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Journal Name

COMMUNICATION

Selective colonization mechanism of *Shewanella putrefaciens* in dyeing wastewater outlet

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Large amounts of dyeing wastewater are discharged to rivers and oceans without appropriate treatment, especially in developing countries. It is imperative to control and administer these wastewater discharges to prevent ecological contamination of rivers and oceans. However, most of the mechanisms of bacterial colonization in contaminated rivers and oceans are unknown, especially in dyeing wastewater outlets. We found *Shewanella putrefaciens* to be the primary bacteria in the dyeing wastewater outlets around Ningbo City, China. Therefore, in this study, we utilized a combination of differential proteomics, metabolomics, and real-time fluorescent quantitative PCR techniques to investigate the selective colonization mechanism of *S. putrefaciens* and to excavate related functional proteins and metabolites to provide a theoretical basis for the biological treatment of dyeing wastewater. We found 26 different proteins were filtrated by 2-DE, referring to the synthesis of fatty acids and amino acids, sulfur metabolism, RNA degradation, energy metabolism, two-component signal transduction, oxidative stress, siderophore transport, ABC transport, etc. Furthermore, 14 candidate genes in mRNA expression levels were researched by qRT-PCR, and the results showed that 9 genes were up-regulated, 3 genes were down-regulated, and 8 genes presented consistency in protein and gene expression levels. Additionally, 57 different metabolites of 8 classes were detected. Most metabolites were up-regulated with the highest up-regulated ratios being thymine (61.13), then ethanol (28.61) and putrescine (20.74). Arginine, AMP and malate were down-regulated though. These metabolites are involved in twin-arginine transduction systems and two-component signal transduction systems, which may be related to the adaptability of *S putrefaciens* to dyeing wastewater. This work can help researchers understand the biological mechanism pathway of *S. putrefaciens* in dyeing wastewater. In our future research, we will look to apply this strain for dyeing wastewater treatment.

Introduction

The textile industry, an economically important and rapidly developing industry in Zhejiang Province and China as a whole, produces a large amount of printing and dyeing wastewater through synthetic dyes every year. This wastewater is often slightly acidic or slightly alkaline, has a low ratio of BOD5/COD (5 days biochemical oxygen demand to chemical oxygen demand, around 20%), and can contain dyestuff, high salt, chloride or bromide, high chroma, fiber, inorganic compounds, and dyeing additives.[1-3] In China, various dye-containing wastewaters constitute nearly 30% of industrial effluents.[4] Regardless of initial outfall location, the dying wastewater is ultimately discharged to marine environments, impairing the quality of seawater and posing a threat to marine organisms. Moreover, negative impacts are associated with various bacteria in dyeing wastewaters such as *Shewanella putrefaciens*, which may possibly bring about infections in humans and animals in beaches and mariculture zones.[5]

S. putrefaciens, a *psychrotolerant* Gram-negative non-fermentative oxidative motive bacillus previously known as *Pseudomonas putrefaciens*, was first isolated by Derby and Hammer.[6] Upon its discovery, it has been regularly found in water-related environments such as freshwater, seawater, lakes, rivers, sewage, fish, and soil.[7, 8] *S. putrefaciens*, an important food spoilage microorganism grown on chilled marine fish,[9, 10] and is also a rare pathogen in humans.[11] It can cause soft tissue infections as an opportunistic pathogen, and it can lead to liver cirrhosis, splenic abscess, diabetes mellitus, osteomyelitis, necrotizing fasciitis, abscess of the lower extremity and cerebellar abscess.[12-16] Moreover, the bacteria provide a valuable reference in terms of discoloration and degradation of dyeing wastewater.[17, 18] For example, Khalid studied the potential of *S. putrefaciens* AS96 for discoloration of four structurally different azo dyes at different concentrations of NaCl (AR-88, DR-81, RB-5 and DO-3).[19] In addition, it was demonstrated by Kamilaki that 85% Remazol Black B was removed in 6.5 h and 80% colour was removed from textile wastewater in 24 h using *S. putrefaciens*.[20]

In our previous research, we collected samples from 10 terrestrial sewage outlets in coastal areas of Ningbo (Fig. S1) and detected *S. putrefaciens* by using the 454 sequencing technique in March, May, August and October.

