Genome-wide identification and expression pattern of sugar transporter genes in salt tolerant fungus Aspergillus sydowii H-1

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Abstract

Aspergillus sydowii is a kind of fungus with rich metabolic capacity, halophilic and can cause coral disease. In this paper, 173 sugar transporter genes in *A. sydowii* H-1 genome were identified and divided into 9 subgroups by bioinformatics method. The transcription levels of these genes were analyzed under different fermentation time and different salt concentration. Combined with protein interaction network analysis, we identified that a glycerol transporter AsSTL1 gene interacted with five MAPK cascade genes: HOG, SSK22, STE11, PBS2 and FUS3. The abundant sugar transporter genes may be an important condition for *A. sydowii* to survive in extreme marine environment, and also indicate that these sugar transporters respond to low carbon source stress caused by prolonged fermentation cycle and salt stress.

1. Introduction

Aspergillus sydowii is a filamentous fungus with strong environmental adaptability and wide distribution. It has been reported from the sea below 4450 meters[1], land plants[2], animal skin and respiratory tract, humus soil to the cold Antarctic region[3]. In recent years, it has been reported that *A. sydowii* produces quinazolone, laccase, cellulose degrading enzyme and other abundant secondary metabolites and enzymes[4–7] and *A. sydowii* of marine origin is pathogenic to coral[8–11]. In addition, this species is called halophilic fungus because of its salt tolerance[12, 13].

Carbohydrates such as glucose, sucrose, fructose, etc. can be used as energy substances or signal molecules, but they can't directly cross the membrane. They need sugar transporters (STs) to transport them from outside the cell membrane to inside the membrane. The STs family is a member of the major facilitator superfamily (MFS), and generally has 12 α transmembrane helix [14]. Sugar transport is the first step of sugar metabolism, which is of great significance for the fermentation of industrial microorganisms. Overexpression of HXT1 in *Saccharomyces cerevisiae* can increase the yield of ethanol[15]. However, overexpression of pentose transporter could accelerate the fermentation of mixed sugars[16]. STs are not only an important way for cells to obtain energy, but also help to resist abiotic stress. Under high salt stress, Apple could enhance the expression of MDSUT2.2 through protein phosphorylation to improve salt tolerance, and the salt tolerance of MDSUT2.2 transgenic plants was enhanced[17]. It has been reported that sugar transporter STL1 controls glycerol uptake by yeast and plays an important role in physiological processes such as cell osmotic pressure regulation and glycerol catabolism [49] In *S.cerevisiae*, the expression of STL1 is inhibited by glucose, but HOG1 will activate the expression of STL1 under hypertonic conditions[18, 19].

The research on yeast related STs is relatively clear, but there is still a lack of systematic research on filamentous fungi. With the progress of sequencing technology, we can understand and mine more STs through genomics and transcriptomics, so as to provide a theoretical basis for later experimental verification. In this study, we excavated the STs in the *A. sydowii* H-1 genome and preliminarily obtained the possible functions according to the phylogenetic relationship. Through the analysis of the expression

of STs in different fermentation periods (Fermentation for 2d and 8d) and different salt concentrations (0M, 0.5M, 2M NaCl), we obtained 44 and 63 differentially expressed STs respectively. These genes are differentially expressed in low carbon sources and high salt concentrations, which is indicated that it may help the strain to cope with abiotic stress. Then we analyzed the protein regulatory interaction network between the differentially expressed genes in high salt concentration and the genes in the MAPK cascade pathway, and obtained a hub sugar transporter AsSTL1. This protein is clustered with the STL1 gene in the phylogenetic tree, which connects multiple proteins in the MAPK cascade pathway and interacts with the other sugar transporter, indicating that AsSTL1 responds to salt stress and is of great significance for cells to cope with stress.

2. Materials And Methods

2.1 Identification of Sugar Transporters in A. sydowii H-1

The whole genome of A. sydowii H-1 has been sequenced. All proteins were searched against PFAM database (Pfam 33.1) by hmmsearch (version: 3.1b1)[20]. Protein which hits to PFAM ID “PF00083” were considered as STs.

In order to find the location of ST genes on chromosomes, we use MapChart (version:2.3.2) [21]to visualize the location of these genes on chromosomes according to the gene annotation results.

