salinivibrio costicola subsp. alcaliphilus, S. kushneri	7	
	Salinivibrio costicola subsp. alcaliphilus, S. sharmensis	1	
	Salinivibrio kushneri, Salinivibrio proteolyticus	96	
	Salinivibrio kushneri, Salinivibrio sharmensis	1960	
	Salinivibrio proteolyticus, Salinivibrio sharmensis	1057	
	S. costicola subsp. alcaliphilus, S. kushneri, S. sharmensis	164	
	Salinivibrio kushneri, S. proteolyticus, S. sharmensis	88	
	S. costicola subsp. alcaliphilus, S. kushneri, S. proteolyticus	2	
	Salinivibrio costicola subsp. alcaliphilus	21	
	Salinivibrio kushneri	139	
	Salinivibrio proteolyticus	70	
	Salinivibrio sharmensis	148	
	Salinivibrio kushneri, Salinivibrio proteolyticus	5	
	Salinivibrio kushneri, Salinivibrio sharmensis	57	
	Salinivibrio proteolyticus, Salinivibrio sharmensis	30	
	S. costicola subsp. alcaliphilus, S. kushneri, S. sharmensis	2	
	Salinivibrio kushneri, S. proteolyticus, Salinivibrio sharmensis	3	
Streptomyces	*Streptomyces tritici*	6	6
Virgibacillus	*Virgibacillus dokdonensis*	4	5
	Virgibacillus dokdonensis	1	
Total		22294	22294

Supplementary Table 4. Enriched cultures at the genus level from CP-Sampang (SE). Red highlights indicate the sequence identity of (≥98.65%); and blue highlights indicate the sequence identity of 97.05-98.64%

Genus	Closest sequences	Reads	
Enterobacter	*Enterobacter mori*	2	3
	Enterobacter mori	1	
Halanaerobium	*Halanaerobium praevalens*	3841	3947
	H. saccharolyticum subsp. senegalense	2	
	Halanaerobium praevalens, H. saccharolyticum	1	
	Halanaerobium praevalens	103	
Halobacillus	*Halobacillus profundi*	1	1

Supplementary Table 4. Continued

Genus	Closest sequences	Reads	
Halomonas	*Halohasta litorea*	2	13
	Halomonas denitrificans	1	
	Halomonas halophila	3	
	Halomonas smyrnensis	5	
	Halomonas halophila	1	
	Halomonas smyrnensis	1	
Haloplanus	*Haloplanus natans*	1	1
Nocardioides	*Nocardioides zeae*	1	1
Psychroflexus	*Psychroflexus salarius*	1	1
Rhodosalinus	*Rhodosalinus sediminis*	1	1
Salinigranum	*Salinigranum salinum*	1	1
Salinivibrio	*Salinivibrio costicola subsp. alcaliphilus*	298	6469
	Salinivibrio kushneri	1358	
	Salinivibrio proteolyticus	2615	
	Salinivibrio sharmensis	1557	
	S. costicola subsp. alcaliphilus, S. proteolyticus	7	
	Salinivibrio kushneri, Salinivibrio proteolyticus	5	
	Salinivibrio kushneri, Salinivibrio sharmensis	496	
	Salinivibrio proteolyticus, Salinivibrio sharmensi	31	
	Salinivibrio costicola subsp. alcaliphilus	28	
	Salinivibrio kushneri, Salinivibrio sharmensis		
	Salinivibrio costicola subsp. alcaliphilus	4	
	Salinivibrio kushneri	13	
	Salinivibrio proteolyticus	27	
	Salinivibrio sharmensis	28	
	Salinivibrio costicola subsp. alcaliphilus, S. proteolyticus	1	
Streptomyces	*Salinivibrio kushneri, Salinivibrio sharmensis*	1	
	Streptomyces tritici	22	23
Vibrio	*Streptomyces tritici*	1	
	Vibrio alginolyticus	3	9
	Vibrio hepatarius	1	
	Vibrio xuii	1	
	Vibrio alginolyticus	4	
Total		10470	10470



Supplementary Figure 1. The distribution of shared operational taxonomic units (OTUs) at the genus-level among uncultured microorganisms and enriched microbial cultures in CP-Tuban and CP-Sampang. Note: TU, uncultured microorganisms in CP-Tuban; SU, uncultured microorganisms in CP-Sampang; TE, enriched microbial cultures in CP-Tuban; and SE, enriched microbial cultures in CP-Sampang

Supplementary Table 5. Alpha-diversity and richness indices of genera identified in Tuban and Sampang samples

