Research Article

Condition-Specific Molecular Network Analysis Revealed That Flagellar Proteins Are Involved in Electron Transfer Processes of Shewanella piezotolerans WP3

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Received 7 March 2021; Revised 28 May 2021; Accepted 20 July 2021; Published 31 July 2021

Academic Editor: Hafiz Ishfaq Ahmad

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Because of the ability to metabolize a large number of electron acceptors such as nitrate, nitrite, fumarate, and metal oxides, Shewanella species have attracted much attention in recent years. Generally, the use of these electron acceptors is mainly achieved through electron transfer proteins and their interactions which will dynamically change across different environmental conditions in cells. Therefore, functional analysis of condition-specific molecular networks can reveal biological information on electron transfer processes. By integrating expression data and molecular networks, we constructed condition-specific molecular networks for Shewanella piezotolerans WP3. We then identified condition-specific key genes and studied their potential functions with an emphasis on their roles in electron transfer processes. Functional module analysis showed that different flagellar assembly modules appeared under these conditions and suggested that flagellar proteins are important for these conditions. We also identified the electron transfer modules underlying these various environmental conditions. The present results could help with screening electron transfer genes and understanding electron transfer processes under various environmental conditions for the Shewanella species.

1. Introduction

Cellular biological processes are not maintained by a single molecule but rather the interactions between genes, proteins, metabolites, and many other biological molecules in potential molecular networks [1, 2]. It is generally believed that molecular networks are highly modular, and different network modules can be used to perform different cellular functions. Therefore, identifying and extracting functional modules in a network does not only help to understand the structural and functional relationships of molecular networks but also help to discover the hidden biological rules in the networks. This has many important practical applications, such as inferring function-related or coregulated gene sets [3]. Researchers have proposed many methods to predict functional modules in networks [4].

Since the complex characteristics of organisms are not caused by a single mutation or genetic variation, by changes in the functions of the molecular network, the functional modules within the network will change under different environmental conditions, biological states, and cellular processes [5]. Therefore, it is difficult to obtain condition-specific functional modules through general interaction data alone. Functional modules of molecular networks should be studied by combining condition-specific expression data and molecular networks [6, 7].

Due to their metabolic versatility, Shewanella species have attracted much attention, and they have been widely used in many fields, such as energy production, wastewater treatment, bioremediation, biosensor, and chemical synthesis (see, e.g., [8, 9]). It is generally believed that the use of a variety of electron acceptors is mainly achieved through
electron transfer proteins (such as multihaem c-type cytochromes) and their interactions [10–12]. To reveal the electron transfer mechanism of Shewanella species in different environments, the deep-sea bacterium Shewanella piezotolerans WP3 (S. piezotolerans WP3) was selected in the present work, since it is considered to be a good candidate for studying the adaptation of Shewanella species to a variety of environmental conditions (low temperature, high pressure, etc.) [13–15]. Therefore, we integrated expression data and molecular networks of this bacterium under various environmental conditions to construct condition-specific molecular networks and identified the key genes and functional modules in the networks and finally investigated their roles in electron transfer processes (Figure 1). These results will have important significance for screening electron transfer genes and understanding different electron transfer processes in response to various environmental conditions for the Shewanella species.

2. Materials and Methods

2.1. Gene Expression Data. To study electron transfer processes of S. piezotolerans WP3 under various conditions, we searched S. piezotolerans WP3 in the GEO (gene expression omnibus) database (https://www.ncbi.nlm.nih.gov/geo/) [16] and obtained four time-series expression datasets for the S. piezotolerans WP3 wild-type strain under cold shock (GSE82259), heat shock (GSE82264), high pressure (GSE82267), and salt shock (GSE82254) [17, 18]. Each of these datasets contains expression profiling of S. piezotolerans WP3 for 30 minutes, 60 minutes, and 90 minutes under the experimental shocks, which could be used for the subsequent differential expression analysis.

2.2. Differentially Expressed Genes. In contrast to the comparison of differential expression between two groups of experiments, comparative studies from multigroup data should provide a much better understanding of differential expression [19]. Therefore, comparative studies of multiple groups (three groups of data with 6 replicates for all conditions) were considered in this study. The web tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) [16] was used to compare these multigroup datasets to identify differentially expressed genes (DEGs) under these conditions. The default parameters of GEO2R were used, and P < 0.01 and FDR (false discovery rate) < 0.05 were used as the cut-off values.

2.3. Construction of PPI Networks. Because proteins do not function independently in cells, but generally form complexes with other proteins, that is, protein-protein interaction (PPI) networks, the PPIs stored in STRING (https://string-db.org/) [20] were used for the construction of PPI networks. We first downloaded the full interaction information for S. piezotolerans WP3, and to enhance the reliability of the interactions, we extracted the high confidence interactions (combined_score ≥ 0.7, as suggested by STRING) to construct the background PPI network for S. piezotolerans WP3. Condition-specific networks can be further obtained by combining this background network with differentially expressed nodes (i.e., the proteins that are encoded by the DEGs).

