Research Article

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Effect of sodium chloride on the expression of genes involved in the salt tolerance of *Bacillus* sp. strain "SX4" isolated from salinized greenhouse soil

Abstract: Salt stress is one of the important adverse conditions affecting bacterium growth. How bacteria isolated from greenhouse soil cope with salt stress and regulate the genes responsible for salt tolerance are still unclear. We conducted RNA transcriptome profiling of genes contributing to the salt tolerance of a *Bacillus* sp. strain ("SX4") obtained from salinized soil. Results showed that NaCl effectively regulated the growth of "SX4" in terms of cell length and colony-forming unit number decrease. A total of 121 upregulated and 346 downregulated genes were detected under salt stress with reference to the control. The largest numbers of differential expression genes were 17 in carbon metabolism, 13 in the biosynthesis of amino acids, 10 in a two-component system, and 10 in ABC transporter pathways for adapting to salt stress. Our data revealed that cation, electron and transmembrane transport, and catalytic activity play important roles in the resistance of bacterial cells to salt ions. Single-nucleotide polymorphism and the mutation of base pair T:A to C:G play potential roles in the adaptation of "SX4" to high NaCl concentrations. The findings from this study provide new insights into the molecular mechanisms of strain "SX4" and will be helpful in promoting the application of salt-tolerant bacteria.

1 Introduction

Land degradation by salts is one of the major threats to sustainable crop production in the world [1], and soil salinity is one of the most harmful conditions not only in field agriculture production but also in greenhouse cultivation [2,3]. Despite that biotic and abiotic environmental factors greatly affect plant growth and yield, salt stress is still considered one of the major stress factors that seriously inhibit plant growth and microbe survival [4]. Salinized soil is widely distributed in the world and hinders the crop growth and productivity [5]. Approximately one billion hectares of salt-affected soils are scattered all over the world [6], and approximately 100 million hm^2 saline-alkali soils are distributed in China [7]. In recent years, owing to rapid developments in protected vegetable cultivation in China, a large number of chemical fertilizers and pesticides have been used. Despite the high yields and benefits, diseases affecting vegetable crops have increased and seriously degraded soil quality [8]. Currently, the proportions of secondary salinization of soil in protected vegetable fields are extensively high, and the problem of soil salinization has become one of the main obstacles to vegetable production [9].

The total salt content of greenhouse vegetable soil is much higher than that in open field conditions because of the excessive application of chemical fertilizers, which are the main causes of secondary soil salinization in greenhouse vegetable fields [10,11]. Soil salinization significantly reduces the diversity and abundance of microbial communities. However, soil enzyme activity is strongly related to soil microbial biomass and community abundance [12]. Therefore, soil microorganisms, including phosphate solubilizing bacteria (PSB), can be used as a biological indicator for salinized soil quality restoration and evaluation. Salt and nutrient accumulation and secondary salinization are common in

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greenhouse vegetable soil [13]. Salt ion composition related to secondary salinization soil in vegetable soil includes eight major ions (HCO_3^-, Cl^-, SO_4^{2-}, NO_3^-, Ca^{2+}, K^+, Mg^{2+}, and Na^+) [10]. The high content of soluble salts in greenhouse soil not only has become the main limiting factor for vegetable production [10] but also affects the distribution of bacterial communities [14].

Dissolved organic matter (DOM) is an important component of soil organic matter [15,16]. More importantly, ions such as Na^+, Ca^{2+}, K^+, and Mg^{2+}, the important inorganic components of DOM [17], facilitate the accumulation of ions in soil. The effects of salt stress on plant or microbe growth are ion toxicity by sodium or chloride and imbalanced nutrition [18,19]. Increase in Na^+ and Mg^{2+} ions cause structural damage to plant or microbes' cells [20]. Despite that the ability of plants to tolerate salt stress can be regulated by multiple biochemical pathways, a large amount of soil salt can cause toxic intermediate products and result in the weakening of crop or bacteria cell metabolism [21,22]. Both plants and microorganisms have to adapt to salt stress and form their own unique way to survive in saline soils.

The expression of salt-tolerant genes in bacteria effectively improves the chance of bacteria to survive. For example, an *Azospirillum* strain obtained from saline soil exhibits high salt tolerance [23]. Therefore, an in-depth study of the regulation of salt on bacterial genes is useful in improving the application of beneficial bacteria because salt-adapted strains may have higher potential to alleviate saline stress [23,24]. Beneficial bacteria in the soil can survive under high salt conditions and potentially promote plant growth under salt stress. In our previous study, a large number of salt-tolerant bacterial strains were isolated from salinized vegetable soils [25], and some of them showed an ability to dissolve phosphorus and fix nitrogen. The purpose of this study was to analyze the expression and regulation of the salt tolerance gene of strain "SX4" by NaCl. Our findings will be conducive to the application of beneficial bacteria and soil improvement.

2 Materials and methods

2.1 Bacterial strain and cultivation

Bacillus sp. strain "SX4" with accession number MF431750 was previously isolated from salinized greenhouse soil [25]. The salt tolerance of "SX4" was determined. A nutrient agar plate with yeast extract, 5.0 g L^{-1}; peptone, 10.0 g L^{-1}, agar, 15.0 g L^{-1} for solid, and 10% NaCl at pH 6.9–7.1. The survival of *Bacillus* sp. "SX4" at various salt concentrations (up to 20%) was examined using a subculture on a nutrient liquid medium at 25 ± 2°C for 3 days. Cells in a nutrient solid agar plate were counted. The individual morphology of bacteria was visualized with a light microscope. Fresh cells were stored with 40% glycerol at −80°C for subsequent analyses.

2.2 Induction of salt tolerance and collection of bacteria

Two kinds of culture methods were used in activating bacterial cultures in solid media. The media containing salt (10%) and that without salt (SX4.CK) were used. Initially, the concentrations of the bacteria were adjusted to 10^8 colony-forming units (CFU) mL^{-1}, and the bacteria were grown on an agar plate for 3 days at 25°C. Then, strain growth was observed. The growing bacteria were collected and transferred to a 1.5 mL tube, frozen with liquid nitrogen, and stored at −80°C until further analyses.

2.3 RNA extraction

Fresh "SX4" cells from two treatments were used in preparing RNA. Total RNA was extracted from the cells with TRIzol® Reagent (Invitrogen, Shanghai, China) according to the manufacturer's instructions. Genomic DNA was carefully removed using DNase I (TaKara, Dalian, China). RNA purity was determined using ND 2000 (NanoDrop Technologies, Wilmington, USA). The obtained high-quality RNA samples were selected according to the following criteria: OD260/280 = 1.8–2.2, OD260/230 ≥ 2.0, RIN ≥ 6.5, and 28S:18S ≥ 1.0. Finally, the purified RNA was used in constructing a sequencing library.