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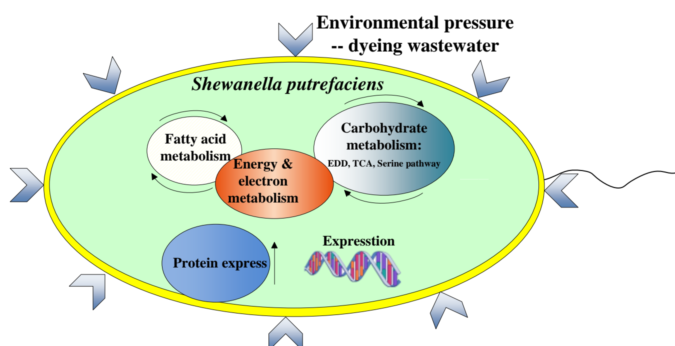

Figure 1 Schematic diagram of *S. putrefaciens* responses to the dyeing wastewater

We found that *S. putrefaciens* was present in high abundance at almost every sewage outlet most of the time (Fig. S2). In the dyeing wastewater outlet, the detection frequency of *S. putrefaciens* was also high. *S. putrefaciens* are mostly freshwater bacteria, and its colonization in seawater near terrestrial sewage outlets might affect the safety of aquatic life. Thus, it is important and necessary to understand how *S. putrefaciens* colonizes dyeing waste outlets and then treat contaminated waters accordingly.

To understand how *S. putrefaciens* responds to the harsh environments (dyeing wastewater, Fig. 1), we employed differential proteomics, metabolomics, and real-time fluorescent quantitative PCR techniques to investigate the selective growth mechanism of *S. putrefaciens* and to compare *S. putrefaciens* growth in freshwater and dyeing wastewater. It will be helpful to understand *S. putrefaciens* colonization in dyeing wastewater outlet from the metabolic pathway.

Experimental section

Materials and methods

Materials

S. putrefaciens was isolated from dyeing wastewater collected from the Xiangshan Junxi outlet (Fig. S5) and preserved in our laboratory. The composition of the dyeing wastewater is detailed in Tab. S1. The Total Solids (TS) content of the waste water was 4.49±0.03 g per liter and Total COD (TCOD) was 1376.93±157.2 mg per liter. All other reagents were purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. and BIO-RAD (USA).

Experimental bacteria

S. putrefaciens isolated from the dyeing wastewater outlets were inoculated to the sterilized culture medium (based on Zhang method) in a thermostatic incubator for 24 h at 28 °C.[21] The bacterial culture was aliquoted and stored in glycerol at -80 °C under aseptic condition. The remaining bacteria culture was stored at 4 °C. *S. putrefaciens* was grown in both freshwater as the control group (peptone 1%, beef extract 0.5%, NaCl 0.5%, deionized water) and dyeing wastewater as the experiment group (peptone 1%, beef extract 0.5%, dyeing wastewater). In both growth media, *S. putrefacians* was grown in culture dish triplicate in a thermostatic incubator at 28 °C for 24 h.

Protein sample preparation

Membrane proteins from *S. putrefaciens* were extracted by adding 1-2 mL of lysate buffer (7 M urea, 3 M thiourea, 64 mM DTT, 4% w/v CHAPS, 20 mM Tris (pH 8.8), 0.5% v/v Bio-lyte (Bio-Rad)) and mixing well. The mix was incubated on ice for 1 h with vortexing for 20~30 s every 15 min, then underwent ultrasounds in 5 s intervals for 3 min, followed by centrifugation at 4 °C, 12000 g for 15 min. Acetone (2 volumes of supernatant) was added to the supernatant and the solution was incubated at -20 °C for 6 h. After centrifugation, the supernatant was removed and the protein precipitate was collected.

3 mL of rehydration buffer (7 M urea, 3 M thiourea, 4% w/v CHAPS, 64 mM DTT, 0.5% v/v Bio-lyte) was added to the protein precipitate and was periodically vortexed. The protein concentration was then determined using the protein quantification kit (Sangon Biotech, Shanghai) with BSA standard.