2.4. Network-Based Methods. In general, complex network methods can be used to analyze the structural properties and functions of biological molecule networks. Degree-based centralization analysis was used to study the importance of each protein in the network, with an emphasis on the top 10 key proteins in these networks because proteins generally form complexes with other proteins to perform specific functions, and the ClusterONE method has been shown to be very effective in the recognition of protein complexes [21]. We therefore employed this method to identify the functional modules in these networks. There are several user-friendly implementations of the method (http://www.paccanarolab.org/cluster-one/), and we used the Cytoscape plugin “ClusterONE” to perform the functional module analysis. The minimum size was set to 4, and P < 0.05 was used as the cut-off value.

2.5. Enrichment Analysis. Enrichment analysis can be commonly used to extract biological information from a given gene/protein set [22]. Here, functional enrichment analysis was performed using the STRING enrichment app through the Cytoscape toolkit (https://cytoscape.org/) [23], and the enrichment FDR cut-off was also set to 0.05. Furthermore, we chose the most representative KEGG pathways from the resulting list (i.e., the lowest FDR), and if there were no enriched KEGG pathways, InterPro enrichment was used instead.

3. Results and Discussion

3.1. Identification of the DEGs That Are Responsible for the Various Environmental Conditions. We first used GEO2R to identify the DEGs under the various environments, and overall 763, 1635, 1039, and 1130 unique DEGs were involved in cold shock, heat shock, high pressure, and salt shock conditions, respectively. Because proteins need interactions to perform their functions, we constructed the corresponding PPI networks for the proteins encoded by these DEGs. Subsequently, considering that enrichment analysis can be used to extract biological insight from a given gene/protein set, we first performed KEGG pathway enrichment analysis to reveal the biological functions and differences of the largest connected components in these PPI networks.

Overall, 44, 54, 36, and 16 KEGG pathways were enriched under the abovementioned conditions, respectively. As illustrated in Figure 2, the most common enriched pathways across these environments included metabolism-related (e.g., metabolic pathways, biosynthesis of secondary metabolites, and microbial metabolism in diverse environments) and protein translation-related (e.g., biosynthesis of amino acids, ribosomes, aminoacyl-tRNA biosynthesis, and protein export) pathways, indicating that metabolism largely acclimates to changing environmental conditions.

| Pathway | Conditions | DEGs |
|---------|------------|------|
| Metabolism | Cold shock | 763 |
| Heat shock | 1635 |
| High pressure | 1039 |
| Salt shock | 1130 |

Overall, these results provide a comprehensive understanding of the molecular networks of Shewanella piezotolerans WP3 under various environmental conditions, which could be used as a reference for further studies on the electron transfer mechanism of this bacterium.
Figure 1: Overall view of the study. Step 1: four time-series expression datasets for *S. piezotolerans* WP3 were obtained from the GEO database; Step 2: the DEGs under these conditions were identified; Step 3: the identified DEGs were used to filter the global PPIs to construct condition-specific PPI networks; Step 4: condition-specific key genes and important modules were identified from these networks. GEO: gene expression omnibus; DEGs: differentially expressed genes; PPI: protein-protein interaction.

Figure 2: KEGG pathway enrichment analysis for the DEGs (differentially expressed genes) under the various environmental conditions. False discovery rate <0.05.
(cold shock, heat shock, high pressure, and salt shock here), and, accordingly, *S. piezotolerans* WP3 needs to produce new proteins that acclimate to these environments. These results are in good agreement with previous studies in other *Shewanella* species (see, e.g., [24, 25]).

On the other hand, apart from these commonly enriched pathways, some pathways presented large differences across three of these conditions (there were no unique enriched pathways under salt shock condition; see Figure 2). First, the largest difference was derived from the heat shock condition, and there were 12 enriched pathways exclusively presented under this condition, indicating that *S. piezotolerans* WP3 is more sensitive to heat shock than the other shocks examined in this study. This point is in good agreement with the fact that *S. piezotolerans* WP3 was isolated from West Pacific sediment at a depth of ~1914 m [15], where the most remarkable environmental features are low temperature, high pressure, and high salt, but not high temperature. Second, there were 6 pathways exclusively presented under the cold shock condition, including glutathione metabolism (FDR: $1.35 \times 10^{-5}$), inositol phosphate metabolism (FDR: $8.54 \times 10^{-3}$), beta-alanine metabolism (FDR: $1.62 \times 10^{-2}$), pentose and glucuronate interconversions (FDR: $2.29 \times 10^{-2}$), lysine biosynthesis (FDR: $2.78 \times 10^{-2}$), and fructose and mannose metabolism (FDR: $4.58 \times 10^{-2}$). Third, only one pathway (degradation of aromatic compounds, FDR: $2.86 \times 10^{-2}$) was exclusively presented under the high-pressure condition. Due to microorganisms need to produce different proteins to cope with the changing environmental conditions, these exclusively enriched pathways and the involved proteins will be helpful to understand the different metabolic processes under various environmental conditions in *S. piezotolerans* WP3.

