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Insights into saline adaptation strategies through a novel halophilic bacterium isolated from solar saltern of Yellow sea

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Solar salterns were placed along the coast and were frequently left unattended after use. While many studies have isolated and identified microorganisms from hypersaline environments, their role and adaptation mechanisms are still unclear. Herein, we elucidated the role of halophiles in salt-polluted areas through the recently reported *Halomonas getboli* YJPS3-2 from the abandoned saltern. We analyzed the expression levels of genes in the YJPS3-2 strain to identify its adaptation mechanisms to high salinity environments, by representing the process from tidal flats to abandoned salterns with varying salinity gradients. The YJPS3-2 strain primarily overexpresses genes associated with ABC transport to adapt to hypersaline environments. Interestingly, the *cheA* gene, which recognizes changes in the surrounding, was the most upregulated, and it was also associated with the overexpression of the MS ring and T3SS mechanisms relating to the flagellar activity. The YJPS3-2 recognized the high salt concentration in its surroundings and attempted to accumulate compatible solutes that could withstand high osmotic pressure inside the cell to adapt to the high salinity environment. Furthermore, during this process, the YJPS3-2 strain removed surrounding pollutants and secreted secondary metabolites that could be utilized by neighboring organisms. Our results suggested that this halophilic bacterium has the potential to serve as a pioneering species for thriving the surrounding while adapting to saline environments.

KEYWORDS

halophiles, halomonas, abandoned solar salterns, hypersaline, whole genome sequence, transcriptome

1 Introduction

Coastal tidal flats are a unique ecosystem that is alternately covered and exposed by the tides, and it includes various habitats such as tidal flats and salt marshes (Chen et al., 2016). The intertidal zones, where seawater and freshwater meet, create unique environments due to the presence of salinity, which limits biodiversity. When salt concentrations increase, the biological diversity decreases, however, halotolerant microorganisms often require high-salt environments and can even be found at the highest salt concentrations (Gerday and Glansdorff, 2007). In particular, coastal solar salterns are noteworthy as they utilize these environmental characteristics of tidal flat for salt production. They are man-made, thalassohaline hypersaline environments (Oren, 2007) created by evaporating seawater and are located along the coastline (Paul et al., 2020). These artificial solar salterns create an extremely hypersaline environment that results in biodiversity loss, providing evidence that the region has undergone “environmental degradation” (Rodríguez-Valera et al., 1985).

Hypersaline environments, such as solar salterns and salt lakes, are characterized by high salt levels (> 3.5%, higher than seawater), and are inhabited by halophiles (Ventosa, 2006; Javor, 2012), a group of microorganisms adapted to these extreme conditions. Halophilic bacteria can be classified based on their salt requirement and growth patterns. Slight halophiles grow optimally at 2 – 5% NaCl, moderate halophiles at 5 – 20% NaCl, and extreme halophiles at 20 – 30% NaCl (Irshad et al., 2014). To cope with high extracellular salinity, halophilic microorganisms have adopted various strategies. Bacteria and archaea accumulate K^+ and Cl^- ions while maintaining a low Na^+ concentration in response to external osmotic pressure. Most bacteria and eukaryotes store or synthesize organic solutes, such as glycine betaine, ectoine, and other amino acids and sugar derivatives, to deal with high salinity (Oren, 2002; Roberts, 2005). Halophiles have developed basic biochemical adaptations in their proteins, osmoregulation systems, nucleic acids, and lipids to thrive and reproduce in such high-salt, low-water activity conditions (Javor, 2012).

Getbol, tidal flats in Korean, are located along the west coast of the Republic of Korea and include several manmade salterns, such as Taepyeong salt farms in Sinan-gun, Jeung-do (Lee et al., 2018), and Yubu-do solar saltern in Taeon-gun. The coastal tidal flats have been extensively studied, and numerous microorganisms have been reported and isolated from this ecosystem (Yeon et al., 2005). However, it is worth noting that microorganisms are also being isolated, particularly from abandoned salterns. Among them, the genus *Halomonas* is frequently isolated from high-salt environments such as abandoned salterns (Lim et al., 2004; Lee et al., 2005; Lee et al., 2016). The genus *Halomonas* belongs to the phylum Gammaproteobacteria, class Oceanospirillales, family Halomonadaceae, and is one of the largest halophilic genus so far with over 100 species and diverse salinity growth range.

In a previous study, the comparative evaluation of bacterial diversity and community structure was conducted on three abandoned salterns with different natural restoration periods (Lee et al., 2020). As the

salterns were abandoned for longer periods of time, the microbial community structure became increasingly similar to that of the adjacent natural tidal flats. In a recent study, a novel halophilic bacterial species named “*Halomonas getboli* YJPS3-2” was isolated and reported from the sediment of an abandoned saltern in Yongyu-do, where the difference in microbial community structure between the saltern and the adjacent natural tidal flat was the greatest in previous studies (Lee et al., 2020; Yoo et al., 2022). Therefore, this study was conducted as a follow-up to the previous research to evaluate the ecological role of halophilic bacteria in abandoned saltern, using newly identified halophilic bacteria “YJPS3-2” as a research subject. The specific objectives of this study were: 1) to reveal adaptation strategies to the hypersaline environment by exposing the strain to various salt conditions and 2) to report halotolerant-related genes through the newly identified species.