2.4 Library preparation and Illumina HiSeq sequencing

RNA-seq strand-specific libraries were prepared according to the procedures in TruSeq RNA sample preparation kit (Illumina Inc., San Diego, USA). RNA (5 µg) was used. rRNA was removed using a RiboZero rRNA removal kit

DE GRUYTER

Genes in *Bacillus* sp. "SX4" respond to NaCl ━━ **11**

(Illumina Inc., San Diego, USA) and fragmented using a fragmentation buffer. cDNA synthesis, end repair, and the A-base addition and ligation of Illumina-indexed adaptors were conducted according to Illumina's protocols. Libraries were selected and constructed on the basis of the sizes of cDNA target fragments (200–300 bp) on 2% low range ultra agarose, then amplified through PCR with Phusion DNA polymerase (NEB, Ipswich, USA) for 15 PCR cycles. All the samples were quantified using TBS-380 (Fluorometer, Turner Biosystems, USA). Then, paired-end libraries were sequenced by Biozeron Biotechnology Co., Ltd (Shanghai, China) with the Illumina HiSeq PE 2 × 151 bp read length (Illumina Inc., San Diego, USA).

2.5 Read quality control and mapping

Raw sequenced and paired-end reads were trimmed, and the quality of raw data was controlled according to the criteria. Trimmomatic software with default parameters (http://www.usadellab.org/cms/?page=trimmomatic) was used. Then, clean reads were separately aligned to a referenced genome with orientation mode with Rockhopper (http://cs.wellesley.edu/~btjaden/Rockhopper/). This software is a comprehensive and user-friendly system for the computational analysis of bacterial RNA-seq data. It is commonly used in calculating gene expression levels at default parameters.

2.6 Differential expression analysis and functional enrichment

For the identification of differential expression genes (DEGs) between the two different treatments, the expression level of each transcript was calculated using the fragments per kilobase of read per million mapped reads (RPKM) method. edgeR (https://bioconductor.org/packages/release/bioc/html/edgeR.html) was used for analyzing the DEG level. DEGs between the two treatments were selected using the following criteria: the logarithmic fold change was higher than 2, and false discovery rate (FDR) was less than 0.05. The functions of the DEGs were explored through gene ontology (GO) (https://github.com/tanghaibao/goatools) functional enrichment and kyoto encyclopedia of genes and genomes (KEGG) pathway analysis with Goatools (https://github.com/tanghaibao/Goatools) and KOBAS (http://kobas.cbi.pku.edu.cn/kobas3). Enrichment analysis

was carried out using Fisher's accurate test ($P < 0.05$). False positive rate was reduced by using four multiple test methods (Bonferroni, Holm, Sidak, and FDR) to correct the P value. When the Bonferroni-corrected P value was less than 0.05, DEGs were considered significantly enriched in GO terms and metabolic pathways. Generally, when the P value is less than or equal to 0.05, a GO function is considered significantly enriched. For the analysis of the effect of salt on gene replication, software applications, such as Samtools (http://samtools.sourceforge.net/) and GATK (https://www.broadinstitute.org/gatk/), are used in analyzing the number of candidate single-nucleotide polymorphisms (SNPs).
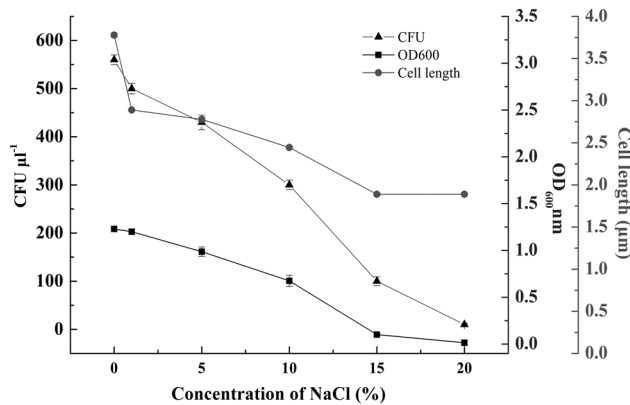
2.7 Statistical analysis

The data in the present study were all subjected to analysis of variance. Meanwhile, the mean values were separated with Fisher's protected least significant difference test, which was performed using SPSS (version 19.0) and Origin version 8.0. Differences with P values of ≤0.05 level were considered significant.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Cell growth under salt stress and sequenced results

Owing to increase in salt concentration, the value of OD_{600nm} and the bacterial number of CFU decreased. The length of "SX4" decreased (Figure 1). The total bases ranged from 1,37,96,68,977 to 1,99,38,78,719 bp, and the error of all samples was less than 0.015%. The Q30 value ranged from 94.28 to 96.66%, and the proportion of GC ranged from 41.98 to 43.85% (Table S1). The six samples showed high saturation (Figure 2), and most of the genes with more than moderate expression amount (FPKM values of genes were above 3.5) were close to saturation (the vertical axis value was close to 1) compared with 40% of sequenced reads, indicating that the overall quality of saturation was high, and the sequencing amount can cover most of the expressed genes (Figure 2).
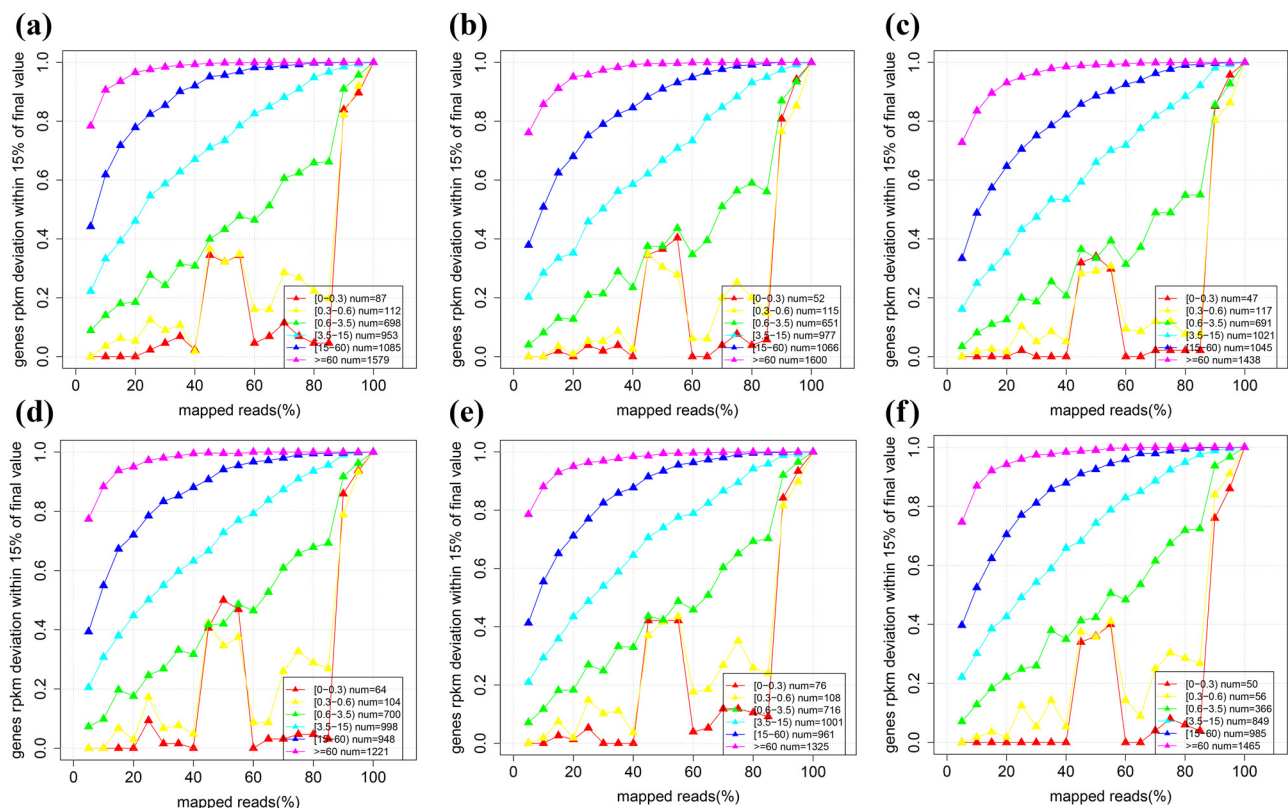
Figure 1: Growth condition of the bacterial strain SX4 under NaCl stress.