Two-dimensional gel electrophoresis (2-DE)

The first-dimension of the 2-DE isoelectric focusing (IEF) was performed in the PowerPac Basic/Mini-Protean® Tetra Cell System (Bio-Rad, USA) according to the manufacturer's instructions. 250 μg of the proteins were diluted in 150 μL of the rehydration buffer and loaded onto a pH 4–7 immobilize dry IPG strip (7 cm, linear, Bio-Rad, USA). The conditions used for IEF were: rehydration and loading of the IPG strips at 50 v for 12 h, 250 v for 1 h, 500 v for 1 h, 1000 v for 1 h, and 4000 V for 3 h. After IEF, strips were equilibrated for 15 min at room temperature in 2.5 mL of SDS equilibration buffer (1.5 M pH 8.8 Tris-HCl 25%(V/V), 6 M urea, 20%(V/V) glycerol, 2% SDS) containing 0.13 M dithiothreitol for the first equilibration, and 0.135 M iodoacetamide for the second equilibration. Strips were then subjected to 12% SDS-PAGE analysis. The protein spots were dyed by Coomassie Brilliant Blue (CBB) and scanned using an Image Scanner GS-800(Bio-Rad, USA). Data and atlas analysis were conducted using the PDQuest 8.0 (Bio-Rad).

Mass spectrometry and protein identification

The protein spots from the 2-DE were accordingly excised from the stained gels. After being washed by MilliQ pure water and bleached by 50% methanol and trypsin digestion (20 ng/μL), the peptides were analysed by matrix-assisted laser desorption/ ionization tandem time-of flight (MALDI-TOF-TOF) mass spectrometer using Autoflex speed™ mass spectrometer (Bruker Dalton). Mass spectrometry conditions were as follows: UV wavelength of 355 nm, repetition rate of 200 HZ , acceleration voltage of 20000 V, optimal mass resolution of 1500 Da, and scanning mass range of 700-3200 Da for signal collection. Baseline peak filtration and signal peaks identification were based on flex analysis (Bruker Dalton) soft.

qRT-PCR

Crude RNA was extracted by liquid nitrogen grinding in combination with RNAiso (TaKaRa). First-strand cDNA synthesis was conducted using M-MuLV First-strand cDNA Synthesis Kits (Sangon) with Moloney Murine Leukemia Virus Reverse Transcriptase (M-MuLV RT) according to the manufacturer's instructions. Different amino acid sequences were acquired from NCBI GenBank, and corresponding qRT-PCR primers were projected by the Primer5 software with reference genes of 16s rRNA. The 2−ΔΔCT method was used to analyze the expression level of candidate genes with Rotor-Gene 6000 series 1.7 software. The data was subjected to one-way analysis of variance (ANOVA). A T-Test was performed for the variance analysis between the control group and experimental group.

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Table 1. List of spots/proteins identified by MS analysis from *S. putrefaciens* by 2-DE

No.	Name of protein	Up/down
1P	Outer membrane porin, Omp35	Down
2P	Porin	Down
3P	Aromatic hydrocarbon degradation membrane protein	Down
4P	OmpA/MotB domain-containing protein	Down
5P	Elongation factor P	Up
6P	3-ketoacyl-ACP reductase	Down
7P	2-oxoglutarate dehydrogenase,	Down
8P	Succinyl-CoA synthetase subunit beta	Down
9P	Cysteine synthase A	Down
10P	Polynucleotide phosphorylase	Down
11P	Phosphoenolpyruvate synthase	Down
12P	Aconitate hydratase 2	Down
13P	Chaperonin GroEL	Down
14P	Trigger factor	Down
15P	TonB-dependent receptor	Up
16P	Phosphoenolpyruvate synthase	Up
17P	Elongation factor Tu	Up
18P	Alkyl hydroperoxide reductase	Up
19P	Elongation factor Tu	Up
20P	ABC transporter	Up
21P	F0F1 ATP synthase subunit alpha	Down
22P	Dihydrolipoamide dehydrogenase	Down
23P	Chorismate mutase	Down
24P	Glu/Leu/Phe/Val dehydrogenase	Down
25P	Malate dehydrogenase	Down
26P	Glycine cleavage system aminomethyl transferasT	Down