2 Materials and methods

2.1 Isolation of bacterial strains and culture conditions

Our previous studies reported the study site (Figure 1) and detailed procedures for the isolation of bacterial strains (Lee et al., 2020; Yoo et al., 2022). Most of the culturable bacteria isolated from Yongyu-do grew well on a basal medium ISP2 (Islam and Hernández, 1966) with seawater (4 g L⁻¹ yeast extract (Difco), 10 g L⁻¹ malt extract (Difco), 4 g L⁻¹ dextrose (Difco), and 10 g L⁻¹ artificial sea salt (Merck), 10 ppt, pH 7.0), as well as marine agar (MA 2216, Difco).

To determine the optimal growth conditions of the strain YJPS3-2 used in this study, it was grown in a basal medium (ISP2, without artificial sea salt) with various concentrations of NaCl (DUKSAN, grade=99.5%), including 0%, 1%, 5%, 10%, 15%, and 20% (w/v), and incubated at 30 °C with shaking at 180 rpm. The concentration of strain YJPS3-2 (OD600) was monitored every 6 h until 96 h using a spectrophotometer (SPARK 10M), with three replicates.

2.2 Extraction of genomic DNA and total RNA of strain YJPS3-2

Strain YJPS3-2 was cultured in a basal medium with 1% NaCl at 30°C for 18 h, and genomic DNA was extracted using the method described in Yoo et al. (2021). Whole-genome sequencing was performed by use of the MiSeq (2 x 300) sequencing platform (Illumina). The sequencing reads were obtained from the paired-end sequencing of a genomic library, with an average insert size of 500 bp. The low-quality reads were trimmed with a quality threshold of Q30. The filtered reads were *de novo* assembled using the SPAdes genome assembler with default parameters (Bankevich et al., 2012).

For total RNAs, strain YJPS3-2 cells (OD600 = 2.0) were grown in a medium containing 1%, 5%, and 15% NaCl, and RNA was



FIGURE 1

Map showing the sampling location of Yongyudo in the Yellow Sea, Korea. (A) Yongyudo coastline, (B) the sampling sites of tidal flats and abandoned saltern, (C) the three sampling sites of abandoned saltern.

extracted using TruSeq Total RNA with Ribo-Zero (Illumina) according to the manufacturer's instructions. Whole-transcriptome sequencing was performed on the HiSeqXten, NextSeq500 sequencing platform (Illumina). Total RNA from one control group (1% NaCl) and three replicates of strains in 5% and 15% NaCl each was prepared for transcriptomic sequencing.

2.3 Genome and transcriptome data processing and differential gene expression analysis

The resulting genomic sequence was submitted to the RAST server (Aziz et al., 2008), which predicted the open reading frames (ORFs), tRNAs, and rRNAs. The predicted ORFs were annotated by querying the SEED (<http://pubseed.theseed.org/>) (Disz et al., 2010) and Kyoto Encyclopedia of Genes and Genomes (KEGG, KO numbers) databases. The PAI Finder (Yoon et al., 2007), a web-based search tool of the pathogenicity island database (PAIDB), was used to identify potential pathogenic islands (PAIs). Biosynthetic gene clusters for secondary metabolites were predicted using the antiSMASH tool (Weber et al., 2015).

For gene expression analysis, transcriptomic reads from the 5% and 15% NaCl samples were aligned to the whole transcriptome sequencing of the 1% NaCl strain of YJPS3-2. *De novo* assembly of the merged data was carried out using Trinity with the default parameters (Grabherr et al., 2011). For the assembled genes, the longest contigs of the assembled contigs were filtered and clustered into non-redundant transcripts using the CD-HIT-EST program (Fu et al., 2012). We defined these transcripts as “unigenes”, which were used for predicting ORFs, annotating against several known sequence databases, and analyzing differentially expressed genes (DEGs). Fragments Per Kilobase Million (FPKM) metrics were used to quantify the total transcription levels of the genes, and genes with expression level P-values of < 0.05 were identified as DEGs (Anders and Huber, 2012). The transcription expression levels were represented using FPKM values evaluated with eXpress. Genes were considered to be differentially expressed if the absolute value 2 of the \log_2FC (Fold change) in FPKM exceeded.

For functional annotation of the unigenes, the Gene Ontology (GO) database, and Kyoto Encyclopedia of Genes and Genomes (KEGG) database were applied to classify the annotated unigenes using BLASTX of DIAMOND with an E-value cut-off of 1.0E5. Classification of the GO terms were subsequently performed using an in-house script. Bi-directional best hit (BBH), which is a widely used method to infer orthology, was used to search against the KEGG database to obtain the KO (reference pathway) number of the KEGG annotation. The KO number of the transcriptome was also obtained according to the KEGG annotation.

