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ORIGINAL ARTICLE

Transcriptome analysis reveals phenanthrene degradation strategy of *Pseudomonas stutzeri LH-42*

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Abstract

Toxic polycyclic aromatic hydrocarbons (PAHs) are often released into the environment during the combustion and processing of fossil fuels and are capable of causing significant pollution to people and the environment. One of the representative substances of PAHs is phenanthrene, which is often studied as a model compound for PAHs treatment. In this study, we compared the results of transcriptome analysis of *Pseudomonas stutzeri LH-42* in two different culture conditions under phenanthrene-induced culture (test group) and glucose-induced culture (control group), and analysed the key enzymatic mechanisms of *Pseudomonas stutzeri LH-42* in the biodegradation of phenanthrene. In our experiments, the transcriptome results showed that a total of 380 genes were more than twofold differentially expressed in the test group, of which 187 genes were significantly up-regulated in expression under Phenanthrene induction. Among the 380 differentially expressed genes, 90 genes were involved in Phenanthrene biodegradation, mainly including genes involved in biometabolism, cellular chemotaxis, substrate transport, signal induction and other related processes. Based on the transcriptome sequence analysis of *Pseudomonas stutzeri LH-42* at the time of phenanthrene induction, a total of 25 dioxygenase genes were identified, and the related genes were mainly concentrated in two relatively concentrated clusters of PAHs biodegradation genes. The transcriptome analysis resulted in a complete set of enzyme genes related to the phenanthrene biodegradation pathway. The analysis of key enzymes led to the inference of a possible phenanthrene biodegradation pathway: the salicylic acid degradation pathway. The results of this study provide a theoretical basis for in situ remediation of PAHs-contaminated environments using *Pseudomonas stutzeri LH-42*.

Keywords Polycyclic aromatic hydrocarbons · Phenanthrene · *Pseudomonas stutzeri* · Transcriptome

Introduction

Polycyclic aromatic hydrocarbons, a class of ubiquitous persistent organic pollutants, are aromatic hydrocarbon compounds formed by two or more benzene rings arranged in linear angular or clustered form, and due to their persistence in the environment, bioaccumulation and carcinogenicity, they are considered to be one of the most serious pollutants One of the most serious pollutants (Dandajeh et al. 2019). Although studies have shown that PAHs can be degraded by microorganisms, their low solubility and high hydrophobicity and lipophilicity make them difficult and slow to be degraded by microorganisms under natural conditions, and the potential hazard of PAHs to human health and ecological safety has made them a public concern (Inam et al. 2018). Based on their relative toxicity, abundance, exposure and levels in environmental samples, 16 of the PAHs are listed as environmental priority pollutants in the US and EU (as shown in Fig. 1), while China also lists naphthalene, phenanthrene, fluoranthene, benzo (β) fluoranthene, benzo[κ]fluoranthene, benzo[α]pyrene, and benzo[1,2,3-cd] pyrene, as environmental priority control pollutants (Sucai et al. 2018). Although studies have shown that PAHs can be degraded by microorganisms, the high hydrophobicity of PAHs makes them difficult to be degraded by microorganisms under natural conditions. The potential hazard of PAHs to human health and ecological safety has made them of public concern (Segura et al. 2017).

At present, there are many techniques to deal with PAHs (Wang et al. 2015). PAHs pollution remediation techniques can be classified into physical, chemical and bioremediation