3.2 GO annotation of differential gene

A total of 121 upregulated genes and 346 downregulated genes (Figure 3a) were obtained in the scatter plot. By analyzing Pearson correlation coefficient, the results show that the samples with the same processing were clustered together and the clustering degree was relatively high

(Figure 3b). In biological processes, upregulated and downregulated genes were involved in cellular component organization or biogenesis (↑2, ↓2), cellular process (↑17, ↓64), localization (↑1, ↓18), growth (↑8, ↓1), metabolic process (↑30, ↓64), and response to stimulus (↑9, ↓13). Only downregulated genes were involved in biological regulation (↓6), detoxification (↓1), developmental process (↓2), locomotion (↓4), multi-organism process (↓2), reproduction (↓1), and signaling (↓2) (Figure 4 and Table 1).

In cellular components, upregulated and downregulated genes were involved in the membrane (↑16, ↓50), extracellular region part (↑1, ↓1), organelle part (↑1, ↓1), organelle (↑2, ↓2), cell part (GO:0044464, ↑33, ↓74), cell (GO:0005623, ↑33, ↓74), protein-containing complex (↑4, ↓10), and extracellular region (↑3, ↓6). Only downregulated genes were involved in the membrane part (↓7) (Figure 4 and Table 1). In molecular functions, upregulated and downregulated genes were involved in binding (↑5, ↓11) and catalytic activity (↑16, ↓33). Only downregulated genes were involved in transporter activity (↓15), molecular transducer activity (↓1), transcription regulator activity (↓1), and structural molecule activity (↓1) (Figure 4 and Table 1).

Figure 2: Trend lines of sequenced saturation curve. (a–f) represent SX4-CK-1, SX4-CK-2, SX4-CK-3, SX4-1, SX4-2, and SX4-3, respectively.
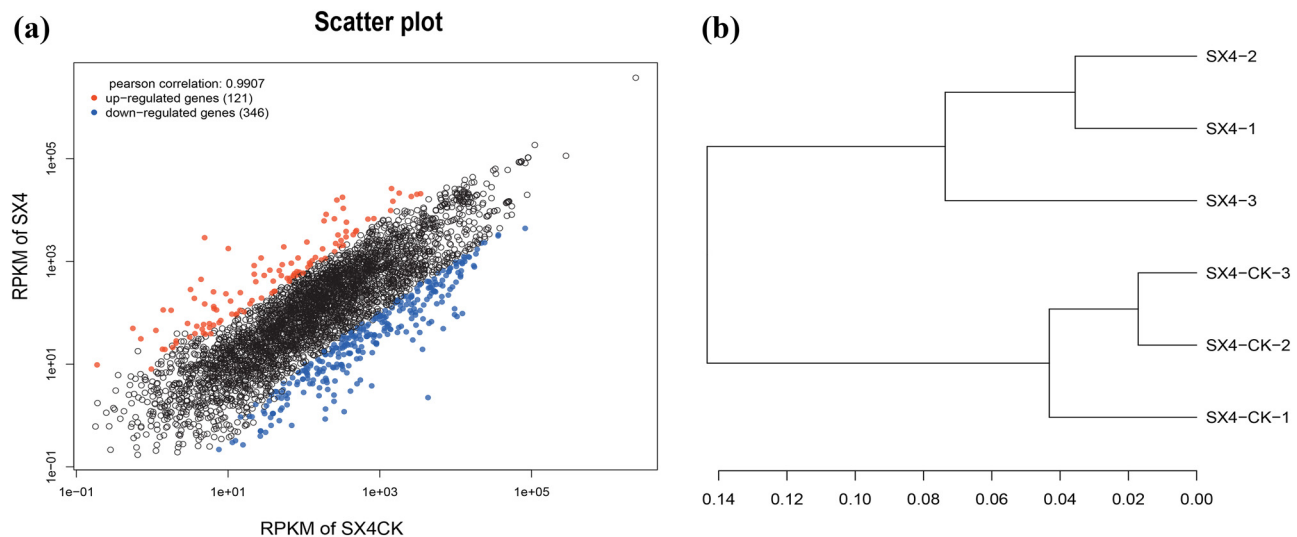
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Genes in *Bacillus* sp. "SX4" respond to NaCl — 13

(a)

(b)

Figure 3: Scatter plot (a) and hierarchical cluster analysis (b).