3 Results

3.1 Bacterial isolation and selection of the experimental strain

In our recent study, we examined the sediment microbiome from Yongyudo and isolated a total of 104 culturable bacteria (Lee et al., 2020) (Table S1). Of these isolates, 73 strains (23 genera and 61 species) were obtained from tidal flats, while 31 strains (16 genera and 27 species) were obtained from abandoned salterns. This result indicated lower biodiversity in salterns, which is consistent with our previous research (Lee et al., 2020). We conducted a salt-tolerant test and found that nine bacterial species were able to grow in a slightly halophilic environment (5% NaCl), while four of them were able to grow in a moderately halophilic environment (15% NaCl) (Table 1). The NaCl concentration gradient used for the salt-tolerant test was selected based on the standards for classifying halophilic bacteria, as well as the salinity of tidal flats, oceans (around 32 – 33‰) (Choi et al., 2021), and solar salterns (Song et al., 2022). Among them, strain YJPS3-2 was newly reported, which was belonging to the genus *Halomonas* with a 16S rRNA gene similarity of 98.2% (Yoo et al., 2022). The genus *Halomonas* is classified within the family Halomonadaceae in the class Gammaproteobacteria and is also well-known as halophilic bacteria, in which the cells are Gram-negative and chemoorganotrophic (Ventosa et al., 2015). Therefore, this strain was selected as the experimental strain in the present study, as it was considered to be appropriate for revealing the mechanism of adaption to halophilic environments.

TABLE 1 List of salt-tolerant tests for halophilic and halotolerant bacteria isolated from the study area.

No.	Strain	Name	Similarity (%)	5% NaCl	10% NaCl	15% NaCl
1	YJ-S2-02	<i>Novosphingobium aureum</i>	97.7	++	w	-
2	YJ-S3-02	<i>Marinobacter halodurans</i>	96.7	+	+	-
3	YJPT1-2	<i>Cobetia amphilecti</i>	99.7	+++	+++	++
4	YJPT2-2	<i>Cobetia marina</i>	99.8	+++	+++	++
5	YJPS1-1	<i>Microbulbifer</i> sp. YJPS1-1	97.8	++	++	-
6	YJPS1-3	<i>Halomonas elongata</i>	99.7	+	+++	++
7	YJPS1-5	<i>Kushneria avicenniae</i>	99.9	++	+++	-
8	YJPS2-1	<i>Kushneria marisflavi</i>	99.2	++	++	-
9	YJPS3-2	<i>Halomonas</i> sp. YJPS3-2 ^T	98.2	++	+++	++

The following results were obtained, where +++ represents heavy growth, ++ for reasonable growth, + for slight growth and - for no growth.

3.2 Growth properties of strain YJPS3-2 under various salt conditions

Strain YJPS3-2 showed a minimum requirement of 1% NaCl for survival, with no bacterial growth observed in either 0% or 20% NaCl conditions (Figure 2). Growth curves revealed a higher growth rate in 1% NaCl compared to 5%, 10%, or 15% NaCl between 3 h and 24 h. These results indicate that NaCl is necessary for growth and that the strain prefers a halophilic environment, classifying it as a halophilic bacterium.

3.3 Genomic features and transcriptomic profiles of strain YJPS3-2

The whole-genome sequencing of strain YJPS3-2 resulted in the annotation of a total of 1,348 genes (Figure 3A), which were categorized as follows (>10%): amino acids and derivatives (19.40%); protein metabolism (12.73%); cofactors, vitamins, prosthetic groups, and pigments (10.59%); and carbohydrates (10.59%) (Figure 3B).

Genomic analysis of strain YJPS3-2 revealed that it is incapable of carbon fixation and instead utilizes glycoside hydrolase, peptidase, and gluconeogenesis to obtain carbon sources. The Embden-Meyerhof pathway and Entner-Doudoroff pathway are engaged during glycolysis, with the TCA cycle being utilized. YJPS3-2 is classified as a mixotroph, capable of using nitrogen via dissimilatory nitrate reduction when oxygen is not available as an electron acceptor. Furthermore, the genomic data indicated that YJPS3-2 can degrade pollutants by utilizing the *aroD* gene for quinate degradation, and *benK*, *benD*, *benB*, and *pobA* for benzonate degradation. Additionally, it can decompose aromatic compounds via *hmgA*, *fahA*, *hppD*, *maiA*, *hppD*, and *phhA*.

The PAI Finder was used to compare the PAI regions of *Halomonas elongata* and YJPS3-2. Consequently, nPAIs (PAI-like regions not overlapping genomic islands) but not PAI-like regions were present in YJPS3-2. The detected nPAI region, HELO_2920 gene was present in the genome of YJPS3-2. However, there was no significant expression in the transcriptome under 5% and 15% NaCl

conditions. In addition, an ectoine synthase (*ectC*) gene cluster encoding enzymes involved in the ectoine transport system was identified by antiSMASH (node 827,601 – 827,993 bps) and showed 75% similarity with the ectoine biosynthetic gene cluster from *Methylomicrobium kenyense*. The siderophore biosynthesis protein (*lucA/lucC*) gene cluster was identified by antiSMASH (node 79,902 – 81,779 bps) and showed 66% similarity with the desferrioxamine cluste from *Streptomyces argillaceus*. Also, RiPP-like (Ribosomally synthesized and post-translationally modified peptides) enzyme gene cluster was identified by antiSMASH (node 421,288 – 433,486 bps) and showed 17% similarity with fengycin biosynthetic gene cluster from *Bacillus velezensis* FZB42 (Figure 4).