2.2 Phylogenetic Analysis of STs

Total 173 STs in A. sydowii H-1 were used to construct phylogenetic tree. Another 61 fungal STs were collected for subsequent analysis with reference to Mao Peng et al, 7 STs from Arabidopsis thaliana were used as outgroups[22]. Muscle (version: 3.8.31)[23] is used for multiple sequence alignment. The phylogenetic tree is constructed by RAxML (version: 8.2.11)[24], and the bootstrap repetitions is set to 100. Tree was visualized by iTOL (version:6, https://itol.embl.de/).

MEME (version:5.4.1 https://meme-suite.org/meme/tools/meme) [25] is used to predict motifs that may be included in the sequence according to specific algorithms.

2.3 Expression Profiles of ST Genes at Different Growth Stages and Different Salt Concentrations

The mycelia fermented for 2 days and 8 days of A. sydowii H-1 were used to extract RNA for transcriptome sequencing analysis. The specific data are in NCBI SRA database (BioProject: PRJNA542911)[26]. Another transcriptome data (BioProject: PRJNA587059) is from strain A. sydowii BMH-0004 which grown up on the medium containing different salt concentrations (0M,0.5M,2.0M)[27].

Use Trimmatic (version:0.39)[28] to filter the low-quality data in the row data. And then the clean data was mapped to A. sydowii H-1 genome by STAR (version: 2.7.10a)[29]. Next, the gene expression levels were calculated and normalized via the expectation maximization method with RSEM (version:1.3.3)[30]. The

expression levels of concerned genes under different conditions are displayed by using TBtools (version:1.098745)[31] to draw heatmap.

2.4 Protein-Protein Interaction Network

Studying the interaction network between proteins is helpful to mine the core regulatory genes. After analyzing the expression pattern of STs, we found that a large number of STs are differentially expressed under different salt concentrations. In order to understand whether these genes play a role in coping with salt stress, we use STRING database (version:) to explore whether there is protein interaction between STs and genes in MAPK cascade pathway.

3. Results

3.1. Characteristic of Sugar Transporters in A. sydowii H-1

Through HMMER (version: 3.1b1), 173 proteins containing “PF00083”in the genome of *A. sydowii* H-1 were found. In order to find the location of ST genes on chromosomes, we use MapChart (version:2.3.2) [21]to visualize the location of these genes on chromosomes (Fig. 1). A total of 26 contigs from the genome assembly of H-1, and we could see that 173 STs were randomly distributed on the first 15 contigs. Contig00001 contains the largest number of STs with 28.

3.2. Phylogenetic Analysis of ST Genes

In order to understand the possible division of these 173 STs, we constructed a phylogenetic tree to divide these proteins into 9 groups (Fig. 2), This grouping is consistent with that described before [22]. In group A, Frt1_bcin was found to be a high affinity proton coupled symporter specific for fructose[32], Fsy1_spas was also an enzyme found in yeast that does not accept glucose as substrate and actively mediates fructose transport[33], and The ITR1_scer and ITR2_scer play a primary and secondary role in the transport of inositol in the medium containing the lowest content of glucose, respectively[34].So the other 8 STs may have the ability to transport fructose or inositol because they are more close to these proteins. In group B, GalA_bcin and GalA_ncra were proven to transport d-galacturonic acid[35], Qa_ncra was demonstrated the ability to transfer quinic acid[36], STs in this group was thought to have the function of transporting d-galacturonic acid and quinic acid. In group C, XltA_anig[37], XltB_anig[37] and XltA_anig[38] can transport xylose, and XltA_anig’s expression was able to restore growth on xylose, glucose, galactose, and mannose as single carbon sources, indicating that this transporter accepts multiple sugars as a substrate, therefore SUTs in this group may have ability to transport xylose and hexose. In group D, HGT-1_ncra, HGT-2_ncra, Glt1_ncra were were identified as the key components of the glucose dual-affinity transport system, which plays diverse roles in glucose transport and carbon metabolism[39]. MstA_anid was defined as a high-affinity glucose transporter expressed in germinating conidia, and MstA_anid as a high-affinity glucose transporter that operates in vegetative hyphae under conditions of carbon limitation[40]. SNF3_scer and RGT2_scer serve as glucose receptors that generate the signal for induction of genes involved in glucose uptake and metabolism[41, 42]. XYT1_ncra was