### 3.2. Construction of the PPI Networks That Are Involved in the Various Environmental Conditions

It is generally believed that the global protein network defines the collective function of cells. In contrast, a condition-specific PPI network contains a lot of interactions that are enriched for processes relevant to the specific condition and thereby may be more relevant for characterizing specific biological processes, such as identifying protein complexes or stimulus-response processes [26, 27].

To construct the PPI networks that are involved in the various environments, we first identified condition-specific protein-coding genes under these conditions by using a Venn diagram. As shown in Figure 3, 167, 647, 330, and 428 unique DEGs were involved in the abovementioned environments, respectively. These gene sets were then used to construct condition-specific PPI networks from the background PPI network for *S. piezotolerans* WP3.

As a result, although there were many isolated proteins in the network, each network contains many interactions that form several interconnected parts. Numerically speaking, all of these condition-specific networks have significantly more interactions than were expected (the PPI enrichment $P$ values were $1.66 \times 10^{-2}$, $7.21 \times 10^{-4}$, $2.74 \times 10^{-2}$, and $9.96 \times 10^{-3}$, respectively). Since proteins mainly carry out biological functions through interactions, it is generally considered that the hubs (i.e., proteins with a high degree value) in the PPI network are more important. To this end, we used the degree value to rank the importance of the proteins in the networks and thereby identified the top 10 key proteins in each network (Table 1). As shown in Table 1, these key proteins are mostly linked to the electron transport processes of *S. piezotolerans* WP3 under various environmental conditions (except for the heat shock condition; see below).

First, the top 10 key proteins under cold shock were mostly relevant to flagellar proteins, including flagellar biosynthesis sigma factor (swp_5124), flagellar basal body rod protein (swp_5105, swp_5110), flagellar basal body L-ring protein (swp_1504, swp_5111), flagellar hook-associated protein FlgL (swp_1508), and flagellar ATPase FilI/YscN (swp_5095). A transcriptional study suggested that the flagellar operon of *Escherichia coli* is inducible in response to cold shock [28]; in addition, a proteomic study showed that flagellar biosynthesis and motility proteins presented significant changes under cold shock [29]. Therefore, the key proteins of *S. piezotolerans* WP3 under cold shock are mostly flagellar proteins and are expectable. Furthermore, the correlation between oxidative electron transport and anaerobic flagellum assembly of *Escherichia coli* was reported long ago [30], and, more importantly, several recent experimental studies from different groups showed that flagellar proteins were involved in the electron transfer activity in *Geobacter sulfurreducens* and *Shewanella oneidensis* (e.g., [31–33]), raising a speculation that flagellar proteins may be also involved in the electron transfer activity in *S. piezotolerans* WP3.

Second, the top 10 key proteins under high pressure also included several flagellar proteins, such as flagellar biosynthesis protein (swp_1531) and two flagellar motor switch proteins (swp_1521, swp_1527). Motor switch proteins have been shown to be directly associated with some energy-linked enzymes and thereby lead to higher rates of ATP synthesis, ATP hydrolysis, and electron transport [34]. Other important
proteins in this environment are also electron transfer-related, including a flavocytochrome c (swp_4352) and a flavoprotein (swp_0430), which are homologous proteins for periplasmic fumarate reductase FccA (63% identity) and quinol:fumarate reductase FAD-binding subunit FrdA (88% identity) from *Shewanella oneidensis* MR-1, respectively. Formate dehydrogenase subunits swp_4312 and swp_2139 are homologous proteins for molybdopterin-binding oxidoreductase (SO_0988, 67% identity) and formate dehydrogenase cytochrome b subunit FdhC (47% identity) from *Shewanella oneidensis* MR-1. These proteins play an important role in electron transfer processes of *Shewanella oneidensis* MR-1 [35]. For example, periplasmic fumarate reductase FccA can promote the periplasmic electron transfer process by rapid transient interactions [36].

Third, the most important proteins that are listed in the top 10 key proteins under salt shock are several mannose-sensitive haemagglutinin-(MSHA-) related proteins, including MSHA pilin protein (swp_0495) and MSHA biogenesis proteins (swp_0501, swp_0486), which have been shown to be involved in electron transfer processes in *Shewanella oneidensis* MR-1 [37]. Other notable proteins are Cbb3-type cytochrome oxidase (the oxidase subunit swp_4145 and the cytochrome c subunit swp_4144), which are homologous proteins for Cbb3-type cytochrome c oxidase subunit CcoN and Cbb3-type cytochrome c oxidase subunit CcoO from *Shewanella oneidensis* MR-1. These proteins have previously been shown to be involved in electron transfer in various organisms [38], which made swp_4145 and swp_4144 probable candidates for electron transfer studies in *S. piezotolerans* WP3.

Finally, the top 10 key proteins under heat shock conditions were mainly metabolism-related, including glutamate synthase subunit (swp_3803), pyruvate kinase II (swp_2388), oxaloacetate decarboxylase (swp_1379), multifunctional fatty acid oxidation complex subunit (swp_3139), 2-isopropylmalate synthase (swp_2213), isopropylmalate isomerase small subunit (swp_2216), chorismate mutase (swp_3758), Na+/transporting methylmalonyl-CoA:oxaloacetate decarboxylase subunit (swp_1380), sodium pump decarboxylase subunit (swp_1378), and malate dehydrogenase (swp_0481). These results are consistent with previous KEGG enrichment results, indicating that cell metabolism undergoes tremendous changes (from their native living environment) under the heat shock condition. These metabolic proteins may be helpful for identifying which forms of metabolism are more important in response to heat shock.