Transcriptomic analysis of strain YJPS3-2 under varying NaCl conditions was performed by *de novo* sequencing. A total of seven samples were analyzed, including one control strain (1% NaCl) and three replicates each in 5% and 15% NaCl conditions, resulting in six designed comparison analyses. The raw reads were generated, on average, for 1% (22 Mbps), 5% (24 Mbps), and 15% NaCl (24 Mbps) conditions, respectively (Table S2). To avoid biased analysis, only 570 contigs were used for statistical analysis after excluding 1,838 out of 2,408 contigs that had more than one read count value of 0 (Figure S1).

3.4 Halotolerance-related genes from differential expression analysis

The different expression levels of genes are presented as a heat map (Figure 5) and notable metabolic pathways in the saline conditions were predicted based on the DEG analysis data and KO number annotations (Figure 6; Tables S4–S6). Compared to the condition with 1% NaCl, both 5% and 15% NaCl conditions had a significant impact on membrane transporter-related genes. The majority of genes were related to ATP-binding cassette (ABC) transporters, two-component systems, and the Na⁺ symporter, as shown in Figures 5, 6. Among the affected genes, *afuA* (iron complex), *phoR* and *phoD* (phosphate), *glnL*, *glnG*, and *glnD* (glutamate), *modB* (molybdate), *aapJ*, *aapQ*, and *aapP* (general L-

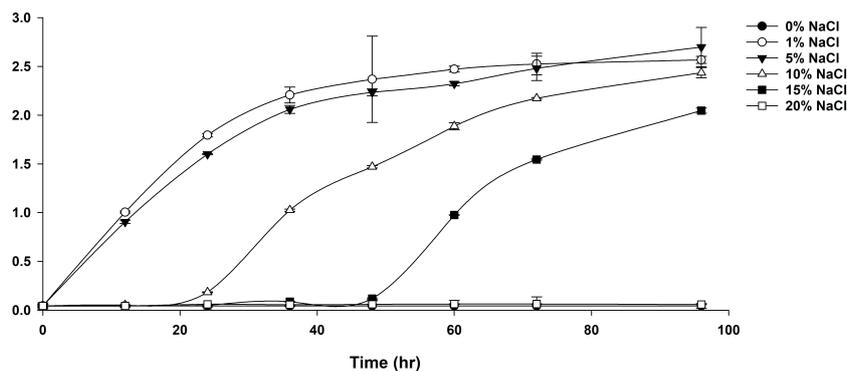


FIGURE 2 Growth curve of strain YJPS3-2 in media containing various concentrations of NaCl, including 0%, 1%, 5%, 10%, 15%, and 20% (w/v) NaCl.

amino acid), and *livM* (branched-amino acid) are involved in ion transport, and *malk* (maltose), *thuF*, *thuG*, and *thuK* (trehalose), *smoK* (mannitol), and *araG* (L-arabinose) are genes involved in solutes compatible with the ABC transporter. In the ABC transporter *smoK*, *thuF*, *thuG*, *thuK*, *malk*, *modB*, *araG*, and *phoD* were upregulated, and *glnL*, *glnG*, and *phoR* were downregulated under 5% NaCl conditions.

Figure 6 shows the differential expression levels of genes involved in the glycolysis pathway under high salinity concentrations. This pathway involves complex genes, such as those related to the pentose phosphate pathway; alanine, aspartate, and glutamate metabolism; valine, leucine, and isoleucine biosynthesis; galactose metabolism, and citrate cycle (TCA cycle) functions. Furthermore, the flagellar assembly and pathway-related genes (*cheA*, *flaG*, *filF*, *fliI*, and *flhA*) from DEG analysis were upregulated in both 5% and 15% NaCl conditions except for *filK*. Notably, the DNA repair pathways, including nucleotide excision repair, mismatch repair, DNA replication, and

homologous recombinations, were significantly affected by saline conditions. Among these pathways, *mutS* and *mutL* were downregulated, while *ruvB*, *uvrA*, and *ligA* were upregulated in saline conditions.

Through DEG analysis, the upregulated genes were sorted under both 5% and 15% NaCl conditions (Table S4). Among the upregulated genes, *cheA*, which encodes taxis sensor histidine kinase for a two-component system, exhibited the highest expression followed by *cslC*, *fruB*, *filF*, and ABC transporter-related genes. On the other hand, the genes that showed downregulated expression under both 5% and 15% NaCl conditions included *nirD*, *aapP*, *aapM*, *nasA*, *mutS*, *dgt*, *pcnB*, *aapJ*, and *ynjC*, as well as the majority of the transporter-related genes and DNA repair-related genes indicated in Figure 6. The genes that were upregulated only under 5% NaCl conditions were *lepA*, *ligA*, *algC*, *patB*, *glmS*, *thuG*, *sugarB*, *gadB*, *gadA*, *AllaA*, *malZ*, *araG*, and *ugpB*. In contrast, the *ntrC*, *phoR*, *uvrA*, *ilvC*, and *metG* genes were downregulated only in 5% NaCl conditions (Table S4).