Extraction of intracellular metabolites and 1H NMR appraisal

Intracellular metabolites were extracted from *S. putrefaciens* using the method of liquid nitrogen grinding in combination with freeze drying. After filtration by prewash Millipore Amicon membranes (Millipore Amicon® ULTRA 3 KDa), 500 µl of ultrapure water was added to the membrane and centrifuged (13000 g, 5 min) at room temperature. This washing and centrifugation of a sample was done 5 times. We utilized ACDSS reagent to extract intracellular metabolites with the detection of Bruker AV III 600 MHz spectrometer. The 1H NMR parameters employed are as follows: temperature: 298 K, magnet frequency: 600.13 MHz, transients/scans: 128, recycle delay: 1, frequency domain size: 65536, spectral width: 12019.231, time domain size: 32768, and pulse sequence: noesypr1d/noesygppr1d. The data processing and analysis were conducted by Chenomx 1H NMR suit (version 7.7, Chenomx, Edmonton, Canada) software with DSS-d6 peak as the standard.

Results and discussion

2-DE and mass spectrometry results

Based on 2-DE and mass spectrometry technology, 26 differentially expressed proteins were identified (Fig.2). A comparison of growth on experimental and control media demonstrated that 7 of these proteins were up-regulated (P5, P15, P16, P17, P18, P19, P20) and the other 19 proteins were down-regulated by dyeing wastewater. The GO analysis

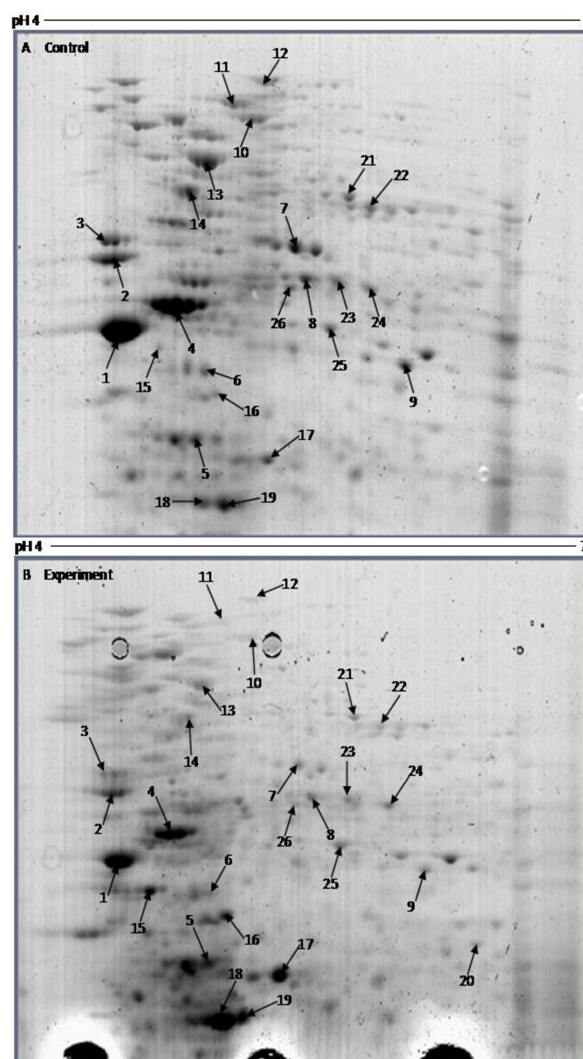

Figure 2. Protein expression patterns of *Shewanella putrefaciens* under different culture conditions: freshwater (A); dyeing wastewater (B). Arrows and spot numbers refer to differentially expressed proteins

demonstrated that these proteins participate in metabolic pathways and the biosynthesis of fatty acids, amino acids and secondary metabolites. These proteins are also involved in the TCA cycle, sulfur metabolism, RNA degradation, methane metabolism, energy metabolism, signal transduction, oxidative stress defense, transport of siderophores, etc. The detailed information and involved metabolic pathways of proteins are shown in Table 1, and more details are shown in Table S2.