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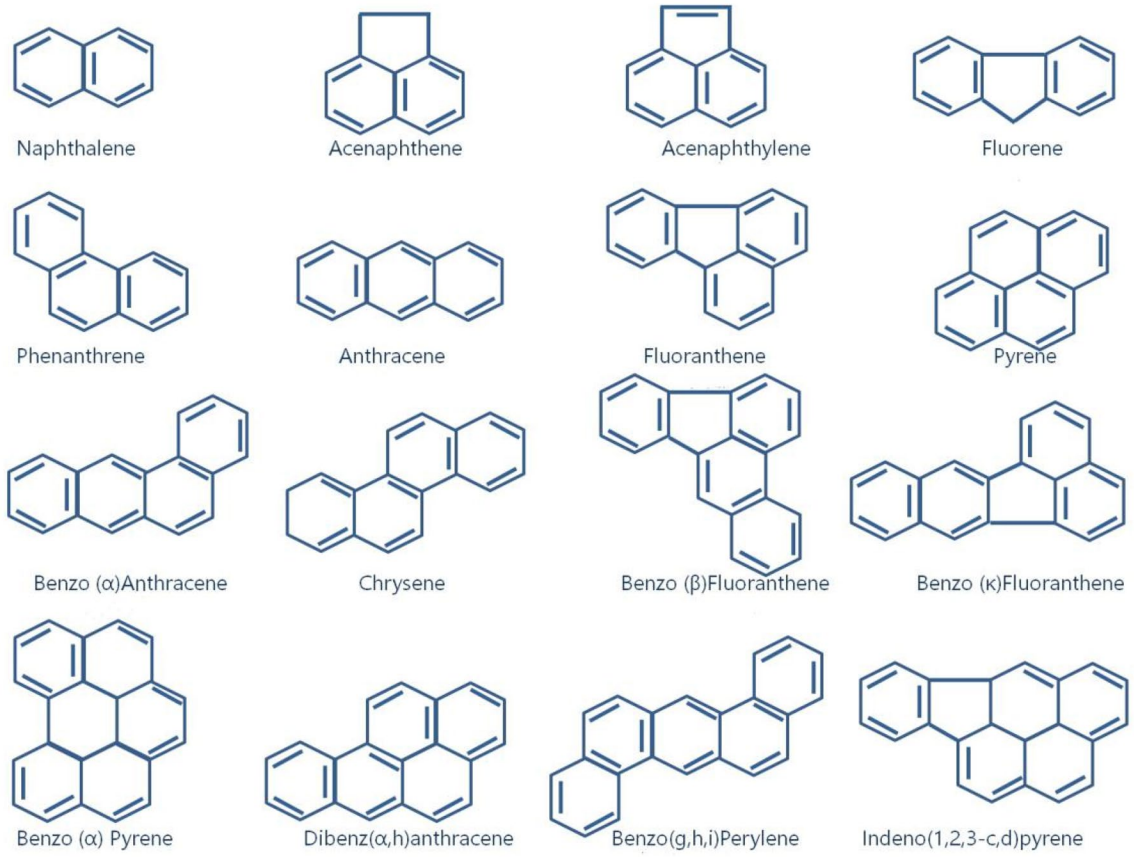

Fig. 1. 16 PAH pollutants prioritised for control by the US EPA

methods according to their principles (Wang et al. 2016). Physical methods mainly use physical processes to separate or remove PAHs from soil sediments, including material adsorption, organic solvent extraction, transfer burial and flocculation and precipitation (Zhang and Chen 2017). Physical methods have the advantage of being relatively simple and inexpensive to operate, but the method can only transfer contaminants and cannot completely eliminate them, and needs to be used in combination with other methods to remove them (Hu et al. 2017). Chemical methods mainly use chemical reaction processes to degrade PAHs, mainly including oxidation and incineration (Lee and Dong 2010). Chemical methods can degrade pollutants, reduce their toxicity and treat them quickly, but the method requires high equipment requirements and is highly damaging to the soil structure. Bioremediation of contaminated soil mainly includes phytoremediation techniques and microbial remediation techniques (Xiao et al. 2008). The method is low cost, has no secondary pollution to the environment and can remediate contaminated soil in situ. The biological method is suitable for treating large areas and low concentrations of PAHs contamination. Phytoremediation is a relatively new method of in situ remediation. PAHs are accumulated, metabolised, transformed or immobilised in plants using plants and their co-existing environmental systems (Wang et al. 2021). Microbial remediation of PAHs involves the use of microorganisms to degrade and metabolise PAHs present in the environment into water and carbon dioxide or other non-toxic compounds, thereby reducing or eliminating the toxicity of contaminants in the ecosystem. Compared with plants, microorganisms have the advantages of easy cultivation, short growth cycle and abundant resources. Microbial remediation technology has gradually become the main means of bioremediation technology for PAHs pollution (Xie et al. 2017).

In recent years, research work on microbial remediation of PAHs has mainly focused on: (1) screening and modification of strains for efficient degradation of PAHs; (2) research on the metabolic pathways and metabolic mechanisms of microorganisms for PAHs; and (3) optimization of microbial remediation processes and reactors for PAHs (Rodrigues et al. 2017). At present, how to obtain strains with efficient degradation ability and how to maintain the degradation activity of the strains in practice are key difficulties in the application of microbial remediation technology (Seguraa et al. 2017).