3.3 Pathways enriched in GO and KEGG analysis

The top GO terms were enriched in proton transmembrane transport (GO:1902600), cytochrome complex (GO:0070069), aerobic electron transport chain (GO:0019646), and cytochrome or ubiquinol oxidase complex (GO:0009319). In this study, GO terms were also enriched in oxidoreduction-driven active transmembrane transporter activity (GO:0015453) and aerobic respiration (GO:0009060). Moreover, cation transmembrane transport (GO:0098655), electron transport coupled with proton transport (GO:0015990), cytochrome bo3 ubiquinol oxidase activity (GO:0009486), energy coupled proton transmembrane transport, and against electrochemical gradient (GO:0015988) were enriched under salt treatment (Figure 5).

The top KEGG pathways were enriched in carbon metabolism (ko01200), citrate cycle (ko00020), pentose phosphate pathway (ko00030), glycolysis/gluconeogenesis (ko00010), arginine and proline metabolism (ko00330), and oxidative phosphorylation (ko00190). Other pathways, such as pantothenate and CoA biosynthesis (ko00770), bacterial chemotaxis (ko02030), carbon fixation in photosynthetic organisms (ko00710), and ascorbate and aldarate metabolism (ko00053), were also enriched (Figure 6). Our results showed that the largest numbers of DEGs in metabolic pathways were 17 in carbon metabolism (ko01200), 13 in the biosynthesis of amino acids (ko01230), 10 in a two-component system (ko02020), and 10 in ABC transporter (ko02010) pathways.
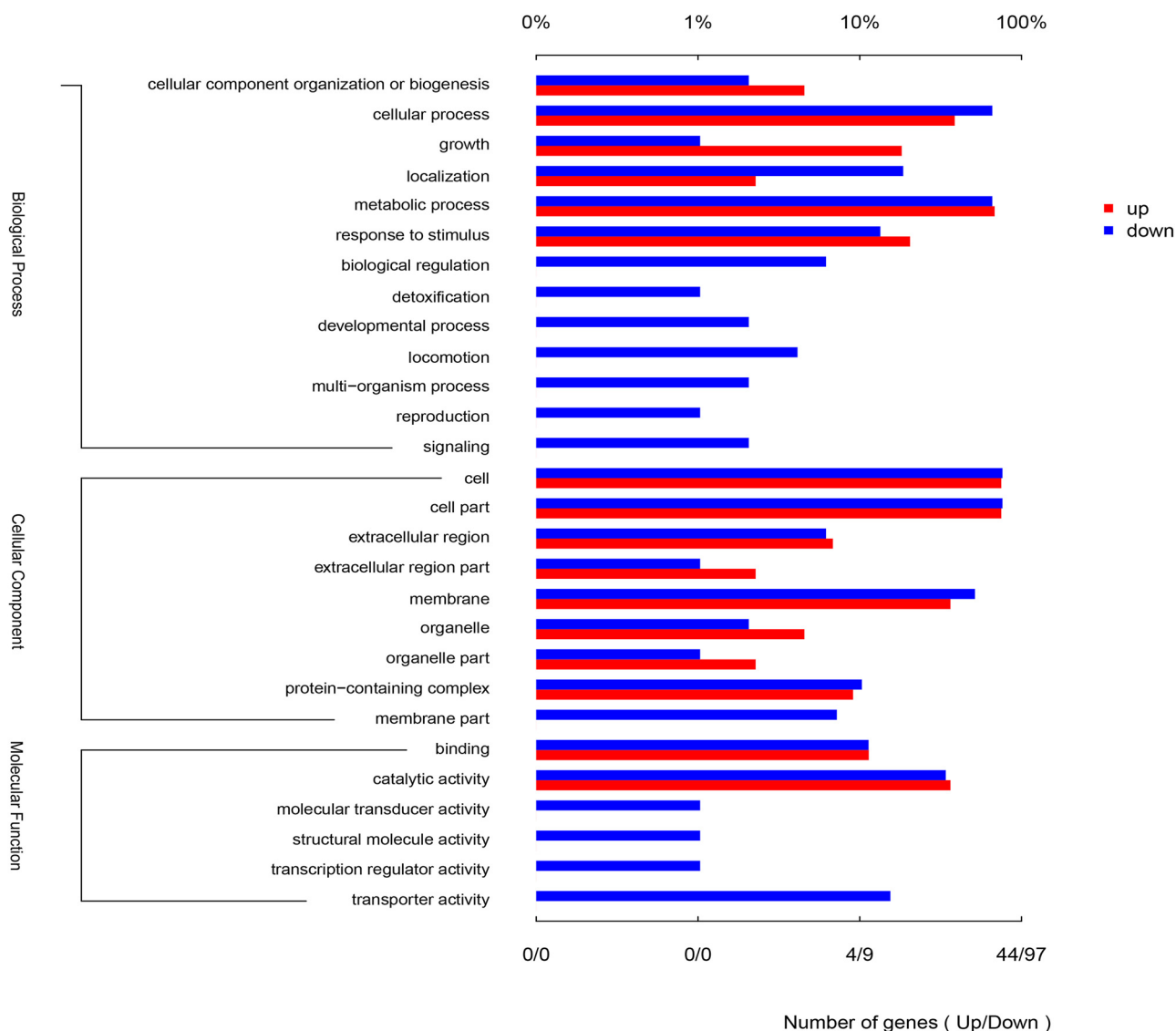
3.4 Clustering heatmap and SNP analysis

The results from the clustering heatmap analysis of DEGs showed that most of the genes that are unregulated under salt free conditions (SX4.CK) were downregulated under salt stress (Figure 7). Through the analysis of branches, a changing trend of genes was detected. In subcluster 1, the expression trend of 329 genes decreased under salt stress. In subcluster 2, 116 genes were upregulated under salt induction (Figure 8). The number of SNPs and indels under salt stress was significantly higher than that in CK treatment (Table 2). Salt stress affected the replication of genes in the bacterial strain "SX4" and resulted in the deletion of gene loci. Among the base mutation types, the highest number was detected from T:A to C:G, followed by that from C:G to T:A (Table S2).

4 Discussion

4.1 Cell growth of strain "SX4" under salt stress

Soil microorganisms including bacteria play pivotal roles in various types of soils due to the mineralization of organic matter, providing available nutrients for plant growth [26]. However, salt and ions, such as Cl^-, SO_4^{2-}, Mg^{2+}, and Na^+, can adversely affect plant and bacterium growth and development due to the induction of ion toxicity [27]. Bacteria have to regulate various genes and

Figure 4: Chart summary of GO enrichment analysis under NaCl treatment. Three categories, namely, biological processes, cellular components, and molecular functions, are shown.

pathways to survive under salt stress, which provides important insight into how soil bacteria can be applied [28,29]. Our data showed that not only the cell number but also the cell length of "SX4" decreased under higher salt conditions (Figure 1). Salinity-tolerant soil microbes, such as bacteria or fungi, offset osmotic stress by synthesizing osmolytes, which maintain cell metabolism [26]. *Bacillus* sp. decreases cell length under salt stress and adapts to salt stress in its natural habitats by undergoing alterations, which has enabled it to survive in saline water in the absence of nutrients [30,31]. However, *Bacillus* sp. is a common species that can tolerate salt, drought, and other inconvenient conditions [32–34]. In this study, strain "SX4," which exhibits a remarkable

ability to tolerate salt stress [25], was isolated from greenhouse salinized soil. Along with salt ions increase in the soil, strain "SX4" regulates different genes to change cell shape or metabolic pathway to increase its capacity for salt adaptation.