### 3.3. Important Functional Modules Underlying the Various Environmental Conditions

First, what interested us was that the different flagellar assembly modules appeared in three of these conditions (Tables 2–5; Figure 4). As we discussed in Section 3.2, flagellar assembly-related proteins are of great concern under cold shock and high-pressure conditions. Although flagellar assembly-related proteins are not key proteins under the heat shock condition, the flagellar assembly module identified here suggests that these proteins should also be important under this condition. As mentioned earlier, flagella are important for biofilm formation, energy production, and electron transfer. The results reported here further illustrate that proteins of flagellar assembly systems will, in fact, form interacting flagellar assembly modules to achieve their purpose. Although they play an important role in a variety of environments, the bacteria can use different flagellar assembly modules under diverse environments, suggesting that diversified flagellar systems are required under diverse environments. In addition, the flagellar assembly module under cold shock contains an important polar flagellum gene (swp_1508), and the flagellar assembly module under high pressure contains a lateral flagella gene (swp_3616). These results coincide with the fact that *S. piezotolerans* WP3 can express a polar flagellum and multiple lateral flagella systems with distinguishing characteristics [39], which can partly explain how the expression of these systems is differentially regulated under low temperature and high pressure conditions [40].

Furthermore, since the use of a variety of electron acceptors is the most important feature of the *Shewanella* species, we then focus on electron transfer processes, and we found several electron transfer-relevant modules in these various environmental conditions (Figure 5).

First, we identified a *molybdenum cofactor biosynthesis module* under the cold shock condition (Figure 5(a)), which could be linked to the fact that some molybdenum cofactor–included enzymes (such as sulfatase) can be used to modulate cold stress-responsive gene expression [41]. Furthermore, the molybdenum cofactor has also been shown to be a redox active prosthetic group, which is an essential component of numerous enzymes for facilitating electron transfer, such as dithiolene complexes [42], aldehyde oxidoreductase [43], and dimethyl sulide dehydrogenase [44].

Second, a *multihaem cytochrome module* was identified under the heat shock condition (Figure 5(b)). Multihaem...
### Table 2: The most representative KEGG pathway that was enriched in the modules identified from the cold shock condition.

| Cluster | Size | Density | P value | KEGG Pathways | Members |
|---------|------|---------|---------|---------------|---------|
| 1       | 8    | 0.96    | 1.87E−04 | Flagellar assembly | swp_5105, swp_5124, swp_5111, swp_5110, swp_1504, swp_5095, swp_5087, swp_1508 |
| 2       | 7    | 0.52    | 8.66E−04 | Ribosome | swp_1665, swp_2026, swp_1999, swp_1429, swp_3015, swp_2462, swp_1221 |
| 3       | 7    | 0.52    | 1.90E−03 | Oxidative phosphorylation | swp_0794, swp_4826, swp_4824, swp_2950, swp_2059, swp_2948, swp_5156 |
| 4       | 5    | 0.50    | 3.54E−03 | Molybdenum cofactor biosynthesis | swp_5032, swp_5036, swp_4980, swp_4983, swp_0101 |
| 5       | 5    | 0.50    | 7.98E−03 | Propanoate metabolism | swp_3440, swp_4179, swp_3386, swp_0423, swp_1949 |
| 6       | 5    | 0.60    | 2.94E−02 | Aminoacyl-tRNA biosynthesis | swp_3015, swp_2462, swp_3260, swp_3938, swp_0586 |

Note: the bold enrichment term indicates that there was no KEGG enrichment and InterPro enrichment was used alternatively.

### Table 3: The most representative KEGG pathway that was enriched in the modules identified from the heat shock condition.

