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2-DE Compared with iTRAQ-based Proteomic Analysis of the Functional Regulation of Proteins in Rhodococcus sp. BAP-1 Response to Fluoranthene

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Abstract. Although the degradation pathways of Polycyclic aromatic hydrocarbons (PAHs) have been extensively studied in many bacteria, the variations in the expression levels of the key functional regulation of proteins during catabolism are still not quantitatively understood. In this study, we compared two proteomic methods, that one is two-dimensional gel electrophoresis (2-DE), a traditional widely used way and the other is isobaric tags for relative and absolute quantization (iTRAQ), an innovative approach, in order to analyze the functional regulation at the protein level in high effective fluoranthene-degrading bacteria named Rhodococcus sp. BAP-1. The number of differentially expressed proteins identified using iTRAQ is much larger than employing 2-DE. Response to fluoranthene, the key over expressed proteins in BAP-1 were NADPH-dependent FMN reductase, 30S ribosomal protein S2, S-ribosylhomocysteinase, etc.; the significant down-regulated proteins were cytochrome ubiquinol oxidase subunit, NAD(P) transhydrogenase subunit alpha, 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, et al.

1. Introduction
In the past few years, with the rapid development of chemical industry and auto industry, oil, one of the most important non-renewable energy resources, has been widely used as a kind of important fossil energy. During oil exploitation and petroleum transportation, oil spill occurs inevitably and makes serious damage for soil, water, air and other natural resources. Polycyclic aromatic hydrocarbons (PAHs), which could lead to toxicity, mutagenicity and carcinogenicity to human being, are primary pollution components among oil. The principle processes for their successful removal is generally considered microbial remediation. In our previous study, Rhodococcus sp. BAP-1 had a good degradation capacity in the presence of 20 mg/L of fluoranthene. Also, in Ahmed's research [1], Rhodococcus sp. CMGCZ was found to be capable to degrade high concentration of fluoranthene. Although the degradation pathways of PAHs have been extensively studied in many bacteria, the variations in the expression levels of the key functional regulation of proteins during catabolism are still not quantitatively understood.

Comparative proteomics, which detects the relative abundance of differentially expressed proteins under different conditions, focuses on up- or down-regulated proteins to analyze the characteristics of the expressed proteins among different samples under different conditions. Two-dimensional gel electrophoresis (2-DE), a traditional proteomic technology, separates thousands of proteins according to their isoelectric point by isoelectric focusing (IEF) in the first dimension and according to their molecular weight (MW) by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in the second dimension. Differential proteomics, which uses Isobaric tags for relative and absolute quantization (iTRAQ) to detect the abundance of differential expressed proteins, pay more attention to
up or down regulated proteins in order to analyze characteristics of expressed protein among different samples under different scenes [2].

2. Materials and methods
Rhodococcus sp. BAP-1 (GenBank entry: JX683682, CGMCC ID: 7.68) in duplicates were cultured and harvested at specific time points (1d and 3d). Protein extraction from two biological replicates was done consecutive day of sampling. Proteins were extracted from BAP-1 treated with fluoranthene using the Total Protein Extraction Kit (BestBio Company, China) following the manufacturer’s protocol. After undergoing digestion, proteins were separated by 2-DE or iTRAQ, separately. Then, peptides experimented fractionation and liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS/MS) proteomic analysis.

3. Results

3.1. Differential protein plots by 2-DE
Proteins were extracted from Rhodococcus sp. BAP-1 induced by fluoranthene (3mg/L) using the Total Protein Extraction Kit (BestBio Company, China). The differential proteins’ expression of Rhodococcus sp. BAP-1 after the first and the third day was separated by 2-DE (Fig.3-1), and then selected 11 significantly differential proteins pots were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Fig.3-2)

![Figure 3-1](image1)

**Figure 3-1.** Two-dimensional electrophoretic map of *Rhodococcus* sp. BAP-1 induced by fluoranthene on the first day (a)

![Figure 3-2](image2)

**Figure 3-2.** Two-dimensional electrophoretic map of *Rhodococcus* sp. BAP-1 induced by fluoranthene on the third day (b)
3.2. Differential protein plots by iTRAQ

iTRAQ labels 113, 114, 115 and 116 have been used to label fluoranthene-exposed time of 1d’, 1d”’, 3d’ and 3d”’, respectively. We set 115/113 (3d’/1d’) and 116/114 (3d”’/1d”’) as comparison clusters and then took the average as 3d/1d. We filtered the results by applying the statistical criteria of only protein a fold-change higher than 50% (ratio of either <0.66 or >1.5, p<=0.05) with at least two unique peptides in all two replicate experiments as the significantly altered relative levels. Then, we were able to detect 591 proteins, including 400 up-regulated and 191 down-regulated proteins. The number of proteins with increased levels were much more than proteins with decreased levels, demonstrating that Rhodococcus sp. BAP-1 exposure to fluoranthene preferred to induce more new or low-expressed proteins compared with before in order to adapt to new situation, rather than restrain or weaken original proteins which play the role of sustaining life activities.
Table.3-1. The Top10 up-regulated Differentially Proteins in BAP-1 Induced by Fluoranthene