The transportation of a specific amino acid to the translating ribosome is indispensable to protein synthesis. During the process of mRNA translation, the binding of aminoacyl-transfer RNA to the ribosome is catalysed by EF-Tu, which is a GTP-binding protein crucial for protein biosynthesis.[22, 23] In this study, EF-Tu expression within dyeing wastewater environments was identified as having an obvious up-regulated expression pattern, which indicates the need for vigorous synthesis of relative proteins against dyeing wastewater. Ramiah reported that the up-regulated EF-Tu played some role in *L. plantarum* 423 adherence to Caco-2 cells, and may be regarded as a multifunctional protein that assisted *L. plantarum*

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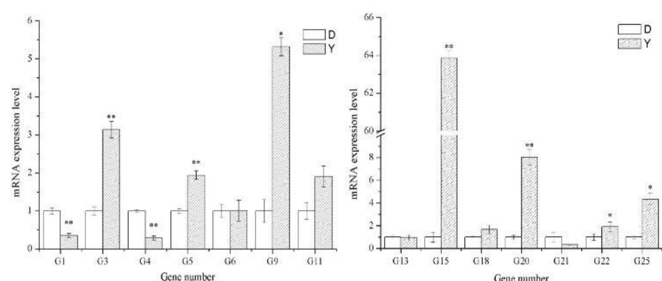

Figure 3 mRNA expression levels of the 14 differentially expressed proteins detected by qRT-PCR; freshwater (D) and dyeing wastewater (Y)

423 in adapting to environmental changes.[24] Ristic showed that the accumulation of chloroplast EF-Tu (up-regulation), induced by heat stress, conferred heat tolerance by acting as a molecular chaperone and protecting chloroplast proteins from thermal aggregation and inactivation.[25] This suggests that the expression of EF-Tu, whether gene or protein, was up-regulated in *S. putrefaciens* extracted from dyeing wastewater.

AhpC, a member of the peroxiredoxin family, which is known to detoxify H_2O_2, organic peroxides, and peroxynitrite, is responsible for protecting cells from the toxic effects of reactive oxygen species generated during normal aerobic metabolism processes and during times of oxidative stress.[26] Euna reported[27] the *ahpC* mutant of *Campylobacter jejuni* exhibited increased accumulation of total reactive oxygen species (ROS) and lipid hydroperoxide (LPO) compared to the wild type. Moreover, Charoenlap[28] demonstrated the *Helicobacter cinaedi ahpC* mutant, compared to the wild-type strain, exhibited diminished resistance to organic hydroperoxide toxicity and significantly reduced cecal colonizing ability in BALB/c and BALB/c interleukin-10−/− mice. Cosgrove[29] concluded as well that *ahpC* was required for nasal colonization of *Staphylococcus aureus*. In vivo, *ahpC* is responsible for oxidative-stress resistance and has broad-spectrum activity against H_2O_2, organic peroxides, and peroxynitrite. The substantial up-regulation of *ahpC* in the dyeing wastewater may imply that the protein contributes to the defence mechanisms of *S. putrefaciens*, as well as to bacterial survival and persistence.

The TonB-dependent receptor, classified as a transport and binding protein, is responsible for the transport of siderophores into the periplasm in Gram-negative bacteria to satisfy nutrient requirements under iron-limiting conditions.[30] The expression level of TonB-dependent receptors is modulated under heavy stress. For example, TonB-dependent receptors, as iron-regulated outer membrane virulence protein of *Shewanella oneidensis* were induced and identified as up-regulated during chromate-treatment conditions and strontium stress.[31, 32] Thompson[33] also confirmed that the TonB-dependent receptor showed increased abundance in response to Cr(VI) doses exposures. Additionally, ABC transporters embedded within the cytoplasmic membrane of Gram-negative bacteria and that consist of an outer membrane receptor, a periplasmic binding protein, and a complex of cytoplasmic membrane proteins with an associated ATP-binding cassette, are responsible for the transport of siderophore into cytoplasm.

Under dyeing wastewater, EF-Tu, *AhpC*, ABC transporters, and TonB-dependent receptors all showed increased expression patterns, indicating that expression of these proteins is an important feature of the response of *S. putrefaciens* to dyeing wastewater.

Some stress proteins, including molecular chaperones and heat shock proteins, are easily induced in response to a wide range of stress challenges, such as heavy metals, pH levels, oxidation, and unsuitable temperatures. GroEL is also known as a heat-shock protein (HSP) and has an essential role in mediating protein folding.[34] Trigger factor (TF), the ribosome-bound protein associated with nascent polypeptides, displays chaperone and prolylisomerase activities in vitro. Nishihara demonstrated that TF over-expression had marked effects on the production of these proteins in soluble forms, presumably through facilitating correct folding. Nishihara further suggested that TF and GroEL-GroES play synergistic roles in vivo.[35] This protein was expressed in the dyeing water group, possibly reflecting a series of adaptive changes when faced with environmental stress.