A total of 121 upregulated genes and 346 downregulated genes were marked as significant regulated genes under salt treatment (Figure 3a). Our data suggested that "SX4" downregulates more genes under salt stress. GO-term enrichment analysis was performed for comparing abundances in the dataset [35]. We found that in biological processes, the largest number of changed genes was located in metabolic process and cellular process. Most genes in cellular component term were regulated,
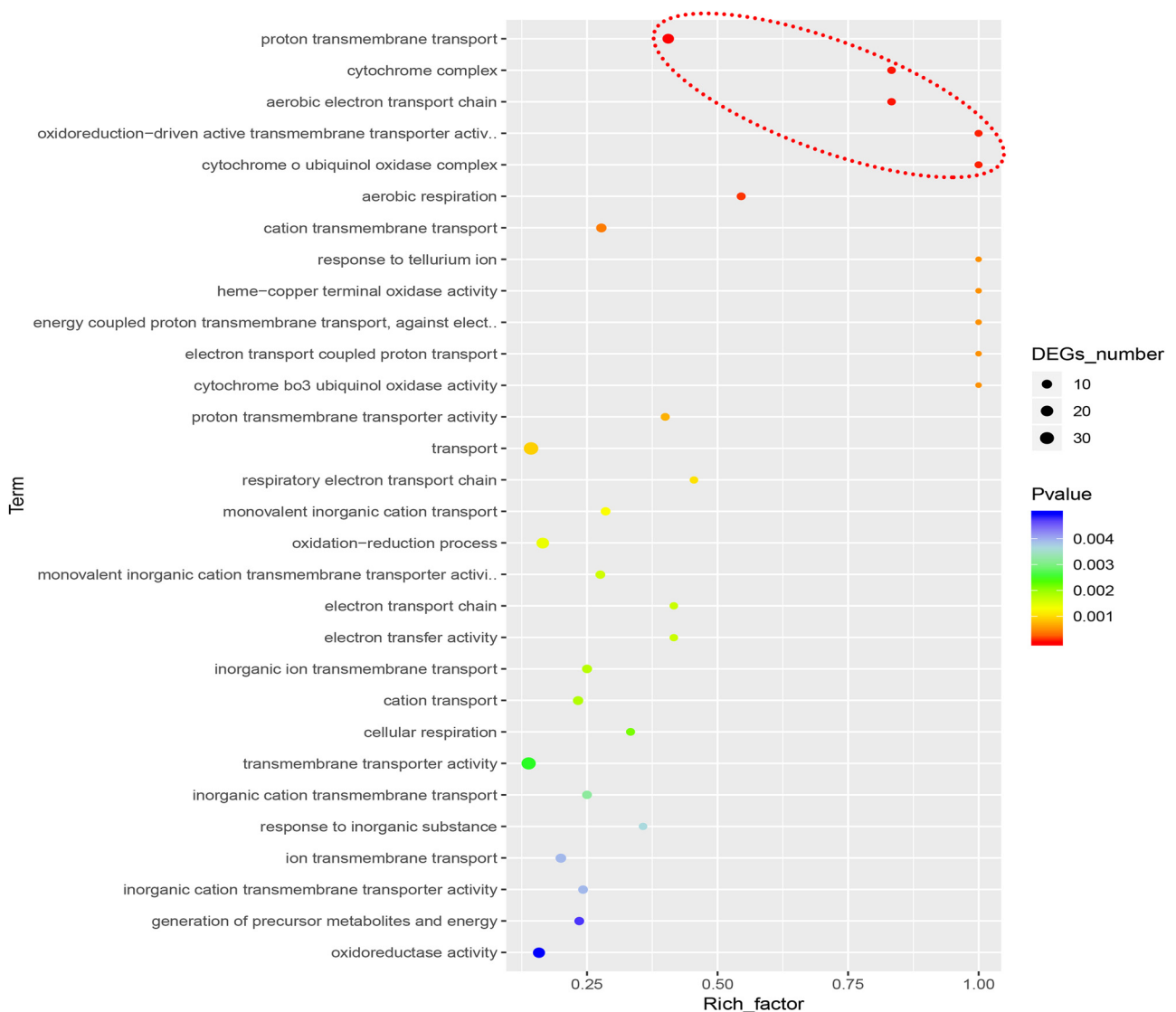
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Genes in *Bacillus* sp. "SX4" respond to NaCl — **15**

Table 1: GO functional analysis in three groups: control (SX4-CK) vs salt-treated (SX4) GO enrichment

GO group namespace	Description	Up/down	Number	Id
Biological process	Biological regulation	Down	6	GO:0065007
	Cellular component organization or biogenesis	Up	2	GO:0071840
		Down	2	
	Cellular process	Up	17	GO:0009987
		Down	64	
	Detoxification	Down	1	GO:0098754
	Developmental process	Down	2	GO:0032502
	Growth	Up	8	GO:0040007
		Down	1	
	Localization	Up	1	GO:0051179
		Down	18	
	Locomotion	Down	4	GO:0040011
	Metabolic process	Up	30	GO:0008152
		Down	64	
	Multi-organism process	Down	2	GO:0051704
	Reproduction	Down	1	GO:0000003
	Response to stimulus	Up	9	GO:0050896
		Down	13	
	Signaling	Down	2	GO:0023052
Cellular component	Protein-containing complex	Up	4	GO:0032991
		Down	10	
	Organelle part	Up	1	GO:0044422
		Down	1	
	Organelle	Up	2	GO:0043226
		Down	2	
	Membrane part	Down	7	GO:0044425
	Membrane	Up	16	GO:0016020
		Down	50	
	Extracellular region part	Up	1	GO:0044421
		Down	1	
	Extracellular region	Up	3	GO:0005576
		Down	6	
	Cell part	Up	33	GO:0044464
		Down	74	
	Cell	Up	33	GO:0005623
		Down	74	
Molecular function	Binding	Up	5	GO:0005488
		Down	11	
	Catalytic activity	Up	16	GO:0003824
		Down	33	
	Molecular transducer activity	Down	1	GO:0060089
	Structural molecule activity	Down	1	GO:0005198
	Transcription regulator activity	Down	1	GO:0140110
	Transporter activity	Down	15	GO:0005215

especially in the cell part. Only one group was marked as downregulated genes and was involved in the membrane part (↓7) (Figure 4 and Table 1). Our data revealed that bacteria regulate genes during cellular and metabolic processes to cope with salt stress, suggesting that these processes are important to the resistance of the bacterial strain "SX4" to salt stress. Bacteria can not only alter plant metabolic response to salt stress but also reduce

metabolic load and increase nutrient availability to living cells [36,37]. Our data also showed that genes involved in metabolic or cellular processes support the survival of "SX4" under high NaCl concentrations.