Studying the degradation mechanisms of PAHs by microorganisms is a prerequisite for applying microbial techniques to environmental contamination by PAHs, and helps to understand the ability of a given microorganism to transform PAHs and the impact of these metabolites on the environment. Transcriptome analysis (Lin et al. 2020) techniques provide an effective new way to study bacterial metabolic mechanisms (Simon et al. 1993). Transcriptome results can reflect the number and expression levels of all expressed genes in bacterial cells under certain physiological conditions. Through comparative transcriptome analysis, differences in the expression of relevant genes in physiological activities such as substrate transport, signal transduction and metabolism can be obtained (Ha et al. 2017), leading to speculation on the functions and metabolic mechanisms of bacterial relevant genes (Venomics and Gland 2017).

The oxygenated oxidation of PAHs is the key rate-limiting step of the degradation reaction, and the main oxygenases that catalyse the opening of the benzene ring are: initiating dioxygenase, catechol dioxygenase and protocatechuic acid 3,4-dioxygenase (Wang et al. 2009b). These oxygenases play a role in catalyzing the opening of benzene rings during the degradation of PAHs. Dioxygenases are multicomponent enzymes, usually consisting of a reductase, a ferric oxide reducing protein and a terminal oxygenase subunit (Chandra et al. 2017).

Bicyclic naphthalenes are often used as model compounds for the study of microbial degradation processes of PAHs. Naphthalene-degrading bacteria are ubiquitous in nature and numerous reports document the degradation of naphthalene by bacteria, including the elucidation of pathways, enzymatic mechanisms and genetic regulation. The naphthalene degradation pathway is first catalysed by naphthalene dioxygenase to form 1,2-dihydroxy-1,2-dihydronaphthalene, which is then dehydrogenated by dihydrodiol dehydrogenase to form 1,2-dihydroxynaphthalene (Lai et al. 2017). After further metabolism, it finally forms salicylic acid, which then enters downstream metabolism and enters the TCA cycle.

The enzymatic mechanism of bacterial naphthalene degradation has been resolved with the full characterization of the naphthalene catabolic metabolism gene in plasmid NAH7 in *Pseudomonas aeruginosa G7* (Liao et al. 2017), where the naphthalene catabolic metabolism gene (nah) consists of two manipulators: the nal manipulator containing the upstream pathway enzyme gene involved in the conversion of naphthalene to salicylate and the sal manipulator containing the downstream pathway enzyme gene in the conversion to pyruvate and acetaldehyde. manipulators are positively regulated by the co-regulator NahR (Pacifico and Davis 2016), a LysR-type positive transcriptional regulator that is widely distributed in bacteria.

Phenanthrene is one of the representatives of PAHs and is the simplest polycyclic aromatic hydrocarbon with both a bay and K region (Fig. 2). The bay and K regions are used as model structures for the study of oncogenic PAH metabolism (Schlipalius et al. 2018). Therefore, phenanthrene is often studied as a model compound in the study of PAHs (Ma et al. 2018). The degradation process of phenanthrene generally goes through the metabolic pathway of naphthalene. Studies on the metabolic mechanisms of phenanthrene degradation by microorganisms have made some research progress. Currently, the aerobic degradation pathways for bacterial degradation of phenanthrene are well-defined, mainly the salicylic acid pathway and the phthalic acid pathway. The degradation of phenanthrene is generally initiated by an aerobic catalytic reaction catalyzed by a double oxygenase (Huang et al. 2017). At the C3 and C4 positions of phenanthrene, two oxygen atoms are added in the presence of dioxygenase to form 3,4-dihydroxyphenanthrene, which is then further cleaved to form 1-hydroxy-2-naphthoic acid, and finally the benzene ring is oxidatively cleaved to form catechins, salicylic acid and other substances to enter the TCA cycle for microbial growth and metabolism (Huang et al. 2018).

In this study, the transcriptome of *Pseudomonas stutzeri LH-42* under phenanthrene induction (test group) and glucose culture (control group) will be compared, and the key enzyme and degradation mechanisms is expected to be discovered in the process of phenanthrene degradation (Pang et al. 2017).