The outer membrane is located outside the cytoplasmic membrane (inner membrane) and peptidoglycan, encompassing the entire bacterium, which is a unique component for the cell wall of Gram-negative bacteria. Outer membrane proteins are the main component of outer membranes of Gram-negative bacteria. They not only play an important role in substance transport, morphology maintenance and synthesis of relevant substances, but also in the adaptation of the organisms to the external environment.[36] Omp35 is a 35kDa outer membrane protein, which

Table 2. Primers used in the present study

No.	Sequence (5'-3')	Size/ bp	protein
1F	ACCGTTTAGTGAGCGCTCAA	259	outer membrane porin, Omp35
1R	AACTTAGCCTGAGCCACACC		
3F	TAGGTGGGATTGTAGGGGCA	253	aromatic hydrocarbon degradation membrane protein
3R	GCTGTGTAGTGCAACGCAAA		
4F	CGCCTTTTACGTCAGCATCG	195	OmpA/MotB domain-containing protein
4R	ATGTTGGTGCAGGTCTAGGC		
5F	CGCGCTTCTTGAATTCACCA	114	elongation factor P
5R	AAGTGATGAAGCCAGCGACT		
6F	ACGGCAAAGTCACTGATCGT	189	3-ketoacyl-ACP reductase
6R	AAGCCGCGTAGTTTGACTGA		
9F	CACTTGGTGCGAACCTTGTG	249	cysteine synthase A
9R	AGTAACGGCTCACACCTGTG		
11F	ACGACCATTTCACCCAGACC	220	phosphoenolpyruvate synthase
11R	CTATCAAGCACGTGTTCGCG		
13F	AAATCGAAGCGCGTGTAAGC	219	chaperonin GroEL
13R	CAGGAACCACGCCTTCTTCT		
15F	ACCAATGGCATTTGCAGCAG	199	TonB-dependent receptor
15R	TGCAGTGTTTAACGCAGCAC		
18F	AGGCGGCATTGGTCAAGTTA	259	alkyl hydroperoxide reductase
18R	GCCTTTTTCCCAACCTGCTG		
20F	CATTAAAGCCACGGTAGCGC	284	ABC transporter
20R	CCTGCGCGATTTTAACCTCG		
21F	CTTCAGAAGCCGTTGCAACC	188	F0F1 ATP synthase subunit alpha
21R	CGGTGACCGTCAAACTGGTA		
22F	CGTTGATAAAGCCACGCTCG	271	dihydrolipoamide dehydrogenase
22R	TTCGACCAAGTGATCCCTGC		
25F	ATCACGGGCGTTAGCTTCAA	255	malate dehydrogenase
25R	GCGGGTACTGAAGTGGTTGA		

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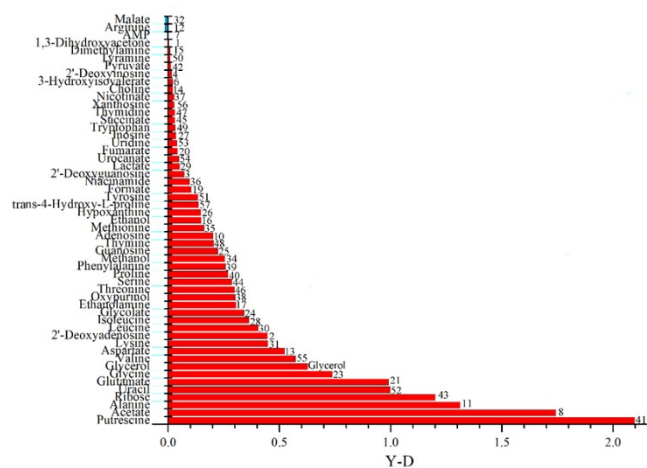

Figure 4. Intracellular metabolites from *S. putrefaciens* detected by 1H NMR. The number on the right side of column is NMR number of ownership spectrum. D: freshwater; Y: dyeing wastewater.

participates in the electron transport on the respiratory chain in the environment of fumaric acid esters, nitrates and iron (III).[37] This protein maybe down-regulated to adapt to the dying waste environment.