pentose transporter from *Neurospora crassa*[43]. The rest are glucose and hexose transporters from *Aspergillus niger*(MstH_anig)[44, 45], *Ustilago maydis* (hxt1_umay)[46], *Aspergillus nidus* (AN10891_anid, AN1797_anid, mstE_anid)[40, 44, 45], and *Saccharomyces cerevisiae* (HXT1_scer, HXT2_scer, HXT3_scer, HXT4_scer, HXT5_scer, HXT6_scer, HXT7_scer, HXT11_scer, HXT13_scer, GAL2_scer)[47–52]. So STs in group D may serve as main mainly 6-carbon sugar transporter. In group E, there are some sucrose transporter (Srt1_umay)[53] and maltose transporter (MAL11_scer, MAL31_scer, MPH2_scer, MPH3_scer, MalP_aory)[54–57]. Proteins in group E may mainly transport polysaccharides. And in group F, there is no known STs, and that the phylogenetic status of this group is between group E and group G, which may have the functions of these two groups and indicate that the STs in this group is still unknown. G group have cellodextrin STs (cltA_anid, CDT1_ncra, CDT2_ncra)[58, 59] and lactose STs (LacpA_anid, LacpB/cltB_anid)[58, 60]. H group also contains glucose STs (Gtt1_thar, hxtA_anid, HGT1_klac,)[45, 61, 62] and some kind of pentose STs such as arabinose (LAT_ncra, LAT_mthe, HGT1_kmar, araT_stip)[63–65], xylose (XltC_anig, HGT1_kmar)[37, 64]. STs in H group may transport glucose and pentose. And finally for I group, Xyp29_psti and NCU00821_ncra xylose specific transporters[65], and some are pentose transporter (XAT1_ncra, LAT2_amon)[43, 66], the others are glycerol transporter (STL1_scer)[19] and glucose transporters (stp1_tree)[67]. In I group, STs may have the ability to transport pentose, glucose and glycerol. Finally, Among the 173 STS, 171 proteins were divided into 9 subgroups, and the remaining two proteins EVM0011030.1 and EVM0006650.1 were not classified. From the perspective of evolutionary tree, the functions of these two proteins may be closer to those of similar subgroups, but they still need experimental verification.

MEME (version:5.4.1 https://meme-suite.org/meme/tools/meme)[2] is used to predict motifs that may be included in the sequence according to specific algorithms. As can be seen from the Figure S1, the number of conservative motifs ranges from 2 to 10. In Group A (Figure S1b), all proteins contain motif1, motif2, motif3, motif5, motif6, motif7. In Group B (Figure S1c), all proteins share only motif1. In Group C (Figure S1d), all but motif9 are shared. In Group D (Figure S1e), all but motif4 are shared. In Group E (Figure S1f), only motif5 is present in all proteins. motif1,2,4 and 5 are contained in all proteins in Group F (Figure S1g). motif1,5 are contained in all proteins of Group G (Figure S1h), and motif8 does not exist in any protein of this group. Except motif10, the protein of Group H (Figure S1i) contains all the other motifs. In Group I (Figure S1j), only motif5 is present in all proteins.

3.3. Expression Profiles of ST Genes at Different Growth Stages and Different Salt Concentrations

At The mycelia fermented for 2 days and 8 days of *A. sydowii* H-1 were used to extract RNA for transcriptome sequencing analysis. And the transcript levels were estimated with RSEM. The expression levels of concerned genes under different conditions are displayed by using TBtools (version:1.098745) [31]. We obtained 44 differentially expressed glycoprotein transport genes at different growth stages (Fig. 3). Among them, 19 STs were highly expressed in the prophase of fermentation, 25 STs were highly expressed in the late fermentation stage. In the preliminary work of the laboratory, the reducing sugar content was high on the second day of fermentation. With the fermentation time, the reducing sugar

could hardly be detected on the eighth day of fermentation[26]. Under the condition of carbon source restriction, the expression of more STs increased, means more STs operates under conditions of carbon limitation, and also indicating that these sugar transporters are trying to maintain the survival of mycelial and help them adapt to the harsh physiological environment.