Materials

Inorganic salt medium (MM medium, g/L): $Na_2HPO_4{\cdot}H_20$, 3.8 g; KH_2PO_4, 1 g; $MgSO_4$, 0.2 g; NaCl, 1 g; NH_4Cl, 0.1 g. Phenanthrene is dissolved in acetone to make a 2 g/L mother liquor, filtered through a 0.22 μm membrane to remove bacteria, and then added to the inorganic salt medium in a certain amount to make Phenanthrene degradation medium. Phenanthrene (> 98% purity), chromatographically pure,

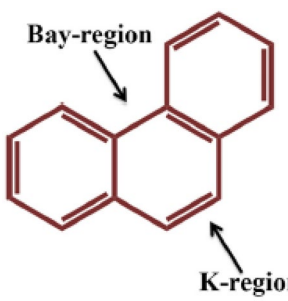

Fig. 2 Structure of phenanthrene

was purchased from Biotech Bioengineering (Shanghai) Co. Bacterial Total RNA Extraction Kit, Reverse Transcription Kit, Novostart® SYBqPCR SuperMix Plus.

Methods

Strains and cultivation

Pseudomonas stutzeri LH-42 was obtained from the enrichment and cultivation of microorganisms in the soil near the oil well in Liaohe Oilfield in the early stage of the laboratory. We carried out a series of studies on *Pseudomonas stutzeri LH-42* morphology and physiology. The results showed that the bacteria were Gram-negative bacteria, and colonies on solid plates are milky white, protruding, irregularly round. Scanning electron microscopy showed that the strain was a short rod-shaped strain with a length of 0.8–1.8 μm, The diameter is 0.4–0.6 μm (see Fig. 3). At present, this strain of *Pseudomonas stutzeri LH-42* has completed genome-wide sequencing and gene function annotation. The genome-wide sequence information of this strain has been submitted to the GenBank database and the authorized accession number (NCKS02000000) has been obtained. The dioxygenase gene associated with the degradation of PAHs was found throughout the genome of the strain. For example, genes such as the initial dioxygenase gene (subunit sequence), protocatechuic acid 3,4-dioxygenase gene, and benzoic acid 1,2-dioxygenase gene (as shown in Table 1). It was proved from the genetic level that this strain has the genetic basis for degrading PAHs.

During incubation, an appropriate amount of bacterial solution was removed from the seed keeping tube and applied to LB solid medium plates and a small amount of Phenanthrene was placed on the medium and incubated at 30 °C. After 2 days, colonies were picked from the solid plate medium and inoculated into MM liquid medium containing phenanthrene and incubated for 1 week. The strains were collected by centrifugation and the DNA of the strains was extracted using the Bacterial Genome Extraction Kit. PCR amplification of the 16S rRNA of the strains was performed using primer 27F (Han et al. 2018) and reverse primer 1492R, and sequence comparison was performed pure cultures of strain *Pseudomonas stutzeri LH-42* will be obtained for subsequent experiments.

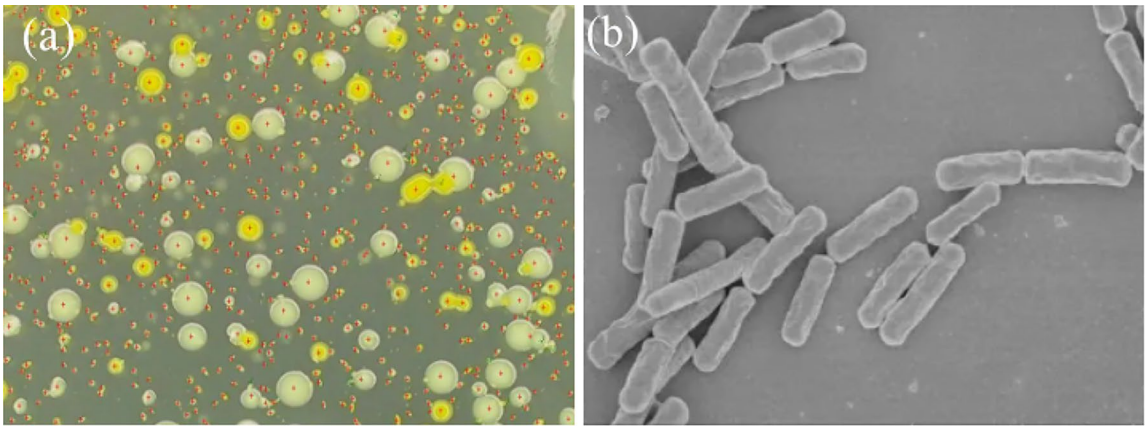

Fig. 3 Morphological characteristics and SEM image of *Pseudomonas stutzeri LH-42*

Table 1 Dioxygenases gene related to PAHs degradation in *Pseudomonas stutzeri LH-42*