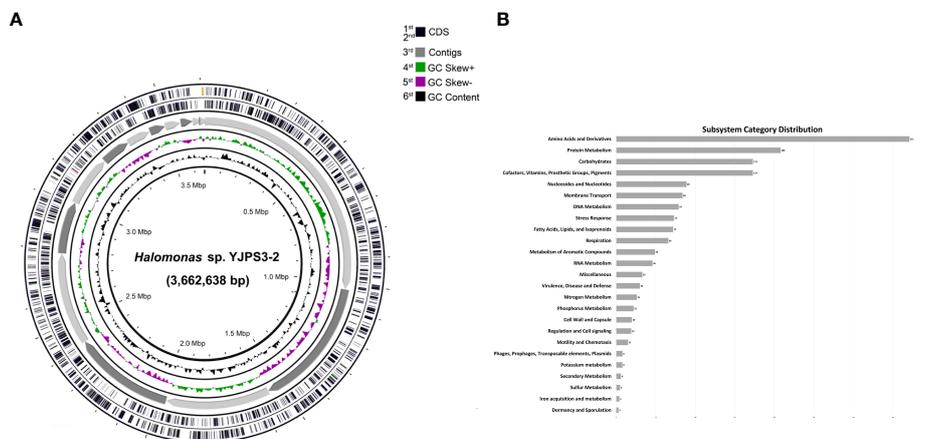


FIGURE 3 Genome map of *Halomonas* sp. YJPS3-2. (A) Rings from the outside are as follows: 1st and 2nd circles, predicted genes on the coding sequence (CDS); 3rd circle, mapping count contigs; 4th circle, GC skew + strand; 5th circle, GC skew - strand; and 6th circle, CG content. (B) Diagram of subsystem category distribution results from whole genome sequencing analysis of strain YJPS3-2.

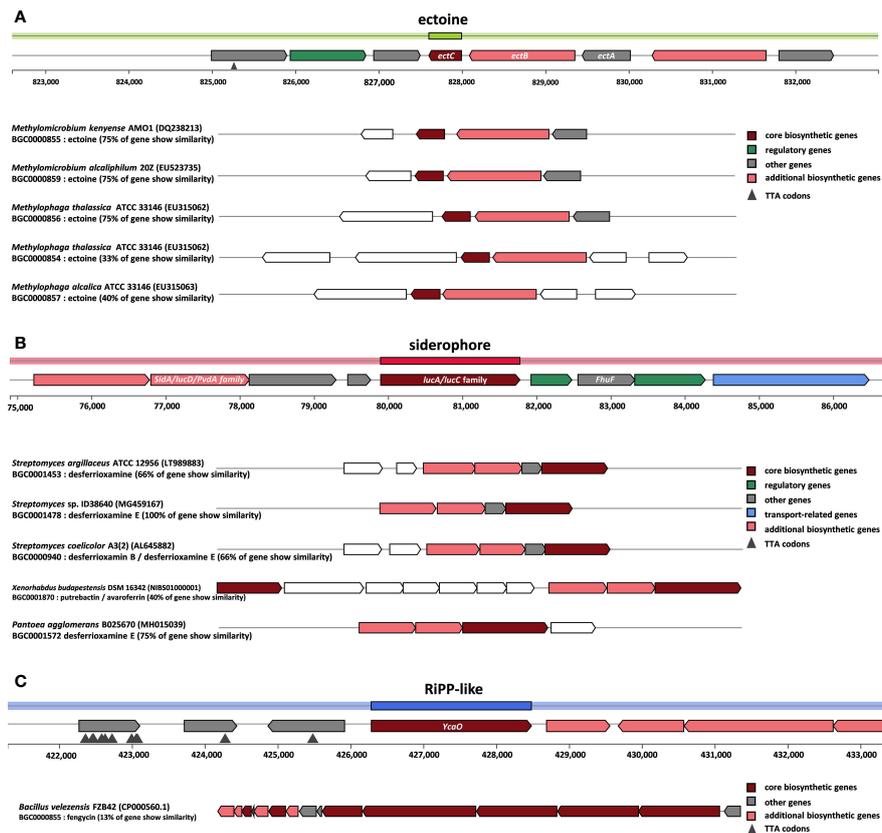


FIGURE 4

Schematic overview of the genomic region containing the strain YJPS3-2 secondary metabolites gene cluster. biosynthetic gene clusters (BGC) comparison of the (A) ectoine synthesis and (B) siderophore synthesis (C) RiPP-like gene clusters of the isolate and related strains as predicted by antiSMASH. Based on sequence similarity and gene function prediction.

Additionally, *ispH*, *tar1*, *tsr*, *flgK*, *futA*, and *araC* were upregulated, and *fdhA*, *yheS*, *tar1*, *pnp*, *htpG*, *phbC*, and *cspA4* were downregulated only under 15% NaCl conditions (Table S5).

4 Discussion

This study focused on halophilic bacteria isolated from abandoned solar salterns, aiming to reveal adaptation strategies under hypersaline environments and designating this halophilic bacterium as a pioneering species in the field. The study utilized the halophilic bacterial strain YJPS3-2, which was found to primarily adapt to the halophilic environment by modulating the ABC transporter to maintain osmotic balance, regulating flagellar assembly, and enhancing the DNA repair pathway (Figure 6). Additionally, the strain also acted as a pioneering species, allowing other organisms to thrive by settling first in hypersaline environments and producing a variety of secondary metabolites (Figure 4).

