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Short Communication

Short Commentary on Label-Free Proteomics Study on *Shewanella putrefaciens*

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ABSTRACT

Shewanella putrefaciens are the spoilage organisms found in aquatic food products stored at low temperature. A brief description of Label-free proteomics of *S. putrefaciens* treated with ε-Poly-lysine was followed in this review, soughting to investigate the anti-bacterial mechanism of *S. putrefaciens* through Gene Ontology (GO), and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. The global changes at protein levels were tentatively identified and this will contribute to further understanding of the putative mechanisms involved in the growth inhibition of *S. putrefaciens*.

Keywords: *Shewanella putrefaciens*; Protein; Mechanisms; Label-free proteomicsc

INTRODUCTION

Shewanella putrefaciens, gram-negative bacteria, are well known for being involved in fish spoilage even stored at low temperature [1]. With the ability of reducing trimethyl-amine-N-oxide (TMO) to trimethylamine (TMA), it produces the "fishy" off-odor of spoiling fish. Furthermore, they can also utilize sulfite/thiosulfate as substrate for the production of volatile sulfides with unpleasant flavour [2]. *S. putrefaciens* has strong putrefaction ability and can produce volatile compounds, such as tetradecanal and 1-hexadecanol, 1-octene-3-alcohol, nonanal and 2- ethylcyclohexanol, and its role has been extensively explored in the spoilage process of aquatic food products [3-6].

Anti-bacterial method has been extensively reported in the past decades, however, the anti-bacterial mechanism involved in the growth inhibition of *S. putrefaciens* have not been elucidated [7-10]. Therefore, a number of researchers have focused on the observation of cell morphology and physiological activities, hoping to identify the antibacterial mechanism. Some theories have been gradually formed, such as damaging the cell wall [11-13], inhibiting enzyme activity and nucleic acid synthesis [14], breaking the biofilm formed [15,16], and influencing the metabolic process [14], but this will need to be confirmed *in vivo* in the future using other advanced technologies, such as Label-free proteomics.

Label-free proteomic analysis offers an unbiased approach to assess the proteinogram of a species under various conditions [17-21]. However, there has been a paucity of studies to investigate the molecular mechanisms involved in the inhibition of spoilage bacteria through label-free proteomic analysis. To identify putative mechanisms involved in the growth inhibition of *S. putrefaciens* treated with ε-PL, we utilized it to determine the global changes at protein levels.

PROTEINS DIFFERENTIAL EXPRESSION

Comparison of bacteria protein between 0.1% ε-PL treatment and control group, a total of 36 differentially abundant proteins were identified. The number of up-regulated and down-regulated proteins was 10 and 26, respectively. Under the condition of proteins were detected at twice in the three biological replications while were not detected in the control group, identified 39 DEPs and the number of up-regulated and down-regulated proteins were 11 and 28, respectively. Furthermore, the DEPs were subsequently subjected to bioinformatics analysis to further analyze the effect of on protein expression.

GO ANALYSIS OF DEPS

GO level analysis was conducted to obtain the significantly enriched functions from three main categories, including Biological Process (BP), Molecular Functions (MF) and Cellular Components (CC) associated with the DEPs. A GO enrichment analysis was

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performed to obtain the metabolic activities affected most by the ε-PL treatment (Figure 1).

(1)MF analysis indicated that the DEPs were generally related to structural molecule activity, structure constituent of ribosome, cyclase activity, and adenylate cyclase activity, etc, in which structural molecule activity and structure constituent of ribosome presented extremely significant difference (P<0.01).

(2)BP analysis revealed that the differentially abundant proteins mostly involved in cell motility process, including cell motility, bacterial-type flagellum-dependent cell motility, movement of cell or subcellular component, and cilium or flagellum-dependent cell motility.

(3)CC analysis indicated that the main DEPs belonged to membrane, cell, macromolecular complex, organelle, membrane part and cell part categories. Further analysis indicated that 8 cellular components, including organelle, non-membrane-bounded organelle, cytoplasmic part, ribosome, intracellular ribonucleoprotein complex, intracellular non-membrane-bounded organelle, ribonucleoprotein complex and intracellular organelle, presented extremely significant difference (P<0.01).