| Protein ID       | Description                                         | Ratio (3d/1d) |
|------------------|------------------------------------------------------|---------------|
| Unigene50806_All | NADPH-dependent FMN reductase                        | 10.00         |
| Unigene14485_All | 30S ribosomal protein S2                             | 10.00         |
| CL276.Contig3_All| S-ribosylhomocysteinase                              | 10.00         |
| Unigene15123_All | iron ABC transporter ATP-binding protein             | 10.00         |
| Unigene44773_All| Nucleoside-diphosphate kinase                        | 10.00         |
| Unigene20021_All| ATP synthase F1 subunit alpha                        | 10.00         |
| CL302.Contig6_All| xylose isomerase domain-containing protein           | 10.00         |
| Unigene3703_All | DinB family protein                                  | 9.87          |
| CL983.Contig2_All| cytochrome ubiquinol oxidase subunit                 | 0.14          |
| CL2066.Contig2_All| 5-methyltetrahydropteroyltrimetilglutamatom-homocysteine methyltransferase | 0.19 |
| Unigene9285_All | formate acetyltransferase                            | 0.19          |
| Unigene9332_All | quinoprotein glucose dehydrogenase                   | 0.20          |
| Unigene21188_All| aldehyde dehydrogenase                              | 0.20          |
| Unigene3186_All | ribonucleotide-diphosphate reductase subunit alpha   | 0.21          |
| Unigene18249_All| acyl-CoA dehydrogenase                               | 0.21          |
| Unigene9514_All | glycerol-3-phosphate dehydrogenase                   | 0.22          |
| Unigene24119_All| pyruvate decarboxylas                                | 0.24          |

We separately selected the top10 changed (up- or down-) expressed proteins for further analysis (Table.3-1 and Table .3-2).

Dehydrogenase plays an important role in the oxidation of n-alkane and ensured an adequate metabolic flux into the downstream catabolic pathway [3, 4]. It is possible that the bacteria increased the expression of isocitrate dehydrogenase and decreased quinoprotein glucose dehydrogenase, aldehyde dehydrogenase, acyl-CoA dehydrogenase and glycerol-3-phosphate dehydrogenase to provide energy in order to enhance its survival in a restricted environment. Substrate-binding protein (SBP)-dependent transporters are extensively present in bacteria and are involved in diverse processes, such as nutrient uptake, quorum sensing and multidrug resistance, which were considered as the primary choices for high-affinity uptake of nutrients[5, 6]. Iron ABC transporter ATP-binding proteins were probably the
members responsible for specific transportation systems for fluoranthene or other PAHs. Ribosomal proteins (RPs) are significant in development and gene expression [7, 8]. The expression of RP genes are largely regulated by the environment and cell’s development. RPs have been found to be involved in DNA repair, cell development, protein biosynthesis, regulation, and cell differentiation [9]. The up-regulation of 30S ribosomal protein S2 helps the bacteria adapting to restricted PAHs conditions, reduce toxicity, and maintain the normal metabolism of cells.

3.3. Comparison between 2-DE and iTRAQ.
In this study, the number of differentially expressed proteins identified using iTRAQ is much larger than employing 2-DE. Owing to abundant data, we could launch functional analysis based on Clusters of Orthologous Groups (COG) categories, the gene ontology (GO) classification system and the Kyoto Encyclopedia of Genes and Genomes (KEGG) database [10].

4. Conclusions
As always, 2-DE, which can provide visual glue map, is a conventional proteomic method. iTRAQ has been increasingly applied in proteomic studies due to the advantages of this methodology over 2D gel electrophoresis. There are several reasons accountable for the superiority of iTRAQ compared with 2-DE. First, iTRAQ allows the analysis of multiple samples (up to eight) simultaneously, whereas 2-DE allows only one sample at a time. iTRAQ greatly improves the processing efficiency as proteins can be extracted from multiple biological replicates and analyzed in a single experiment. The ease of use and its multiplexing capabilities make it an effective approach in many scientific fields [11]. In addition, with iTRAQ labelling, proteins can be easily identified by tandem mass spectrometry automatically, unlike 2-DE, wherein protein spots of interest must be excised from two or three replicated gels depending on the image analysis software being used. Additionally, iTRAQ can identify more proteins, even proteins with low copy number, membrane proteins, or other proteins that are difficult to detect by 2-DE. In the future, combining proteomics with genomics, transcriptome and metabolomics is main directions for our research.

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6. References
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