ABC transporters, which should be initially discussed when studying the halophilic adaptive mechanism of strain YJPS3-2, use the energy of ATP hydrolysis for the uptake and efflux of solutes across the cell membrane. These transporters are crucial for halophiles, which produce or acquire compatible solutes (such as ectoine, trehalose, glycine betaine, and choline) and anions into the

cytoplasm to balance the osmotic pressure (van der Heide and Poolman, 2000; Lewis et al., 2012; Das et al., 2015). Genes related to the ABC transporter for maltose (*malK*), trehalose (*thuF*, *thuG*, and *thuK*), mannitol (*smoK*), and L-arabinose (*araG*) were upregulated in a 5% NaCl environment. However, genes associated with negatively charged ions such as phosphate (PO_4^{3-}), glutamate, and amino acids were downregulated, indicating that the bacterium avoids importing them to adapt to osmotic pressure.

Motility is a common strategy used by various types of bacteria to respond to adversity (Li et al., 1993), and it has been found that it is higher in 25% NaCl environments compared to 0% NaCl (Remonsellez et al., 2018). The bacterial flagellum functions as a motor, protein exporter, and assembly apparatus (Macnab, 2003), and is composed of an MS ring formed by the inner-membrane protein FlfF (Kubori et al., 1992), T3SS (type III secretion system), and a hook-filament junction. Chemotaxis is a mechanism by which bacteria efficiently and rapidly respond to changes in chemical composition using methyl-accepting chemotaxis proteins (MCPs) and *cheA* and *cheY* genes (Bren and Eisenbach, 2000). In response to changes, bacteria use flagella to attach to and invade targets (Bange et al., 2010), promote plant growth (Glick, 1995), and remove contaminants (Omotayo et al., 2013). In the study, genes related to flagellar assembly, including MS ring formation (*filF* and *filI*), T3SS (*flhA* and *flhK*), and attachment (*cheA*), were overexpressed, with the

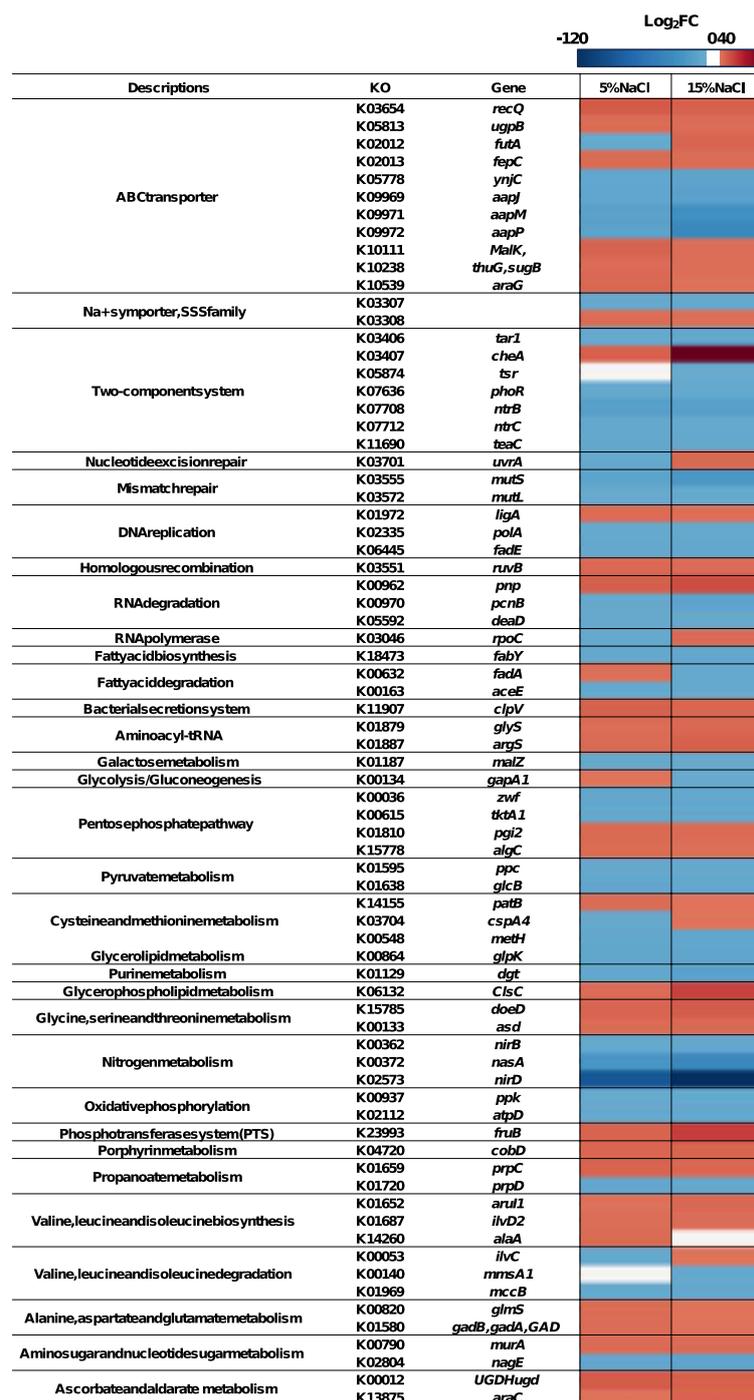


FIGURE 5 The differentially expressed genes (DEGs) of strain YJPS3-2 between 5% NaCl and 20% NaCl conditions. The red columns represent upregulated DEGs, for which the log₂FC of FPKM was higher than 2; the blue columns represent downregulated DEGs, for which log₂FC in FPKM was lower than -2. The genes without significant expression change were colored white.

most highly overexpressed gene being *cheA*, which regulates gene expression in response to changing environmental conditions (Stock et al., 1988). The *cheA* was expressed more in 15% NaCl than in the 5% NaCl conditions, which might be attributed to its role in the bacterial chemotactic system.