The specific colonization mechanism of the dye waste by *S. putrefaciens* seen in the present study is unclear. Decolorization of the dying solution by bacteria could be due to adsorption to microbial cells or due to biodegradation.[38, 39] Our results suggest that dying waste environmental stress effect the protein expression in *S. putrefaciens*.

qRT-PCR

To further characterize the expression of identified proteins at the gene level and understand the effects of dyeing wastewater on *S. putrefaciens*, the expression profiles of 14 differentially expressed proteins potentially involved in physiological processes were investigated by qPCR (Tab. 2 and Fig. 3). These proteins include P1(Omp35), P3(aromatic hydrocarbon degradation membrane protein), P4 (OmpA/MotB domain-containing protein), P5(elongation factor P), P6(3-ketoacyl-ACP reductase), P9(cysteine synthase A), P11(phosphoenolpyruvate synthase), P13(chaperonin GroEL), P15(TonB-dependent receptor), P18(alkyl hydroperoxide reductase), P20(ABC transporter), P21(F0F1 ATP synthase subunit alpha), P22 (dihydrolipoamide dehydrogenase), and P25 (malate dehydrogenase). The expression levels of twelve genes showed significant differences between the two groups. Among them, nine genes, (G3, G5, G9, G11, G15, G18, G20, G22, and G25) were up-regulated by dyeing wastewater–dependent treatment. However, the expression levels of G1, G4, and G21 were negatively consistent with the above 12 genes. No statistically significant change was detected for G6 and G13 between the two groups. Furthermore, eight proteins (P1, P4, P5, P13, P15, P18, P20, and P21) demonstrated absolute consistency at the protein and gene levels.

Metabolomics

A typical 1H NMR spectrum of intracellular metabolites of *S. putrefaciens* after respective cultivation for 24 h in fresh water and dyeing wastewater was shown in Figure S4. 57 metabolites were detected, including a wide range of amino acids (Alanine, Arginine, Glutamine, etc.), nucleic acid components (AMP, Ribose, Thymine, etc.), organic acids (Glutamate, Acetate, Formate, etc.), alcohols (Ethanol, Methanol), amines (Ethanolamine, Dimethylamine, Putrescine), amino acid derivatives (Tyramine, Urocanate), ammoniums compounds (Choline), vitamin/cofactors (Niacinamide, Nicotinate), and Glycerol. The majority of intracellular metabolites under dyeing wastewater were up-regulated; for example, Thymine had the highest up-regulation ratio (61.13), followed by Ethanol (28.61), Putrescine (20.74), Serine (17.75), Leucine(14.35), stc. Conversely, 3 metabolites (AMP, Arginine, Malate) showed the opposite expression pattern (Fig. 4).

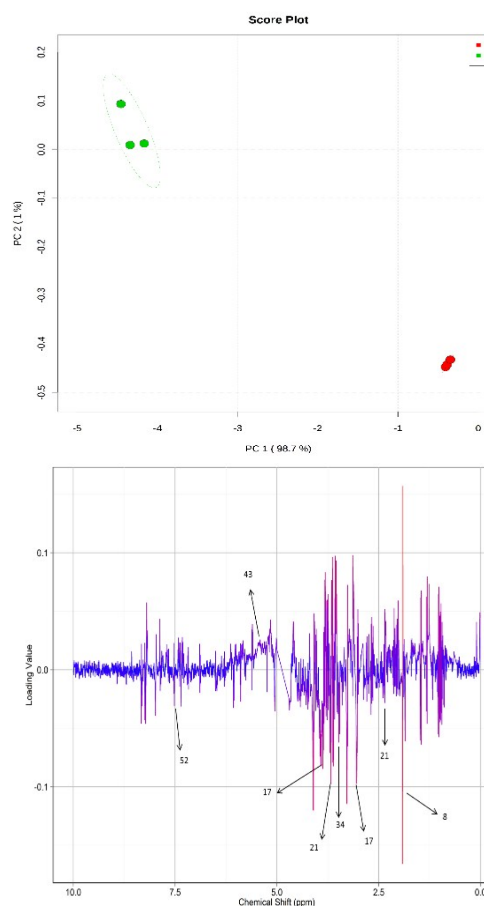

Figure 5. The PCA analysis graphics and 1H NMR spectra of intracellular metabolites from control group (red) and experimental group (green) of *S. putrefaciens*. (8) Acetate, (17) Ethanolamine, (21) Glutamate, (34) Methanol, (43) Ribose, (52) Uracil. D: freshwater; Y: dyeing wastewater.