Sp	∑T	∑R	*K*	*M*	*H`*	*J`*	D_s	1- D_s	Chao1	iChao	ACE
TU	59	1741	7.7725	1.414	2.6081	0.6396	0.1282	0.1282	107.372	109.136	84.113
SU	180	7772	19.9815	2.0418	3.1196	2.6007	0.0982	0.9018	223.607	234.505	241.434
TE	23	22294	2.1973	0.154	0.6981	0.2227	0.591	0.591	24.6	24.694	25.275
SE	12	10470	1.1884	0.1173	0.7007	0.282	0.5238	0.5238	33.998	35.748	43.188

Sp = Samples, TU = CP-Tuban uncultured, SU = CP-Sampang uncultured, TE = CP-Tuban enriched, SE = CP-Sampang enriched, ∑T = total numbers of operational taxonomic units (OTUs), ∑R = total numbers of sequence reads, *K* = Margalef richness index (Margalef 1958), *M* = Menhinick Index (Menhinick 1964), *H`* = Shannon Entropy index (Shannon 1948), *J`* = Pielou evenness (Pielou 1966), *Ds* = Simpson diversity index (Simpson 1949), 1-*Ds* = Gini-Simpson index (Jost 2006). Other diversity indices include Chao1 (Chao 1984; Chao and Yang 1993), iChao1 (Chiu *et al.* 2014), and the abundance coverage estimator (ACE) (Chao and Lee 1992; Chao and Yang 1993; Good 1953; Good and Toulmin 1956)

Supplementary Table 6. Genera (identity ≥97%) detected in CP-Tuban and CP-Sampang, which are considered beneficial to the salt production process

Family	Genus	Read number			
		CP-Tuban		CP-Sampang	
		TU	TE	SU	SE
Halobacteriaceae (Oren 2010, 2014)	*Halorubrum*	143	3	57	
	Haloarcula	2		95	
	Halobaculum	107	2	582	
	Haloferax	-		1	
	Halobellus	138	4	29	
	Haloplanus	93	4	894	1
	Natronomonas	31		3	
	Halosimplex	3		3	
	Halomicrobium	1		3	
	Halogranum	-		1	
	Halolamina	-		2	
	Halonotius	39	2	3	
	Halorientalis	6		54	
Flavobacteriaceae	*Psychroflexus* (Zhong *et al.* 2016)	5		26	1
Rhodobacteraceae	*Rhodosalinus* (Guo *et al.* 2017)	35		507	1
Total		603	15	2260	3

The potential contribution of the genera listed above to the quality of salt produced is due to their possible ability to produce carotenoid pigments (Oren 2010)

Supplementary Table 7. Genera of uncultured microorganisms relatively dominant in CP-Sampang (SU), which were undetected in CP-Tuban (TU). Red highlight indicates ≥98.65% identity, and blue highlight indicates the identity of 97.00 to 98.65%

Genus	Reads	Closest sequences
Albirhodobacter	11	*A. marinus* (11 reads)
Aquicoccus	16	*A. porphyridii* (16 reads)
Acinetobacter	40	*A. baumannii* (11 reads)
Aeromonas	43	*A. caviae* (34 reads)
Aliifodinibius	45	*A. halophilus* (19 reads)
Aliishimia	15	*A. ponticola* (15 reads)
Anoxybacillus	14	*A. flavithermus* (9 reads)
Arhodomonas	33	*A. recens* (33 reads)
Bacillus	29	*B. carboniphilus* (10 reads)
Brevibacillus	127	*B. thermoruber* (127 reads)
Corynebacterium	10	*C. amycolatum* (5 reads)
Defluviimonas	10	*D. nitratireducens* (10 reads)
Gracilimonas	23	*G. tropica* (16 reads)
Halovibrio	13	*H. denitrificans* (13 reads)
Hydrobacter	14	*H. penzbergensis* (14 reads)
Longimonas	13	*L. halophila* (10 reads)
Marinobacter	19	*M. hydrocarbonoclasticus* (2 reads)
Mycolicibacterium	12	*M. aubagnense* (8 reads)
Novosphingobium	26	*N. capsulatum* (16 reads)
Pelomonas	10	*P. saccharophila* (9 reads)
Ralstonia	24	*R. pickettii* (22 reads)
Roseicyclus	13	*R. mahoneyensis* (13 reads)
Ruegeria	19	*R. intermedia* (19 reads)
Salibacter	329	*S. halophilus* (327 reads)
Sphingomonas	16	*S. jeddahensis* (1 reads)
Wenzhouxiangella	13	*W. sediminis* (6 reads)