The overexpression of *ligA* and *ruvB*, which plays an important role in DNA processes (Alomari, 2018) and recombination, respectively, was upregulated in both conditions. Nucleotide repair-related genes in bacteria have previously been reported to be important for bacterial survival under salt stress (Mirete et al.,

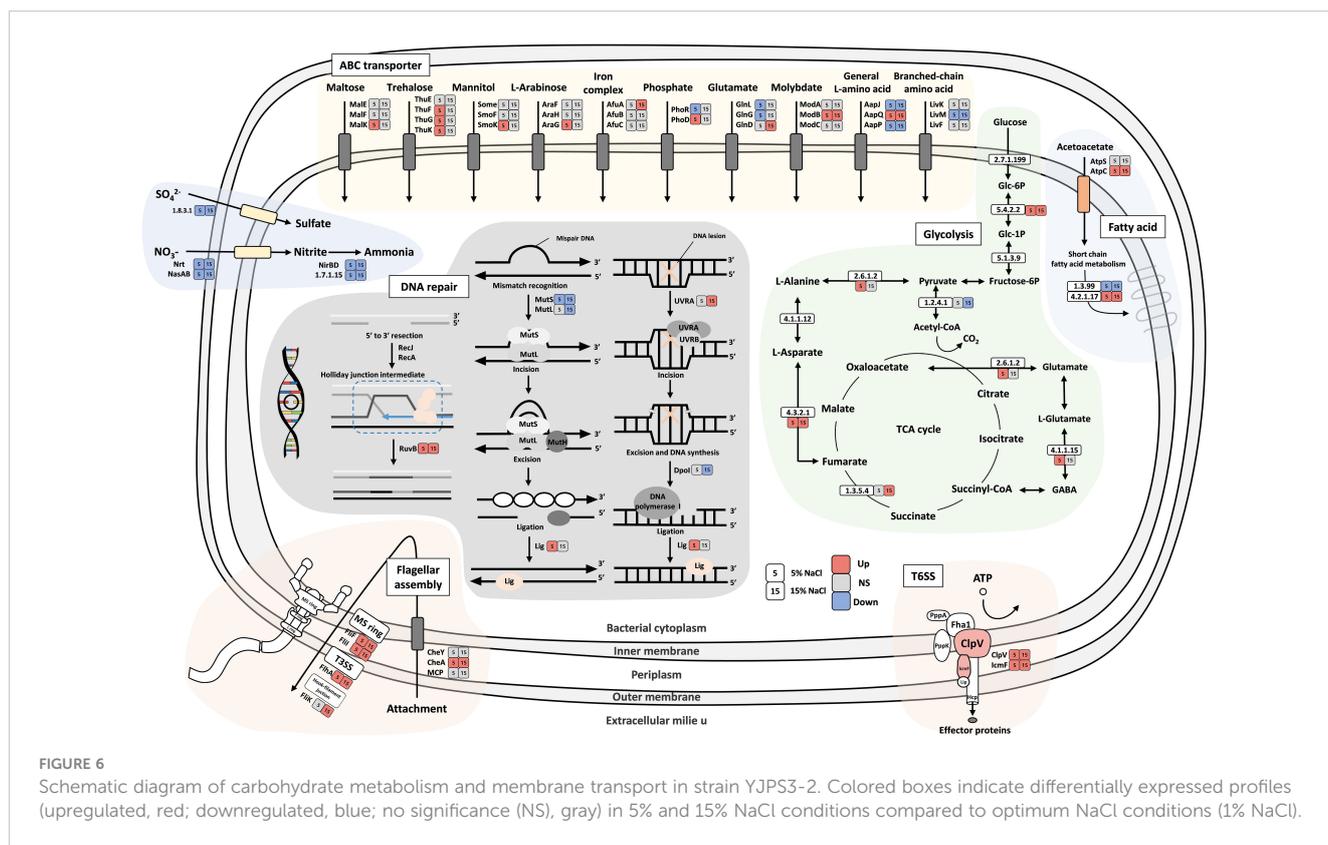


FIGURE 6 Schematic diagram of carbohydrate metabolism and membrane transport in strain YJPS3-2. Colored boxes indicate differentially expressed profiles (upregulated, red; downregulated, blue; no significance (NS), gray) in 5% and 15% NaCl conditions compared to optimum NaCl conditions (1% NaCl).

2015), and their over and under-expression is expected to be common in extreme environments (Puig et al., 2021).