To understand whether STs can help resist abiotic stress, we analyzed a set of salt tolerant transcriptome data. Transcriptome data (BioProject: PRJNA587059) is from strain *A. sydowii* BMH-0004 which grown up on the medium containing different salt concentrations (0M,0.5M,2.0M NaCl)[27]. Using STAR (version: 2.7.10a)[29], we compared nine repeats of three conditions with the *A. sydowii* H-1 genome as a reference, and the average alignment rate was 81. 88%. Next, the gene expression levels were calculated and normalized via the expectation maximization method with RSEM (version:1.3.3)[30]. Genes with differential expression in STs were used TBtools (version:1.098745)[31] to draw heatmap. Finally, we found that 63 STs were differentially expressed at different salt concentrations (Fig. 4). Among them, 10 genes were only highly expressed at medium salt concentration (0.5 M NaCl), and were low expressed at both no salt (0 M NaCl) and high salt conditions (2 M NaCl). The other 10 genes were only highly expressed at no salt condition (0 M NaCl), and were low expressed at both medium ((0.5 M NaCl)) and high salt conditions ((2 M NaCl)). 20 genes were only highly expressed at high salt concentration ((2 M NaCl), indicating that these genes have important functions at the corresponding salt concentration. In particular 20 genes expressed under high salt stress indicate that these glycoproteins may have important functions to help mycelium resist stress. And under different stress conditions, the genes are expressed differently, which also indicates that these genes have division of labor and cooperation in coping with abiotic stress

3.4. Protein Interaction Network of Sugar Transporters

Mitogen activated protein kinase (MAPK) cascade pathway is composed of ser/thr protein kinase, which is activated by extracellular stimulation and is highly conserved in all eukaryotic cells[68]. These kinases activate the genes related to the synthesis of osmotic antagonists by phosphorylation, which can regulate cell osmotic pressure in response to salt stress regulation and other abiotic stresses[69]. In order to further understand whether sugar transporters are involved in carbon stress and salt stress, we constructed the protein-protein interaction network of differentially expressed sugar transporters and differentially expressed MAPK cascade genes under different salt concentration stress (Fig. 5). We found that sugar transporter EVM0009831.1 interacts with genes in multiple MAPK cascade pathways, including HOG1, STE11, PBS2, FUS3 and SSK22, and also interacts with downstream sugar transporters. EVM0009831.1 is highly expressed only at high salt concentration, and a is clustered with STL1_scer gene in the phylogenetic tree. STL1_scer is annotated as a glycerol transporter in *Saccharomyces cerevisiae*[70], Small and uncharged glycerol is an important molecule in yeast metabolism and osmotic adaptation, indicating that in response to salt stress, MAPK cascade may help cells resist salt stress by regulating EVM0009831.1 to accelerate glycerol transport. Finally, we named EVM0009831.1 as AsSTL1.

4. Conclusion And Discussion

We finally found 173 STs in *A. sydowii* H-1 genome. Compared with 127 and 86 STs in *Aspergillus oryzae*[71] and *Aspergillus niger*[72], this number is undoubtedly larger and indicate a high potential for substrate utilization. Through analysis, we obtained a phylogenetic tree similar to the sugar transporters in *A. niger*[72], which were divided into 9 subgroups including hexose, glucose, xylose, galactose, glycerol, inositol, sucrose and other transporter types (Fig. 2). The heat maps of gene expression at different times of fermentation showed that different families of STs may function at the same time, and the same family of STs may also express at different times (Fig. 3). Many studies have shown that STs may be related to cell stress resistance, such as Lily[73], Banana[74], *Saccharomyces cerevisiae*[75], etc. And for *A. sydowii*, under salt stress, there are 63 STs were differentially expressed (Fig. 4). Through protein network interaction analysis, we found that a hub gene AsSTL1 interacting with HOG, SSK22, STE11, PBS2 and FUS3 (Fig. 5). These results indicate that AsSTL1 is an important protein in response to salt stress.

In a word, we systematically analyzed the sugar transporters in H-1, identified 173 sugar transporters through bioinformatics methods, and their chromosomal localization and conserved motifs were draw. Through phylogenetic analysis, these sugar transporters were divided into 8 subgroups, their functions were roughly predicted according to the grouping results, for example, it may have the ability to transport pentose, glucose, or macromolecular sucrose, etc. Then, we analyzed the differential expression of these sugar transporters. There were 44 and 63 differentially expressed STs under different fermentation time (2d and 8d) and different salt concentration (0M, 0.5M, 2M NaCl), respectively. Through protein network interaction analysis, we found that EVM0009831.1, as the center, interacts with proteins in MAPK cascade pathway and other STs, and it can be identified as glycerol transporter through phylogenetic tree, named as AsSTL1.

Declarations

Author Contribution Statement: All authors have read and agree to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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Figures

Figure 1

Chromosomal localization of STs protein.