| Cluster | Size | Density | P value | KEGG Pathways | Members |
|---------|------|---------|---------|---------------|---------|
| 1       | 15   | 0.67    | 2.85E−05 | Pyruvate metabolism | swp_1380, swp_5027, swp_4752, swp_4333, swp_3528, swp_2388, swp_2216, swp_2215, swp_2213, swp_2053, swp_1378, swp_0477, swp_0481, swp_0360 |
| 2       | 12   | 0.56    | 1.56E−04 | Ribosome | swp_2008, swp_2030, swp_4802, swp_3049, swp_2027, swp_2028, swp_2025, swp_2007, swp_0208, swp_1998, swp_0427, swp_1556 |
| 3       | 9    | 0.64    | 6.31E−04 | Peptidoglycan biosynthesis | swp_1499, swp_5085, swp_5084, swp_5083, swp_5082, swp_1519 |
| 4       | 6    | 1.00    | 7.64E−04 | Beta-lactam resistance | swp_1172, swp_2888, swp_2886, swp_2884, swp_1173, swp_2883 |
| 5       | 7    | 0.62    | 2.84E−04 | Flagellar assembly | swp_4806, swp_2182, swp_4116, swp_2918, swp_2183, swp_3403, swp_0613 |
| 6       | 7    | 0.73    | 1.28E−03 | Multihaem cytochrome | swp_1172, swp_2888, swp_2886, swp_2884, swp_1173, swp_2883 |
| 7       | 7    | 0.62    | 1.94E−03 | Aminoacyl-tRNA biosynthesis | swp_3543, swp_4396, swp_0518, swp_0110 |
| 8       | 8    | 0.64    | 2.70E−03 | Lipopolysaccharide biosynthesis | swp_3507, swp_3506, swp_0834, swp_3511, swp_3509, swp_3508, swp_3516, swp_3515 |
| 9       | 5    | 0.70    | 2.79E−03 | — | swp_3204, swp_3981, swp_0083, swp_3979, swp_1828 |
| 10      | 4    | 0.67    | 1.01E−02 | — | swp_2103, swp_3188, swp_3190, swp_3192 |
| 11      | 5    | 0.70    | 1.71E−02 | Glycolipid metabolism | swp_0081, swp_5056, swp_4820, swp_4318, swp_0775 |
| 12      | 5    | 0.60    | 2.91E−02 | Bacterial secretion system | swp_0196, swp_0739, swp_0187, swp_0189, swp_0188 |
| 13      | 4    | 0.67    | 3.34E−02 | — | swp_3543, swp_4396, swp_0518, swp_0110 |

Note: the bold enrichment terms indicate that there was no KEGG enrichment and InterPro enrichment was used alternatively; — indicate that the enrichment analysis returned no results.

### Table 4: The most representative KEGG pathway that was enriched in the modules identified from the high-pressure condition.

| Cluster | Size | Density | P value | KEGG Pathways | Members |
|---------|------|---------|---------|---------------|---------|
| 1       | 9    | 0.58    | 3.49E−04 | Carbon metabolism | swp_3663, swp_5025, swp_4312, swp_1239, swp_2139, swp_2142, swp_3875, swp_3458, swp_0182 |
| 2       | 9    | 0.50    | 9.74E−04 | Oxidative phosphorylation | swp_4058, swp_0854, swp_4352, swp_3589, swp_4940, swp_1424, swp_1425, swp_0430, swp_0429 |
| 3       | 6    | 0.87    | 1.26E−03 | Flagellar assembly | swp_1531, swp_3616, swp_1536, swp_1521, swp_1527, swp_1525 |
| 4       | 4    | 0.67    | 1.01E−02 | — | swp_0679, swp_4217, swp_0678, swp_2705 |
| 5       | 4    | 0.67    | 1.01E−02 | — | swp_0679, swp_0060, swp_0056, swp_1586 |
| 6       | 5    | 0.50    | 2.40E−02 | — | swp_4653, swp_4654, swp_4652, swp_4657, swp_4655 |
| 7       | 7    | 0.52    | 2.19E−02 | Purine metabolism | swp_2623, swp_2495, swp_4933, swp_4301, swp_4936, swp_4935, swp_4940 |
| 8       | 4    | 0.50    | 2.89E−02 | Purine metabolism | swp_1361, swp_4724, swp_2972, swp_2621 |
| 9       | 5    | 0.50    | 4.13E−02 | NrfD family | swp_4653, swp_4654, swp_4652, swp_4655, swp_1240 |
| 10      | 6    | 0.53    | 4.29E−02 | Microbial metabolism in diverse environments | swp_1907, swp_2623, swp_1982, swp_4429, swp_5068, swp_1640 |

Note: the bold enrichment terms indicate that there was no KEGG enrichment, and InterPro enrichment was used alternatively; — indicate that the enrichment analysis returned no results.
cytochromes are major electron transfer proteins in *Shewanella* species. These proteins often contain several closely arranged haem cofactors, which can mediate rapid and long-distance electron transfer by reversible changes between the reduced and oxidized states of iron atoms in haem cofactors [10, 12]. Typically, from the inner membrane through the periplasm and outer membrane to the extracellular space, they can form electron transfer pathways [11]. Recently, we also identified a multihaem cytochrome module in *Shewanella oneidensis* MR-1 under altered O₂ conditions [25]. Therefore, the multihaem cytochrome module of *S. piezotolerans* WP3 identified here should also be responsible for electron transfer processes under this condition. In addition, the nitrite reductase (cytochrome *c552* swp_0613 and swp_3403) can catalyze the reduction of nitrite to ammonia. The reduction of nitrates and nitrites by the shock heating supplies reduced nitrogen under nonreducing conditions, which are necessary for amino acids and nucleic acids, and thus the origin of life under such conditions [45].

Third, an *NrfD family module* was identified under the high-pressure condition (Figure 5(c)), including the formate-dependent nitrite reductase NrfGCD (swp_4654–swp_4652) and the polysulfide reductase NrfD (swp_1240). Periplasmic nitrite reductase Nrf can obtain electrons from other cytochrome *c* molecules located in the inner membrane and then use them to reduce soluble substances such as nitrites, a process that has been widely studied in *Shewanella oneidensis* MR-1 [46]. Polysulfide reductase NrfD could also be used to transfer electrons from the quinone pool.