KEGG PATHWAY ANALYSIS OF DEPS

To determine the metabolic and signaling pathways associated with the DEPs treated by 0.1% ε-PL and further to reveal the critical factors influencing the processes, KEGG pathway annotation and enrichment were performed. Compared with control group, 6 KEGG pathways in *S. putrefaciens* cells were significantly influenced by ε-PL treatment (P<0.05) and the ribosome pathway was especially significant (P<0.001), followed by the pathwats flagellar assembly, toluene degradation and fluorobenzoate degradation, etc. (Figure 2).

In this study, ribosome pathway was significantly affected (P<0.001) by ε-PL in *S. putrefaciens*. Despite the ribosome pathway, the flagellar-assembly pathway was inhibited by ε-PL notably as well (Figure 3). Three DEPs belonging to flagellar protein were involved in the flagellar-assembly pathway in *S. putrefaciens*, including flagellin, flagellar P-ring protein and flagellar M-ring protein. This indicated that ε-PL had a significant effect on *S. putrefaciens* mobility.

PPI NETWORK ANALYSIS FOR S. PUTREFACIENS INDUCED BY E-PL

The Proton Pump Inhibitor(PPI) was lay out by degree values of the nodes, which were equal to the number of proteins connected with these protein nodes. In particularly, 19 DEPs possessed no less than 2 interactions and 15 of these were already annotated. The DEPs possessing degree values>6 included the 50S ribosomal proteins L18, L27, L30, L31 and L33 and the 30S ribosomal proteins S16 and S20. Additionally, a DNA-directed RNA polymerase subunit beta encoded by rpoC (A0A252ERG2) interacted with the 5 DEPs and participated in both purine and pyrimidine metabolic pathways (Figure 4). The ribosomal protein DEPs are essential for protein synthesis and RpoC is an RNA polymerase III β' subunit proteinand necessary for transcription initiation and extension and is also important action site for antibacterial agents. Therefore, the downregulation of these ribosomal proteins represents an interference with essential protein synthetic functions in *S. putrefaciens*.

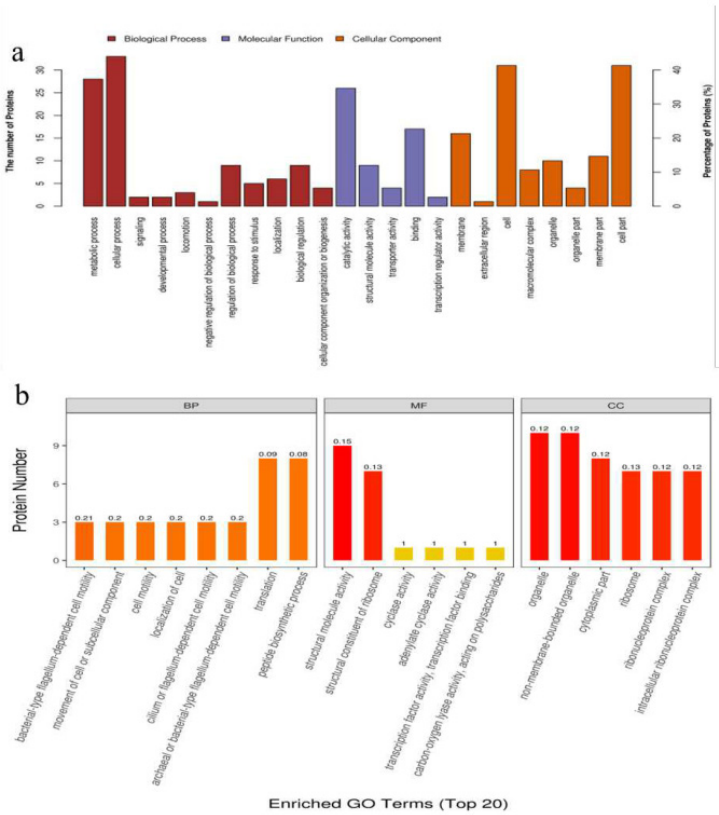

Figure 1: GO enrichment analysis of differentially expressed proteins in 0.1% ε-PL-treated *S. putrefaciens* compared with control groups.