Figure 2

Phylogenetic Analysis of SUT protein. The tree contains 173 putative sugar transporters in *A. sydowii* H-1 and other 61 fungi SUT protein□7 sugar transporters from *Arabidopsis* for tree rooting.

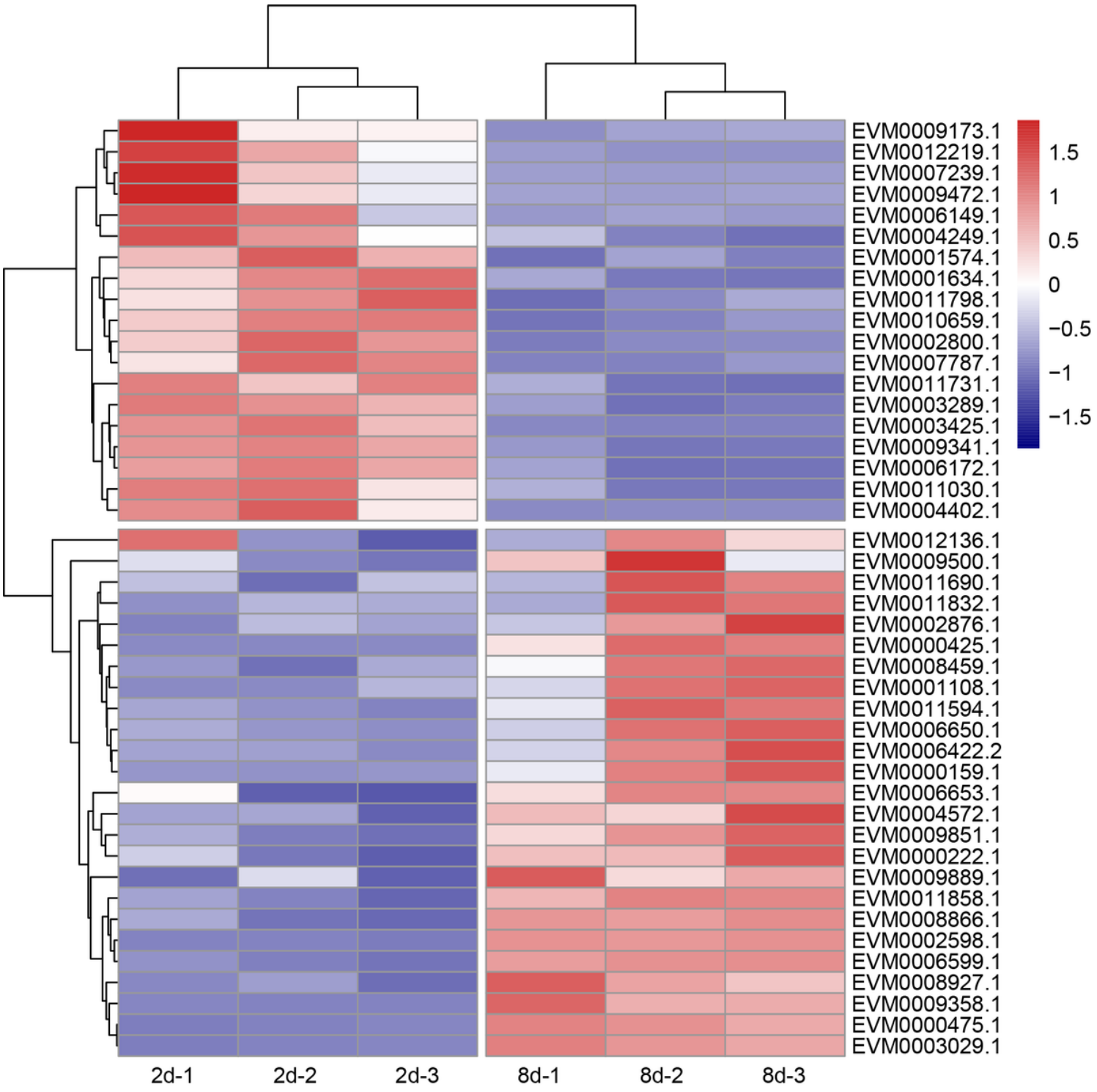

Figure 3

Heat map of the expression of 44 ST genes in *A. sydowii* H-1.2d-1,2d-2,2d-3: Gene expression after 2 days of fermentation. 8d-1,8d-2,8d-3: Gene expression after 2 days of fermentation. The color of the scale bar, ranging from blue to red, represents low and high expressions, respectively.