Finally, we identified a *cytochrome c oxidase module* under the salt shock condition (Figure 5(d)). The module

### Table 5: The most representative KEGG pathway that was enriched in the modules identified from the salt shock condition.

| Cluster | Size | Density | P value | KEGG pathways | Members |
|---------|------|---------|---------|---------------|---------|
| 1       | 4    | 0.67    | 1.01E - 02 | Protein of unknown function DUF3131 | swp_2164, swp_2170, swp_2165, swp_2169 |
| 2       | 4    | 0.83    | 2.35E - 02 | Cytochrome *c* oxidase subunit | swp_2579, swp_4145, swp_4144, swp_4143 |
| 3       | 4    | 1.00    | 3.01E - 02 | — | swp_0495, swp_0501, swp_0486, swp_0485 |
| 4       | 4    | 0.67    | 3.34E - 02 | — | swp_2897, swp_2732, swp_3465, swp_2421 |
| 5       | 4    | 0.67    | 4.75E - 02 | Valine, leucine and isoleucine biosynthesis | swp_2214, swp_2842, swp_0361, swp_2606 |

Note: the bold enrichment terms indicate that there was no KEGG enrichment and InterPro enrichment was used alternatively. — indicate that the enrichment analysis returned no results.
mainly contains two $cbb_3$-type cytochrome $c$ oxidase subunits (swp_4145–swp_4144) and one class I cytochrome $c$ subunit (swp_4143), which are homologous to CcoN, CcoO, and CcoP from Shewanella oneidensis MR-1, respectively. Considering that $cco$ family of proteins plays a key role in both aerobic and anaerobic respiration in Shewanella oneidensis MR-1, and they have also been shown to be involved in the generation of electric current [38], the cytochrome $c$ oxidase module is thereby also considered to be related to these processes in S. piezotolerans WP3.

4. Conclusion

Currently, constructing a network model and then using module detection methods to group genes/proteins is one of the most common methods for genome-scale expression data study [47]. However, networks generally constantly change under different environmental conditions, such as different temperatures and pressures. The study of condition-specific and even dynamic molecular networks (and their functional modules) has become an important topic in bioinformatics researches [6, 7].

To explore the electron transfer mechanism of Shewanella species under various environments, we integrated multiexpression data and constructed condition-specific molecular networks for S. piezotolerans WP3. We then identified the key electron transfer genes and analyzed the functional modules, both of which suggest that flagellar proteins are important under these conditions. Finally, we discussed the condition-specific electron transfer-relevant modules under these various environmental conditions. Further experimental investigation, such as the influence of the respiratory capacity of the cells through flagellar gene deletion mutant, is needed to verify the putative roles of flagellar proteins in S. piezotolerans WP3.

Data Availability

All relevant data are within the paper.

Conflicts of Interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by the Science and Technology Foundation of Jiangxi Education Department (GJJ180852 and GJJ201605).