Replicon accession	Start	Stop	Protein name
NZ_NCKS02000234.1	1878	2755	Catechol 1,2-dioxygenase
NZ_NCKS02000234.1	7606	8094	Benzoate 1,2-dioxygenase small subunit
NZ_NCKS02000234.1	8097	9458	Benzoate 1,2-dioxygenase large subunit
NZ_NCKS02000097.1	1	1483	4-Hhydroxyphenylpyruvate dioxygenase, partial
NZ_NCKS02000240.1	21,919	23,004	4-Hhydroxyphenylpyruvate dioxygenase
NZ_NCKS02000259.1	2050	2769	Protocatechuate 3,4-dioxygenase subunit beta
NZ_NCKS02000259.1	2787	3387	Protocatechuate 3,4-dioxygenase subunit alpha
NZ_NCKS02000045.1	29,349	30,110	Dioxygenase
NZ_NCKS02000246.1	23,232	24,059	Dioxygenase

Extraction of *Pseudomonas stutzeri LH-42* total RNA

The experimental bacterium *Pseudomonas stutzeri LH-42* was first cultured in 100 mL of MM medium at 30 °C and 170 rpm until OD600 = 1.0. The sediment was then collected after centrifugation at 8000 rpm.

Experimental group (T group) and control group (C group): in the experimental group, the collected bacteria were rinsed twice with MM medium, divided equally into three inorganic salt media containing 100 mg/L Phenanthrene, incubated until OD600 = 0.6, centrifuged and collected, rinsed twice, and inoculated into inorganic salt media containing 100 mg/L Phenanthrene for 24 h, and collected to extract total bacterial RNA. The bacteria were divided into three inorganic salt media containing 100 mg/L of glucose, incubated until OD600 = 0.6, centrifuged and collected, rinsed twice, and all of them were inoculated into inorganic salt media containing 100 mg/L of glucose and incubated for 24 h, and the bacteria were collected to extract total RNA. Three parallel replicate experiments were done for both the experimental and control groups.

One copy of the RNA extracted from the experimental and control groups was sent to Wuhan Sage Biological Company for RNA profile checking and transcriptome sequencing, and one copy was reserved for real-time PCR experiments for validation.

Transcriptome sequencing and analysis of *Pseudomonas stutzeri LH-42*

Total RNA of *Pseudomonas stutzeri LH-42* was extracted and sent to Wuhan Sage Biotechnology Co., Ltd. for sequencing using Illumina HiSeq PE150. The raw sequence data were compared with the reference sequence after removal of impurities, and gene expression was annotated. Real-time PCR was used to verify the up-regulation of key genes (Rezek et al. 2008). Primer design (Isaac et al. 2013) is shown in Table S1.

Results and discussion

Extracted bacterial total RNA quality test results

The results of RNA agarose gel electrophoresis are shown in Fig. 4 (1–3 are control samples, 4–6 experimental samples), with clear lane bands, both with two bands (16S and 23S), indicating that the extracted total RNA is of good quality and can be used for subsequent experiments.

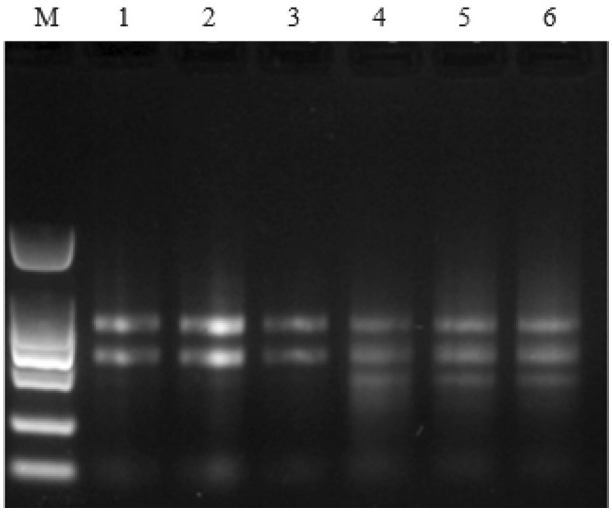

Fig. 4 Results of RNA agarose gel electrophoresis of *Pseudomonas stutzeri LH-42*

Transcriptome sequence overview

The valid sequences of the transcriptome obtained were compared with the reference genes (all *Pseudomonas stutzeri LH-42* genes) and the results are shown in Table 2 under the condition that no more than 5 base mismatches were allowed. 56.80% (mean) of the valid sequences of the experimental group and 50.26% (mean) of the glucose control group were able to match the *Pseudomonas stutzeri LH-42* genes. The proportion of perfect matching sequences reached 43.13% and 40.89% for the Phenanthrene-induced experimental group and the glucose control group, respectively.