In GO terms of molecular function, two groups were marked as having upregulated and downregulated genes that are involved in catalytic activity and binding. Salt stress mainly affects cellular functions, such as catalytic

Figure 5: Top GO-enriched pathways. A large rich factor indicates a high degree of enrichment.
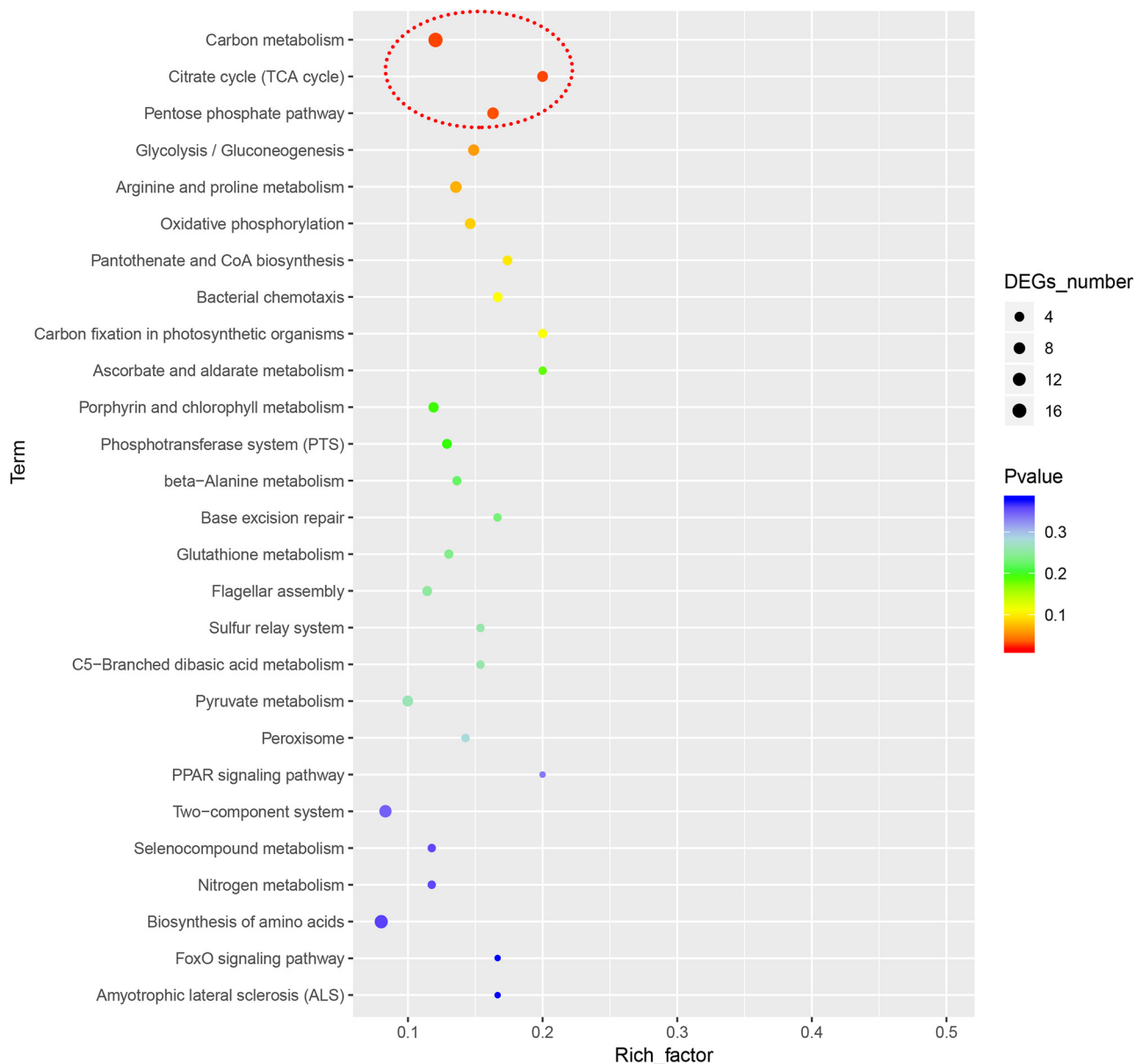
activity, in the leaves and roots of maize [38]. Similar to this conclusion, our data showed that salt ions exhibit the same effect on molecular functions, such as binding and catalytic activity in bacteria. Moreover, that salt bridge related to a single inter-subunit can affect catalytic activity in a bacterial quinone reductase [39], suggesting that catalytic activity is important to bacterial cells related to salt molecules. However, in this study, we found that only four groups were marked as downregulated genes and were involved in transporter, molecular transducer, transcription regulation, and structural molecule activity (Figure 4 and Table 1). Our data suggested that the four processes are negatively regulated under slat stress. Beneficial bacteria can ameliorate salt stress by accumulating osmolytes in their cytoplasm [40], which may regulate

genes involved in the activities of transporters and structural molecules.