References

[1] A. Laddach, J. C.-F. Ng, S. S. Chung, and F. Fraternali, “Genetic variants and protein-protein interactions: a multidimensional network-centric view,” Current Opinion in Structural Biology, vol. 50, no. 1, pp. 82–90, 2018.
[2] S. Yi, S. Lin, Y. Li, W. Zhao, B. Mills, and N. Sahni, “Functional variomics and network perturbation: connecting genotype to phenotype in cancer,” Nature Reviews Genetics, vol. 18, no. 7, pp. 395–410, 2017.
[3] L. Cowen, T. Ideker, B. J. Raphael, and R. Sharan, “Network propagation: a universal amplifier of genetic associations,” Nature Reviews Genetics, vol. 18, no. 9, pp. 551–562, 2017.
[4] W. Saclens, R. Cannoodt, and Y. Saeyes, “A comprehensive evaluation of module detection methods for gene expression data,” Nature Communications, vol. 9, no. 1, p. 1090, 2018.
[5] M. Padi and J. Quackenbush, “Detecting phenotype-driven transitions in regulatory network structure,” NPJ Systems Biology and Applications, vol. 4, no. 1, p. 16, 2018.
[6] M. P. Dobay, S. Stertz, and M. Delorenzi, “Context-based retrieval of functional modules in protein-protein interaction networks,” Briefings in Bioinformatics, vol. 19, no. 5, pp. 995–1007, 2018.
[7] Y. Xu, J. Zhou, J. Zhou, and J. Guan, “CPredictor3.0: detecting protein complexes from PPI networks with expression data and functional annotations,” BMC Systems Biology, vol. 11, no. 1, p. 135, 2017.
[8] B. E. Logan, R. Rossi, A. Ragab, and P. E. Saikaly, “Electroactive microorganisms in bioelectrochemical systems,” Nature Reviews Microbiology, vol. 17, no. 5, pp. 307–319, 2019.
[9] D. Shi, H. Dong, G. Reguera et al., “Extracellular electron transfer mechanisms between microorganisms and minerals,” Nature Reviews Microbiology, vol. 14, no. 10, pp. 651–662, 2016.
[10] M. Breuer, K. M. Rosso, J. Blumberger, and J. N. Butt, “Multi- haem cytochromes in Shewanella oneidensis MR-1: structures, functions and opportunities,” Journal of the Royal Society Interface, vol. 12, no. 1, Article ID 20141117, 2015.
[11] D. W. Ding, J. Xu, L. Li, J. M. Xie, and X. Sun, “Identifying the potential extracellular electron transfer pathways from a $c$-type cytochrome network,” Molecular Biosystems, vol. 10, no. 12, pp. 3138–3146, 2014.
[12] S. Pirbadian, S. E. Barchinger, K. M. Leung et al., “Shewanella oneidensis MR-1 nanowires are outer membrane and periplasmic extensions of the extracellular electron transport components,” Proceedings of the National Academy of Sciences of the United States of America, vol. 111, no. 35, pp. 12883–12888, 2014.
[13] Y. Chen, F. Wang, J. Xu, M. A. Mehmoond, and X. Xiao, “Physiological and evolutionary studies of NAP systems in Shewanella piezotolerans WP3,” ISME Journal, vol. 5, no. 5, pp. 843–855, 2011.
[14] W. F. Wu, F. P. Wang, J. H. Li, X. W. Yang, X. Xiao, and Y. X. Pan, “Iron reduction and mineralization of deep-sea iron reducing bacterium Shewanella piezotolerans WP3 at elevated hydrostatic pressures,” Geobiology, vol. 11, no. 6, pp. 593–601, 2013.
[15] X. Xiao, P. Wang, Z. Xiao, D. H. Bartlett, and F. Wang, “Shewanella psychrophila sp. nov. and Shewanella piezotolerans sp. nov., isolated from west Pacific deep-sea sediment,” International Journal of Systematic and Evolutionary Microbiology, vol. 57, no. 1, pp. 60–65, 2007.
[16] T. Barrett, S. E. Wilhite, P. Ledoux et al., “NCBI GEO: archive for functional genomics data sets-update,” Nucleic Acids Research, vol. 41, no. D1, pp. D991–D995, 2013.
[17] H. Jian, S. Li, X. Feng, and X. Xiao, “Global transcriptome analysis of the heat shock response of the deep-sea bacterium Shewanella piezotolerans WP3,” Marine Genomics, vol. 30, no. 1, pp. 81–85, 2016.
[18] H. Jian, S. Li, X. Tang, and X. Xiao, “A transcriptome resource for the deep-sea bacterium Shewanella piezotolerans WP3 under cold and high hydrostatic pressure shock stress,” Marine Genomics, vol. 30, no. 1, pp. 87–91, 2016.
[19] M. Tang, J. Sun, K. Shimizu, and K. Kadota, “Evaluation of methods for differential expression analysis on multi-group RNA-seq count data,” BMC Bioinformatics, vol. 16, no. 1, p. 360, 2015.

[20] D. Szkarczyk, A. Franceschini, S. Wyder et al., “STRING v10: protein-protein interaction networks, integrated over the tree of life,” Nucleic Acids Research, vol. 43, no. D1, pp. D447–D452, 2015.

[21] T. Nepusz, H. Yu, and A. Paccanaro, “Detecting overlapping protein complexes in protein-protein interaction networks,” Nature Methods, vol. 9, no. 5, pp. 471–472, 2012.

[22] A. Subramanian, P. Tamayo, V. K. Mootha, J. S. Ebert, D. H. Gillette, N. J. Paulovich et al., “Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles,” Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 43, pp. 15545–15550, 2005.

[23] M. E. Smoot, J. Ono, J. Ruscheinski, P. L. Wang, and T. Ideker, “Cytoscape 2.8: new features for data integration and network visualization,” Bioinformatics, vol. 27, no. 3, pp. 431–432, 2011.

[24] S. E. Barchinger, S. Pirbadian, C. Sambles et al., “Regulation of gene expression in Shewanella oneidensis MR-1 during electron acceptor limitation and bacterial nanowire formation,” Applied and Environmental Microbiology, vol. 82, no. 17, pp. 5428–5443, 2016.

[25] D. W. Ding and X. Sun, “Network-based methods for identifying key active proteins in the extracellular electron transfer process in Shewanella oneidensis MR-1,” Genes, vol. 9, no. 1, p. 41, 2018.

[26] T. Ideker and N. J. Krogan, “Differential network biology,” Molecular Systems Biology, vol. 8, no. 1, p. 565, 2012.

[27] J. Wang, X. Peng, M. Li, and Y. Pan, “Construction and application of dynamic protein interaction network based on time course gene expression data,” Proteomics, vol. 13, no. 2, pp. 301–312, 2013.

[28] S. Phadtare and M. Inouye, “Genome-wide transcriptional analysis of the cold shock response in wild-type and cold-sensitive, quadruple-csp deletion strains of Escherichia coli,” Journal of Bacteriology, vol. 186, no. 20, pp. 7007–7014, 2004.