In contrast to T3SS, T6SS (type VI secretion system) is a versatile mechanism that can transfer toxins to both eukaryotic and bacterial cells (Douzi et al., 2016), increasing the survival rate of bacteria by removing risk factors from their surrounding environment. T6SS component genes, namely *clpV* and *lcmF* were found to be upregulated in 5% and 15% NaCl conditions, respectively. The genus *Halomonas* has been studied for its biochemical potential, but some studies have reported its pathogenicity (Rojas et al., 2009; Tsuji et al., 2022) and potential harm to humans (Stevens et al., 2009; Kim et al., 2012). HELO_2920 gene encodes the T6SS cluster protein VasA, which is a macromolecular machine that plays an important role in the pathogenicity of Gram-negative bacteria (Wang et al., 2021) by transferring and secreting effectors (Mariano et al., 2019) and injecting a toxin into eukaryotic cells to hydrolyze the cell walls. The existence of such a potential halophilic human pathogen in abandoned salterns near tidal flats that are easily accessible to people may pose a danger.

During the process of adapting to high salinity conditions, it is crucial to investigate not only the overexpressed genes but also to genes that are downregulated or underexpressed.

In this study, we observed a substantial underexpression of genes associated with nitrogen metabolism, including *nirB*, *nirD*, and *nasA*. Notably, *nirD* exhibited the most pronounced underexpression among all the downregulated genes. This finding is consistent with previous studies reporting a decrease in nitrogen metabolism in bacteria under high salinity environments (Li et al., 2021). The observed decrease in

nitrogen metabolism suggests a strategic energy conservation strategy employed by bacteria to optimize their physiological responses to high salinity stress and allocate resources primarily to essential metabolic pathways required for their adaptation and survival.

The intriguing similarity in gene expression patterns across major pathways is noteworthy, wherein genes that were overexpressed in 5% NaCl conditions were under-expressed in 15% NaCl conditions, and vice versa. Nevertheless, some genes displayed higher expression in 15% NaCl conditions compared to the 5% NaCl conditions (Figure 5). This suggested that the genes that enable adaptation to the halophilic environment may also contribute to survival in a subsequent hypersaline environment.

Halophilic microorganisms, while adapting to hypersaline environments, produce various secondary metabolites that aid the survival of other organisms (Chung et al., 2020). The genomic analysis of strain YJPS3-2 by antiSMASH predicted the production of ectoine and siderophore, which was consistent with the transcriptome data. Ectoine helps the organism tolerate osmotic pressure (Hahn et al., 2016), while the compatible solutes produced by the bacteria are absorbed by algae and plants in a halophilic environment, enabling them to withstand osmotic stress (Rontein et al., 2002; Moghaieb et al., 2006). Moreover, siderophores, which are peptidic chelates excreted by microorganisms to uptake iron (Scavino and Pedraza, 2013), contribute to plant growth and phytosanitary protection (Compant et al., 2005; Maheshwari, 2011). Thus, halophilic strains living in an extremely halophilic environment produce various secondary metabolites that assist other organisms in surviving in saline environments, thereby promoting the recovery these areas through the bloom of algae and plant growth and the increase in biodiversity (Scavino and Pedraza,

2013). This highlights the significance of the “halophiles settlement” phenomenon.

The genus *Halomonas* is known for its ability to degrade aromatic compounds (García et al., 2005; Oie et al., 2007; Mnif et al., 2009), as well as its high tolerance to hypersaline environments. Strain YJPS3-2 also contains genes involved in the degradation of quinate, biphenyl, benzoate, p-hydroxybenzene, and generate. However, these genes were not expressed in either 5% or 15% NaCl conditions, suggesting that aromatic compounds were not supplied to the medium during transcriptomic analysis. This finding implies that this strain has the potential to act as a pioneer species in both salt and oil contamination.

In the present study, we confirmed low microbial diversity in hypersaline environments and investigated the adaptive genes of a halophilic bacterium, with a focus on its potential to serve as a pioneering species in harsh environments. Genomic and transcriptomic analysis of strain YJPS3-2 revealed that this strain adapts to halophilic environments by secreting substances that can be used by surrounding organisms, aiding in the thriving of algae and plants in hypersaline environments.

5 Conclusions

We investigated the adaptive strategies of halophilic bacteria in hypersaline environments, using a newly identified strain YJPS3-2 as a model approach. The transmembrane protein in the cell membrane seemed to play a key role in withstanding osmotic pressure and this bacterium could aid other living organisms to survive in harsh environment by adapting to a salt-stress environment. Although whole-genome sequencing analysis and transcriptomic analysis determined the gene expression levels under various environmental conditions, only known and annotated genes were interpreted. Further research would be necessary to evaluate the newly identified salt-stress-related gene and, particularly identification of the secondary metabolites. Our findings suggest YJPS3-2 would be considered as a pioneer in improving the surrounding by first settling in hypersaline areas near the coast, and elsewhere.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/[Supplementary Material](#).

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Author contributions

YY: Conceptualization, formal analysis, statistical analyses, visualization, writing – original draft, writing – review & editing. HL: Conceptualization, formal analysis, statistical analyses, visualization, writing – original draft, writing – review & editing. JL: Visualization, writing – review & editing. JK: Conceptualization, data curation, writing – review & editing, project administration. J-JK: Conceptualization, writing – review & editing, project administration, funding acquisition, supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor YH declared a past collaboration with the authors YY, J-JK, and HL.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1229444/full#supplementary-material>

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