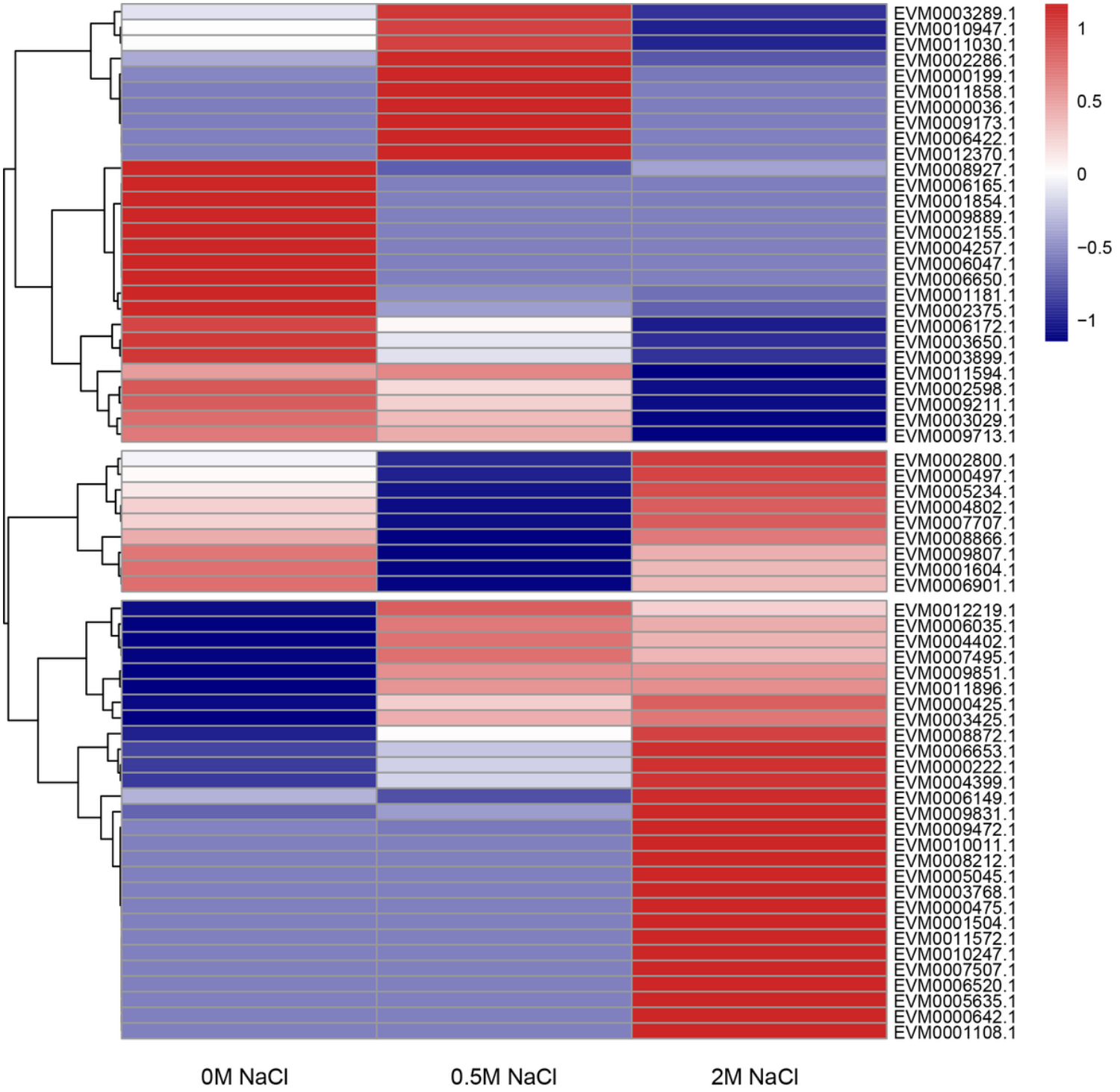

Figure 4

Heat map of the expression of 43 ST genes in *A. sydowii* BMH-0004 which grown up in different salt concentrations (0M, 0.5M, 2.0M). The color of the scale bar, ranging from blue to red, represents low and high expressions, respectively.

Figure 5

A protein-protein interaction (PPI) network between STs and genes in MAPK cascade pathway. The nodes represent proteins, and the edges represent the corresponding PPI. The confidence score was required to be greater than 0.4.

Supplementary Files

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