The randomness analysis of the sequencing results can be expressed in terms of the distribution of the effective sequences on the reference genes. In this experiment, the distribution of effective sequences on the reference genes for the Phenanthrene-induced experimental group (a) and the glucose culture control group (b) are shown in Fig. 5 (only the results of one of the experimental groups and one of the transcript groups are shown in Fig. 5), and the curves are flat and both are relatively uniform, indicating that the mRNA fragmentation before sequencing of the *Pseudomonas stutzeri LH-42* transcript group is effective and the sequencing results have good randomness.

Saturation curves can be used to analyse whether the sequencing volume of a sequenced sample has reached saturation (Wu et al. 2010). The horizontal coordinate indicates the number of genes detected with sequencing volume and the vertical coordinate indicates the number of genes detected by sequencing. However, when the sample sequencing volume reaches a certain value, the growth rate of the number of genes detected slows down and the number of

Table 2 Overview of the *Pseudomonas stutzeri LH-42*

Sample	Total number of sequences	Match rate (%)	Perfect match rate (%)	Multiple matching sequence rate (%)	Mismatched sequence rate (%)
C_1	8,312,887	50.57	40.90	9.53	49.43
C_2	7,982,311	50.65	41.23	5.52	49.35
C_3	7,364,608	49.57	40.56	9.75	50.43
T_1	7,353,175	68.68	45.17	3.861	31.32
T_2	7,616,591	48.43	39.94	8.55	51.57
T_3	7,856,550	53.28	44.29	4.16	46.72

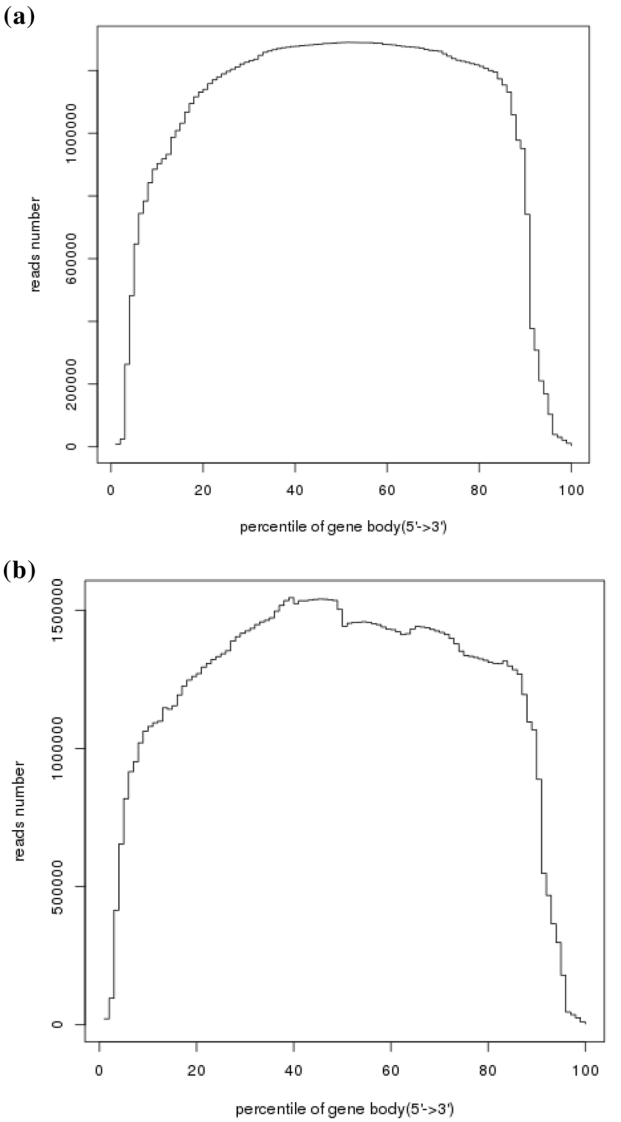

Fig. 5 Randomization analysis of sequencing: test group (**a**); control group (**b**)