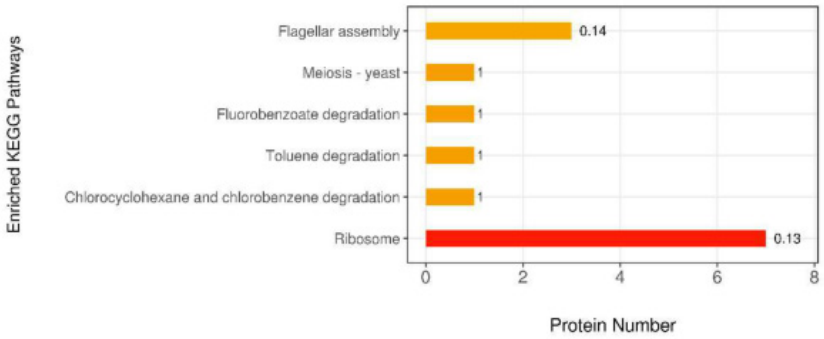

Figure 2: KEGG pathway enrichment analysis of differentially expressed proteins from 0.1% ε-PL *S. putrefaciens* cells.

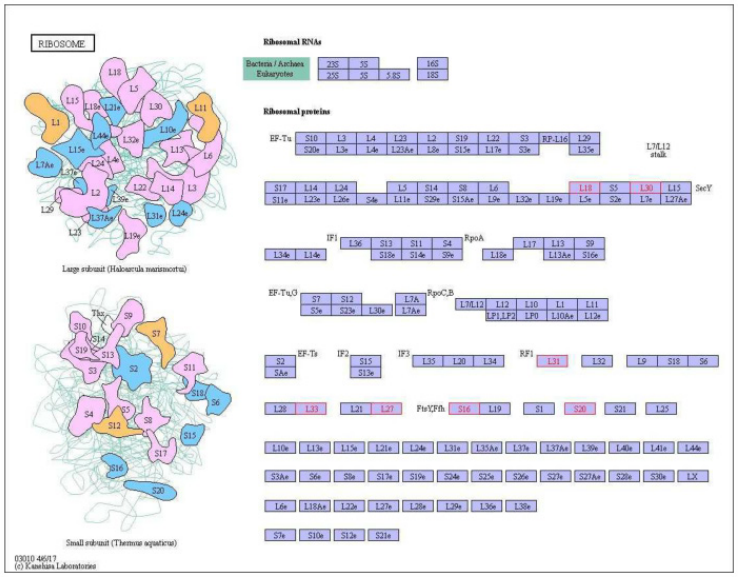

Figure 3: Significantly enriched ribosome KEGG pathway of DEPs from 0.1% ε-PL *S. putrefaciens* cells.

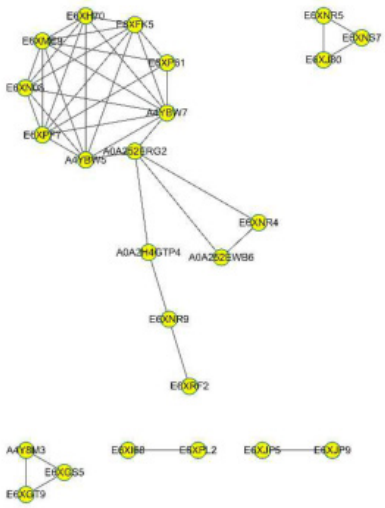

Figure 4: Interactive network diagram of proteins differential expression between the control and 0.1% ε-PL-treated groups.

CONCLUSION

The proteomics methods will provide a new visual angle and method in the search for the molecular mechanisms involved in the inhibition of spoilage bacteria. Up to now, proteomics has been used to explore the influence mechanism of many influencing factors on *S. putrefaciens*, including environment, signal molecules and natural plant ingredients. These results above provided antibacterial mechanism in *S. putrefaciens*, which may be served as the basis for the subsequent study of identifying potential antimicrobial targets to obtain more effective control of this spoilage organism.

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