4.2 Pathways enriched in GO and KEGG analysis

The top GO terms were located, and we found that strain "SX4" regulates genes involved in proton transmembrane transport (GO:1902600), cytochrome complex (GO: 0070069), and aerobic electron transport chain (GO: 0019646). The aerobic electron transport chain is essential in bacteria, such as *Mycobacterium smegmatis*, which can terminate one of three possible terminal oxidase

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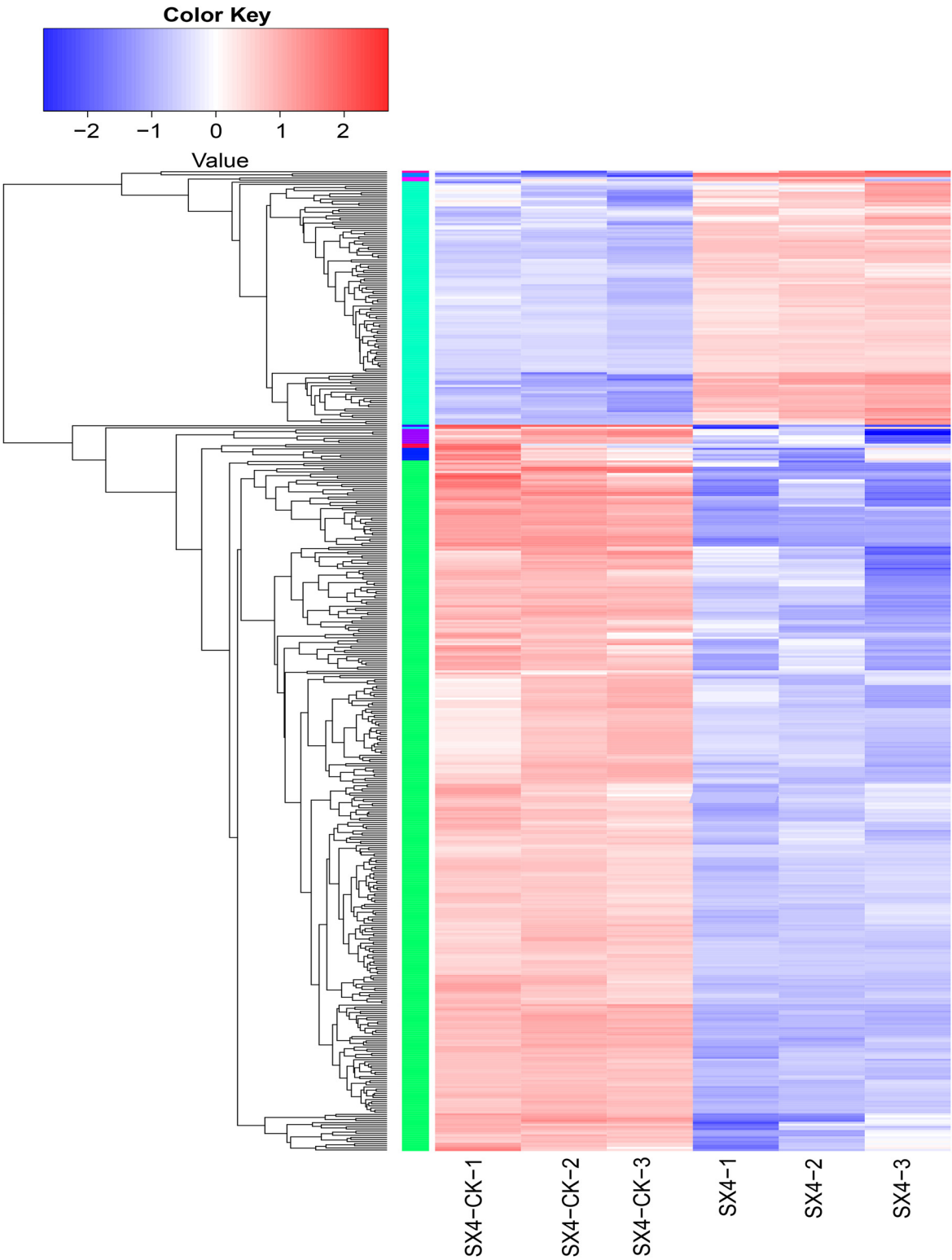Genes in *Bacillus* sp. "SX4" respond to NaCl — **17**

Figure 6: Top pathways enriched in KEGG analysis. A large rich factor indicates a high degree of enrichment.

complexes [41]. Our data revealed that the located GO terms are important to the tolerance of bacteria to salt stress. The movement of ions across the cell plasma membrane and organelle membranes is mainly mediated by several transport proteins [42,43]. However, in this study, other processes, such as cytochrome or ubiquinol oxidase complex (GO:0009319) and oxidoreduction-driven active transmembrane transporter activity (GO:0015453), were marked in "SX4" (Figure 5), suggesting that these processes contribute to resistance against salt stress. Cation transmembrane (GO:0098655), electron transport coupled proton (GO:0015990), energy coupled proton transmembrane, and against electrochemical gradient (GO:

0015988) were also located in "SX4" (Figure 5). Most GO groups in "SX4" are related to ion regulation and transmembrane, indicating that "SX4" regulates most genes to reform its cell membrane system in order to protect itself from Na^+/Cl^- toxicity. Furthermore, cation, electron, and transmembrane transport are important not only to bacteria but also to plant cells, and cation/proton exchangers are antiporters energized by a proton gradient [44,45].
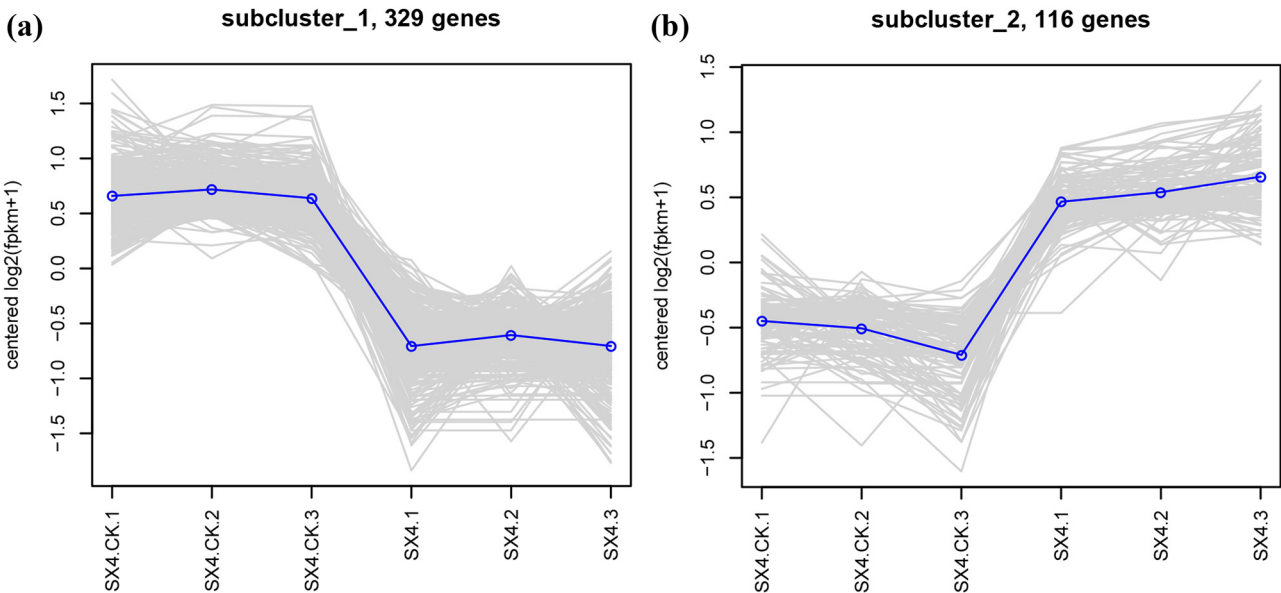
KEGG is considered a knowledge base for systematically analyzing gene functions in terms of the networks of genes [46]. Our data showed that strain "SX4" regulated carbon metabolism (ko01200), citrate cycle (TCA cycle; ko00020), pentose phosphate pathway (ko00030),