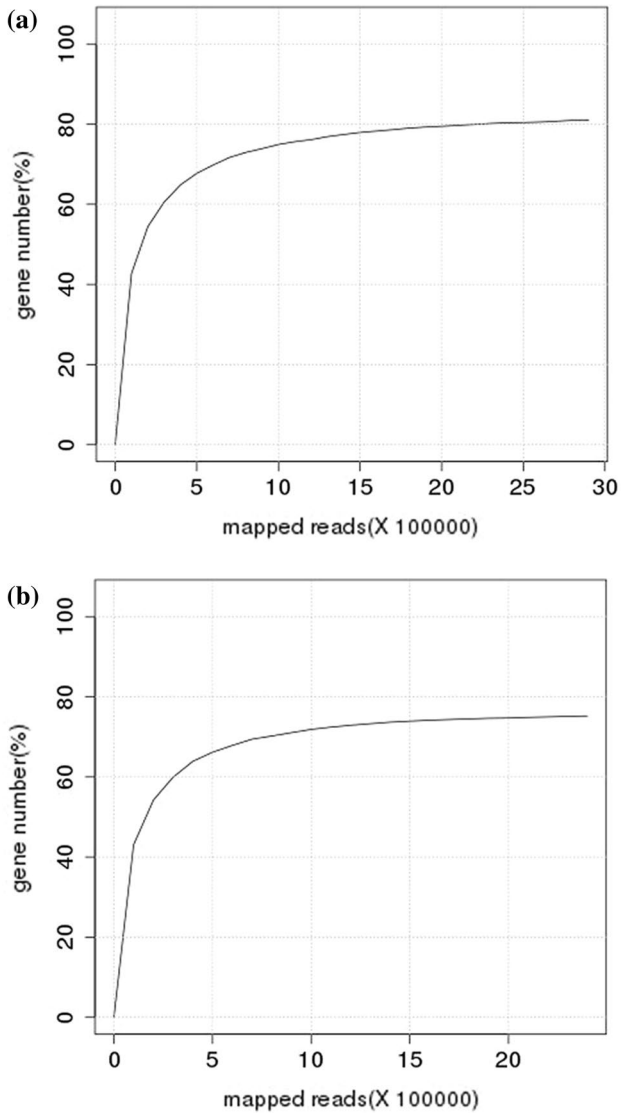

Fig. 6 RNA sequencing saturation curve, test group (**a**), control group (**b**)

genes detected plateaus, indicating that the sequencing volume is saturated. Figure 6 shows the saturation curves of the transcriptome sequencing process of *Pseudomonas stutzeri LH-42*. Only one of the experimental groups and one transcriptome are shown as representative saturation curves in Fig. 6a shows the glucose control group and 6b shows the

Phenanthrene induction culture experimental group, and it can be seen that both groups of samples in this experiment reached saturation in terms of sequencing depth (Wang et al. 2012).

Gene sequencing coverage is the ratio of the number of bases covered by a perfect match sequence (Ghasemi et al. 2011) on a reference gene to the total number of bases in that gene in the sequencing result. The results are shown in Fig. 7, which shows the gene sequencing coverage results of the control group (Fig. 7a) and the experimental group (Fig. 7b), in which the genes that achieved more than 60% coverage accounted for more than 95% and 93% of the total number of reference genes, while the genes with 80% or more gene sequencing coverage reached more than 90% and 85% of the total number of reference genes in the control and experimental groups and, respectively, indicating that *Pseudomonas stutzeri LH-42* transcriptome data had good gene sequencing coverage.

From the evaluation of the above transcriptome data, it is clear that the sample transcriptome valid sequences (Moser and Stahl 2001) have good coverage on the reference genome *Pseudomonas stutzeri LH-42* gene, the transcriptome sequencing results have good randomness and the rRNA sequencing depth reaches saturation, indicating that good and reliable data were obtained from the *Pseudomonas stutzeri LH-42* transcriptome sequencing (Ohlendorf et al. 1988).

Gene differential expression analysis

RPKM (Reads Per Kb per Million reads) is often used to represent gene expression, which can eliminate the effect of

