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High-throughput transcriptome sequencing analysis provides preliminary insights into the biotransformation mechanism of *Rhodopseudomonas palustris* treated with alpha-rhamnetin-3-rhamnoside

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ABSTRACT

Background: The purple photosynthetic bacterium *Rhodopseudomonas palustris* has been widely applied to enhance the therapeutic effects of traditional Chinese medicine using novel biotransformation technology. However, comprehensive studies of the *R. palustris* biotransformation mechanism are rare. Therefore, investigation of the expression patterns of genes involved in metabolic pathways that are active during the biotransformation process is essential to elucidate this complicated mechanism.

Results: To promote further study of the biotransformation of *R. palustris*, we assembled all *R. palustris* transcripts using Trinity software and performed differential expression analysis of the resulting unigenes. A total of 9725, 7341 and 10,963 unigenes were obtained by assembling the alpha-rhamnetin-3-rhamnoside-treated *R. palustris* (RPB) reads, control *R. palustris* (RPS) reads and combined RPB&RPS reads, respectively. A total of 9971 unigenes assembled from the RPB&RPS reads were mapped to the nr, nt, Swiss-Prot, Gene Ontology (GO), Clusters of Orthologous Groups (COGs) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (*E*-value <0.00001) databases using BLAST software. A total of 3360 unique differentially expressed genes (DEGs) in RPB versus RPS were identified, among which 922 unigenes were up-regulated and 2438 were down-regulated. The unigenes were mapped to the KEGG database, resulting in the identification of 7676 pathways among all annotated unigenes and 2586 pathways among the DEGs. Some sets of functional unigenes annotated to important metabolic pathways and environmental information processing were differentially expressed between the RPS and RPB samples, including those involved in energy metabolism (18.4% of total DEGs), carbohydrate metabolism (36.0% of total DEGs), ABC transport (6.0% of total DEGs), the two-component system (8.6% of total DEGs), cell motility (4.3% of total DEGs) and the cell cycle (1.5% of total DEGs). We also identified 19 transcripts annotated as hydrolytic enzymes and other enzymes involved in ARR catabolism in *R. palustris*.

Conclusion: We present the first comparative transcriptome profiles of RPB and RPS samples to facilitate elucidation of the molecular mechanism of biotransformation in *R. palustris*. Furthermore, we propose two putative ARR biotransformation mechanisms in *R. palustris*. These analytical results represent a useful genomic resource for in-depth research into the molecular basis of biotransformation and genetic modification in *R. palustris*.

1. Introduction

Rhodopseudomonas palustris (*R. palustris*) is well known for its characteristic versatile metabolism. This purple photosynthetic bacterium is an alpha proteobacterium and it can be isolated from diverse sources in nature such as stagnant water, earthworm excrement, seabed sediment and swamp pits (Frank et al., 2004). Further, it is non-toxic and harmless to both people and animals (Hu et al., 2002). Recently, several studies have demonstrated that *R. palustris* possesses lead-reducing and anti-oxidative effects (Du et al., 2004; Bai et al., 2007). These characteristics contribute to its application in many fields, particularly in biotransformation technology (Raymond, 2003). *R. palustris* has exceptional metabolic flexibility, with the following four major types of metabolism: photosynthetic

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(using light and carbon as energy sources), photoheterotrophic (using light and organic compounds as energy sources), chemoheterotrophic (using carbon and organic compounds as energy sources) and chemoautotrophic (using inorganic compounds and carbon dioxide as energy sources). Therefore, it has been widely applied in microbial transformation for the production of a number of chemicals (Chongle et al., 2008). In our previous study, alpha-rhamnetin-3-rhamnoside (ARR), one of the active monomers of the traditional Chinese herb *Loranthus tanakae* that has been extensively used as a component in other Chinese herbal treatments, was reliably converted into rhamnetin by *R. palustris* (Niu et al., 2001). ARR has been reported to have little biological activity *in vitro*, but its aglycone, rhamnetin, is involved in highly important processes. Rhamnetin, which has alpha-7-nAChR activity, has anti-inflammatory effects and therapeutic potential in the treatment of neuroinflammatory conditions (Joseph et al., 2014). ARR, as a type of flavonol, has been shown to have less moderate cytotoxicity against cultured human tumor cell lines than rhamnetin *in vitro* (Young-Kyoon et al., 2004). In addition, rhamnetin, a phenolic flavonoid compound, has been widely used to treat several disorders in animal models (Wei et al., 2015). Recently, rhamnetin has been shown to have anti-inflammatory and anti-oxidative activities in some cell and animal models, such as B16 cells, mouse macrophage-derived RAW264.7 cells, and rats with paw edema (Jnawali et al., 2014; Kim, 2013; Mondal and Rajalingam, 2013). Important processes involving rhamnetin have attracted much attention, particularly the conversion of ARR to rhamnetin. Moreover, microbial transformation has attracted widespread attention due to its unique advantages, such as environmental protection, cost effectiveness, convenience and safety, compared with chemical reactions (Chen and Zhu, 2006). Microbial biotransformation technology, which has great prospects for development, has been widely applied in pharmaceutical research, such as drug development, discovery and preparation (Chen and Zhu., 2006). Thus, we used ARR as a metabolic substrate to investigate the intrinsic molecular mechanism underlying biotransformation by *R. palustris*, which could be partially explained by variation in gene expression during the biotransformation process. We examined the corresponding regulation *via* a thorough analysis of differentially expressed genes (DEGs) in RPB versus RPS samples in response to ARR stimulation.