Figure 7: Clustering heatmap analysis of differentially expressed genes.

glycolysis/gluconeogenesis (ko00010), and arginine and proline metabolism (ko00330). *Lactobacillus paralimentarius* and *Lactobacillus alimentarius*, which are commonly used in industrial product named "paocai," are involved in numerous metabolic pathways, such as glycolysis/gluconeogenesis (ko00010) [47], indicating that the bacteria

DE GRUYTER

Genes in *Bacillus* sp. "SX4" respond to NaCl — **19**

(a)

(b)

Figure 8: SX4.CK vs SX4 differentially expressed gene subcluster trend lines. A total of 329 genes were analyzed by Branch 1 (a), and 116 genes were analyzed by Branch 2 (b).

regulate this pathway under adverse conditions. Moreover, arginine and proline metabolism are enriched in early molecular events associated with the shortage of nitrogen in rice seedling roots [48].

KEGG pathway analysis of salt-induced genes revealed that oxidative phosphorylation (ko00190), pantothenate and CoA biosynthesis (ko00770), bacterial chemotaxis (ko02030), carbon fixation in photosynthetic organisms (ko00710), and ascorbate and aldarate metabolism (ko00053) were enriched (Figure 6). Bacteria can sense chemicals and modulate swimming behavior to migrate to favorable environments through bacterial chemotaxis, which is involved in signal transduction [49]. The largest numbers of marked DEGs were 17 in carbon metabolism (ko01200), 13 in the biosynthesis of amino acids (ko01230), 10 in a two-component system (ko02020), and 10 in ABC transporters (ko02010) to adapt to adverse soil environments, suggesting that these pathways are the most

enriched under salt stress and play potential roles in adaptation to ion toxicity. Carbon metabolism is regulated in the roots of sugar beet plants under salt stress [50]. The biosynthesis of amino acids has been considered essential in plants [51], and this pathway also influences the response of "SX4" to salt ions. In cucumber plants, *Enterobacter* sp. can induce the regulation of amino acids and thereby improve salt tolerance [52]. A two-component system is vital not only to plants but also to bacteria, and ABC-type transporters play important roles in bacterial resistance [53–55]. Our data suggested that the aforementioned pathways were related to adapt to salt ions in *Bacillus* sp., which make this genus show potential ability to survive in the saline soils.

4.3 Clustering heatmap and SNP analysis

Salt has a significant effect on the gene expression of "SX4," and most genes under salt-free conditions were upregulated (Figure 7). Although a lower number of genes were upregulated, our data suggested that the upregulated genes are strongly associated with the ability to cope with salt stress. Our data showed that not only the number of SNPs but also that of the indel was significantly higher than that of CK treatment under salt stress (Table 2). Presently, the use of SNPs in microbiological fields has shown some merits for diagnosing bacteria with high homology of their DNA [56]. The adaptation

Table 2: Analysis of SNP in two treatments (SX4 and SX4.CK)

Type	SX4-1	SX4-2	SX4-3	SX4-CK-1	SX4-CK-2	SX4-CK-3
All-snp	4,204	2,890	4,582	1,601	1,541	1,414
hom	589	720	940	800	661	484
het	3,615	2,170	3,642	801	880	930
All-indel	233	176	231	95	121	122
Deletion	112	78	133	49	41	38
Insertion	121	98	98	46	80	84

of *Lactococcus lactis* to high temperatures leads to SNP mutations during cell growth [57]. Our data revealed that more SNP and indel were induced under salt conditions. Furthermore, salt stress regulates the deletion of gene loci. The highest mutation number was detected in replacement of T:A with C:G, followed by C:G with T:A (Table S2), suggesting that the mutation of the base pair T:A to C:G is preferred by "SX4" under salt stress. Bacteria exhibit a wide degree of variations in their overall G/C content and the mutation of C:G to T:A is not easily preferred by bacteria [58]. Although gene mutations or deletions were specifically detected in bacterial survival strategies for salt stress in the current study, the mechanism of these mutations, especially those related to the improvement of the ability of "SX4" to adapt to salt stress, should be further explored. Overall, current progress in this work conveys that future research is still needed to provide insights into strategies for improving not only the quality of greenhouse soil but also the application of salt-tolerant bacteria.

5 Conclusions

The RNA sequencing analysis of bacteria responding to salt facilitates the study of the mechanisms underlying the adaptation of microbes to salt stress and the formulation of potential solutions using salt-tolerant beneficial bacteria for future agricultural plant production. Our results suggested that NaCl effectively regulated the growth of "SX4," particularly in terms of cell length. The OD_{600nm} value and number of bacterial CFU decreased. A total of 121 upregulated genes and 346 downregulated genes were detected under salt stress. The top GO terms were involved in proton transmembrane transport, cytochrome complex, aerobic electron transport chain, and electron transport coupled proton transport. The top KEGG pathways were involved in carbon metabolism, citrate cycle, pentose phosphate pathway, glycolysis/gluconeogenesis, and arginine and proline metabolism. Carbon metabolism, the biosynthesis of amino acids, two-component system, and ABC transporter pathways for adapting to adverse soil environments had the largest numbers of marked metabolic pathways. In addition, the mutation of the base pair T:A to C:G plays a potential role in the adaptation of "SX4" to high salinity. These findings provide a basis for understanding the molecular mechanisms underlying the response of the salt-tolerant strain "SX4" to adverse greenhouse soil conditions.

Abbreviations

PSB	phosphate-solubilizing bacteria
CFU	colony-forming units
FDR	false discovery rate
DEGs	differential expression genes
DOM	dissolved organic matter
GO	gene ontology
KEGG	kyoto encyclopedia of genes and genomes
FPKM	fragments per kilobase of transcript per million fragments mapped
SNP	single-nucleotide polymorphism

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Genes in *Bacillus* sp. "SX4" respond to NaCl — **21**

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