Principal component analysis (PCA) and further analysis on the NMR spectral data of *S. Putrefaciens* is displayed in Fig. 5. The PCA figure revealed two clusters of *S. putrefaciens* with a clear separation between the first principal component (PC1, 98.7%) and the second principal component (PC2, 1%). This resulted in significant ($p<0.01$) differences between the control and the dyeing wastewater-treated groups. In addition, the loading value of NMR data provided key metabolites with significant differences between two groups.

Metabolites are the end products of cellular regulatory processes, and their levels can be regarded as the ultimate response of biological systems to environmental changes, the stimulation of exogenous substances or genetic modification.[40]

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Metabolomics, dynamic response of proteomics on metabolite levels, is closely related to proteomics. In the methane metabolism pathway, for instance, malate dehydrogenase consistently catalyzes oxaloacetate to malate. The enzyme and malate were all down-regulated in our study. The down-regulation of cysteine synthase, which facilitated the conversion of serine to cysteine, was also consistent with the up-regulation of serine.

Formate is an important metabolite in many aerobic bacteria, anaerobic bacteria and some yeasts, and is used in many growth medium. We proposed that *S. putrefaciens* undergo formate metabolism under wastewater stress (Fig. S6). Formate and Glycine were closely related to the biosynthesis of tetrahydrofolate (THF, vitamin B9), which is involved in one-carbon transfer reactions in all living cells and an absolute requirement for the synthesis of purine, thymidylate, formylmethionyl-tRNA, pantothenate, and methionine, and for the interconversion of glycine and serine.[41] The glycine cleavage system aminomethyltransferase T, catalyzing the conversion between 5,10-methylene-THF and THF, was up-regulated. Acetate, derived from sulfide by cysteine synthase catalysis in the sulfur metabolic pathway, participates in carbon fixation pathways in prokaryotes. Pyruvate, as an intermediate product of glycolysis, could repair the cell damage through antioxidation and modulation of energy metabolism. These metabolites were all up-regulated in our study as well.

Amino acids (AA), as cell signalling molecules and regulators of gene expression and protein phosphorylation cascades, were key precursors for the synthesis of low-molecular weight substances; however, elevated levels of AA and their products are pathogenic factors for neurological disorders, oxidative stress, and cardiovascular disease.[42] In our study, the majority of AA displayed up-regulated patterns, which appeared to be a corresponding response to the dyeing wastewater stress. Additionally, L-cysteine was directly related to the biosynthesis of glutathione (GSH), cysteine, and methionine metabolism.[43] Ye considered that the levels of several AA such as leucine, isoleucine, and valine were accordingly increased under temperature stress.[44] Glutamine and arginine was indicated to potentially regulate the utilization and metabolism of serine and aspartate families of AA.[45]

Conclusions

We have studied the changes of proteomics and metabolomics of *S. putrefaciens* under dyeing wastewater environment. The results suggested that dyeing wastewater could induce oxidative stress, and disturbances in energy metabolism, protein synthesis, and metabolites synthesis. Furthermore, Elongation factor Tu, alkyl hydroperoxide reductase, TonB-dependent receptor, molecular chaperones and trigger factor were modulated, which regulated the synthesis of the corresponding metabolites. Metabolites levels, like those found in this study, could serve as an ultimate indicator of proteins. The combination of proteomics and metabolomics have proved to be an effective method to investigate the molecular mechanisms of organisms.

Acknowledgements

This research was supported by the Ocean Public Welfare Scientific Research Project, State Oceanic Administration of the People's Republic of China (Grant No. 201105007). We also would like to acknowledge financial support from the Chinese National Science Foundation (Grant No. 2008C50027). We wish to thank Andrew Kim and Alexander Marzilli for a critical review, and the owner of the boat used to take samples.

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