Although microbial transformation mechanisms have recently attracted a great deal of attention in the application of biotransformation technologies in the pharmaceutical, chemical and medical industries, there are few reports of the uses of important scientific advances, such as high-throughput sequencing, to research the molecular mechanism of biotransformation in *R. palustris*. Therefore, study of the molecular mechanism of biotransformation in *R. palustris* is warranted using these established technologies. In this study, ARR-treated *R. palustris* was used as a model to investigate the mechanism of molecular biotransformation. We identified differentially expressed transcripts in this bacterium by transcriptome profiling using RNA-Seq technology. Recently, significant progress has been made in RNA-Seq, which is a cost-efficient method that has enabled copious amounts of transcriptome data to be obtained from many organisms and tissues (Birol, 2009; Trapnell, 2010). *De novo* transcriptome sequencing can be achieved *via* RNA-Seq, which has been widely applied in many fields due to advances in short-read sequencing technologies such as the Roche 454, SOLiD and Illumina platforms (Wang et al., 2009). The Solexa/Illumina platform is a proven and practical technology that can be used to identify small changes in gene expression and low-abundance transcripts, thereby broadening its scope of application. Furthermore, this platform provides unbiased transcriptome information and does not require the input of the gene sequences to be studied as reference sequences (Hoen et al., 2008). High-throughput transcriptome sequencing is an emerging technique that is used to analyze transcriptome profiles (Pollack, 2009). A great deal of genetic information has been successfully obtained with the use of this valuable technique, enabling the examination of gene expression and discovery of diverse genes that are currently unknown, facilitating the development of nucleic acid therapeutics, better integrating human health-related molecular data and disease information to form a complete picture at the individual level and promoting the development of novel techniques (Kahvejian et al., 2008).

Bacterial genes can be regulated by changing the density of microflora in a cell density-dependent manner. This bacterial behavior is also referred to as quorum sensing and response (QS). Many bacterial species contain QS systems, particularly Proteobacteria. Most of these systems include acyl-homoserine lactone (acyl-HSL) QS circuits (Fuqua and Greenberg, 1998; Waters and Bassler, 2005). QS systems regulate many diverse genes depending on the bacterial species, including bioluminescence genes, protease genes, conjugal transfer genes and antibiotic synthesis genes (Fuqua and Greenberg, 1998; Waters and Bassler, 2005). Many products of QS-regulated gene expression are “public goods” that can be shared by all members of a bacterial group (Hidetada et al., 2011). In this paper, we examined the molecular mechanism of biotransformation in *R. palustris* treated with ARR, observed gene expression changes in response to ARR stimulation and identified the genes annotated to environmental information processes related to QS systems in *R. palustris*. Many studies have shown that *R. palustris* is one type of photosynthetic bacteria that can obtain nutrients from the environmental supply and by internal synthesis to promote its own growth; in addition, it has been shown to be very tolerant to harsh environments due to the changes in internal transcriptional regulation associated with its QS system (Marjolein et al., 2004). We took advantage of these characteristics of *R. palustris* to biotransform active monomers among complicated components of natural products and attempted to improve upon the microbial transformation technique by elucidating the mechanism of biotransformation in this bacterium. Because *R. palustris* is one type of photosynthetic bacteria and can maximally convert substances obtained from various sources into products that can be easily metabolized into energy for growth, we predicted that the mechanism of biotransformation is likely to be partly associated with the four modes of metabolism mentioned above. Thus, the up-regulated and down-regulated genes identified in the RPS and RPB samples may be related to these four modes of metabolism. A comparison of the transcriptome profiles obtained in this study has provided a valuable genetic resource for the further study of biotransformation by *R. palustris*.

2. Materials and methods

2.1. R. palustris *culture and ARR treatment*

In this study, *R. palustris* strains identified and isolated from the laboratory of photosynthetic bacteria at Shanxi University were cultured in conventional medium (Table S1) at 37 °C and 2,500 lux of lighting power for 72 h. In our previous experiment on the growth of *R. palustris*, bacteria cultured for 72 h were in a better state based on determination of viable counts, construction of growth curves and measurement of dehydrogenase activity during the logarithmic phase of growth (Du et al., 2004). In addition, the concentrations of ARR and its product, rhamnetin, were measured at the 0th, 24th, 48th, 72th, 96th and 120th hours under the same culture conditions mentioned above *via* HPLC. The results showed that the 72th time point was the optimal culture time compared with the other time points not only because the conversion rate of ARR