[29] Y. H. Kim, K. Y. Han, K. Lee, and J. Lee, “Proteome response of Escherichia coli fed-batch culture to temperature downshift,” Applied Microbiology and Biotechnology, vol. 68, no. 6, pp. 786–793, 2005.

[30] J. B. Tana, B. J. Howlett, and D. E. Koshland Jr., “Flagellar formation in Escherichia coli electron transport mutants,” Journal of Bacteriology, vol. 130, no. 2, pp. 787–792, 1977.

[31] L. Liu, G. Liu, J. Zhou, and R. Jin, “Energy taxis toward redox-active surfaces decreases the transport of electroactive bacteria in saturated porous media,” Environmental Science & Technology, vol. 55, no. 8, pp. 5595–5568, 2021.

[32] X. Liu, X. Jing, S. Zhuo, and Y. Yuan, “Flagella act as Geobacter biofilm scaffolds to stabilize biofilm and facilitate extracellular electron transfer,” Biosensors and Bioelectronics, vol. 146, no. 1, p. 111748, 2019.

[33] S. Wang, Y. Wu, J. An et al., “Geobacter autogenically secretes fulvic acid to facilitate the dissimilated iron reduction and vivianite recovery,” Environmental Science & Technology, vol. 54, no. 17, pp. 10850–10858, 2020.

[34] G. Zarbiv, H. Li, A. Wolf et al., “Energy complexes are apparently associated with the switch-motor complex of bacterial flagella,” Journal of Molecular Biology, vol. 416, no. 2, pp. 192–207, 2012.

[35] H. H. Hau and J. A. Granick, “Ecology and biotechnology of the genus Shewanella,” Annual Review of Microbiology, vol. 61, no. 1, pp. 237–258, 2007.

[36] G. Sturm, K. Richter, A. Doetsch, H. Heide, R. O. Louro, and J. Gescher, “A dynamic periplasmic electron transfer network enables respiratory flexibility beyond a thermodynamic regulatory regime,” ISME Journal, vol. 9, no. 8, pp. 1802–1815, 2015.

[37] L. A. Fitzgerald, E. R. Petersen, R. I. Ray et al., “Shewanella oneidensis MR-1 Msh pilin proteins are involved in extracellular electron transfer in microbial fuel cells,” Process Biochemistry, vol. 47, no. 1, pp. 170–174, 2012.

[38] H. C. Gao, S. Barua, L. Liang et al., “Impacts of Shewanella oneidensis c-type cytochromes on aerobic and anaerobic respiration,” Microbial Biotechnology, vol. 3, no. 4, pp. 455–466, 2010.

[39] H. H. Jian, H. Wang, X. Zeng, L. Xiong, F. Wang, and X. Xiao, “Characterization of the relationship between polar and lateral flagellar structural genes in the deep-sea bacterium Shewanella piezotolerans WP3,” Scientific Reports, vol. 6, no. 1, p. 39758, 2016.

[40] F. Wang, J. Wang, H. Jian et al., “Environmental adaptation: genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium Shewanella piezotolerans WP3,” PLoS One, vol. 3, no. 1, Article ID e1937, 2008.

[41] L. Xiong, M. Ishitani, H. Lee, and J. K. Zhu, “The arabidopsis LOSS/ABA3 locus encodes a molybdenum cofactor sulfurylase and modulates cold stress- and osmotic stress-responsive gene expression,” Plant Cell, vol. 13, no. 9, pp. 2063–2084, 2001.

[42] E. M. Armstrong, M. S. Austerberry, J. H. Birks et al., “Coupled proton-electron transfer to dithiolene complexes relevant to the molybdenum cofactor of the oxomolybdoenzymes,” Journal of Inorganic Biochemistry, vol. 43, no. 2-3, p. 588, 1991.

[43] L. Krippahl, P. N. Palma, J. Moura, and J. J. G. Moura, “Modelling the electron-transfer complex between aldehyde oxidoreductase and flavodoxin,” European Journal of Inorganic Chemistry, vol. 2006, no. 19, pp. 3835–3840, 2006.

[44] N. L. Creevey, A. G. McEwan, and P. V. Bernhardt, “A mechanistic and electrochemical study of the interaction between dimethyl sulfide dehydrogenase and its electron transfer partner cytochrome c5,” Journal of Biological Inorganic Chemistry, vol. 13, no. 8, pp. 1231–1238, 2008.

[45] D. P. Summers, “Ammonia formation by the reduction of nitrite/nitrate by FeS: ammonia formation under acidic conditions,” Origins of Life and Evolution of Biospheres, vol. 35, no. 4, pp. 299–312, 2005.

[46] H. C. Gao, Z. K. Yang, S. Barua et al., “Reduction of nitrate in Shewanella oneidensis depends on atypical NAP and NRF systems with NapB as a preferred electron transport protein from CymA to NapA,” ISME Journal, vol. 3, no. 8, pp. 966–976, 2009.

[47] D. Chaussabel and N. Baldwin, “Democratizing systems immunology with modular transcriptional repertoire analyses,” Nature Reviews Immunology, vol. 14, no. 4, pp. 271–280, 2014.