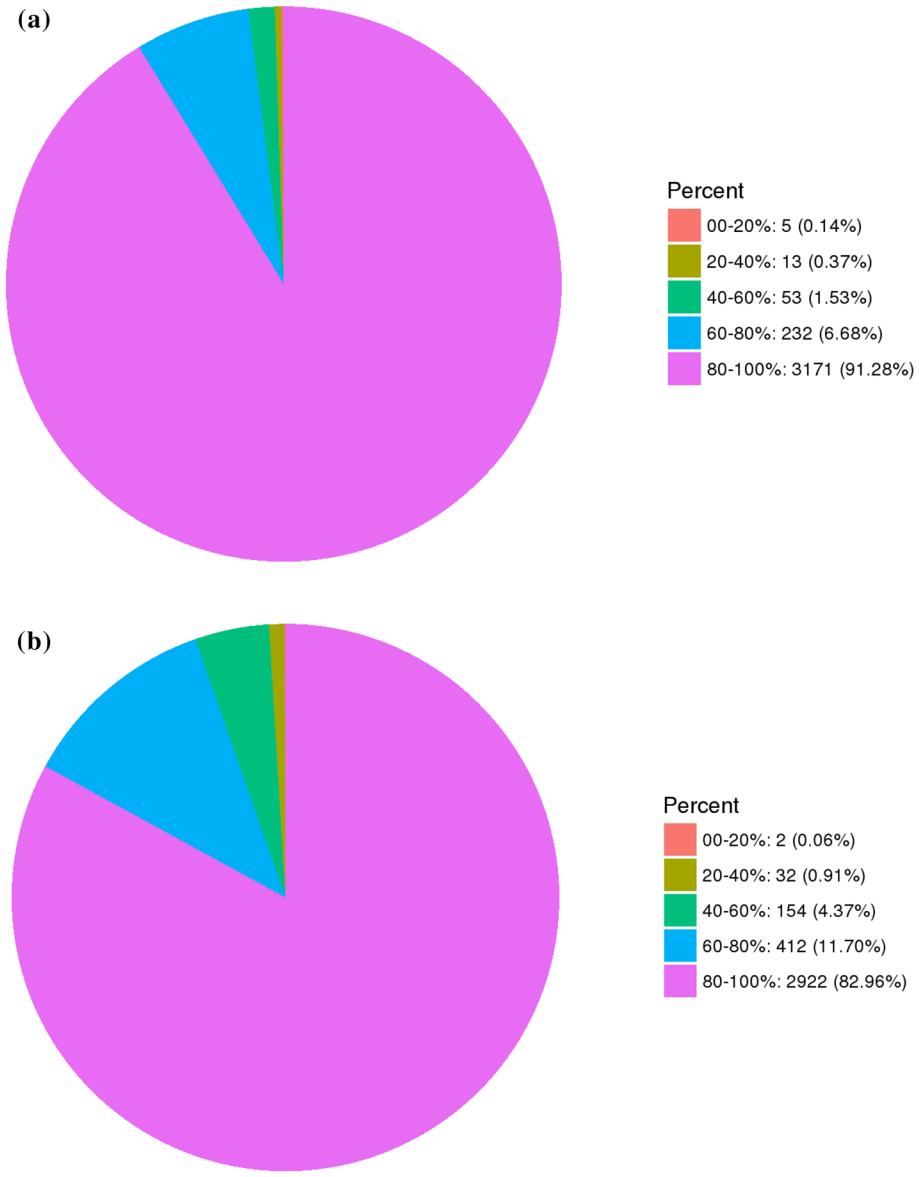

Fig. 7 Gene coverage of transcriptome data, test group (**a**), control group (**b**)

different gene lengths or different transcriptome sequencing volumes on gene expression, and the gene expression calculated using this method can be directly used to analyse gene expression differences between samples. In the differential gene expression analysis of this thesis, the conditions for screening differential expression were that the False Discovery Rate (FDR) of differentially expressed genes was ≤ 0.05 and $|\log2(\text{RPKM-T/RPKM-C})| > 1$.

Analysis of the transcriptome results of strain *P. stutzeri* LH-42 showed that 380 genes (11.26%) were significantly differentially expressed in the experimental group compared to the control group, with 197 genes significantly up-regulated in the strain under Phenanthrene-induced culture conditions (Peng et al. 2010).

GO (Gene Ontology) enrichment analysis, which can be used to describe the properties of genes and gene products in an organism, is an internationally standardised system for classifying gene functions (Silva et al. 2004). GO function analysis includes GO function classification annotation of differentially expressed genes and GO function enrichment analysis. In this study, GO functional analysis was performed on the differentially expressed genes of *Pseudomonas stutzeri LH-42* transcriptome data, and functional classification was performed at the next level of the above three ontologies (Macchi et al. 2018), and the results of the functional enrichment analysis are shown in Fig. 8 (biological processes (14 gene families), cellular components (13 gene families), and molecular functions (9 gene families)). The strain *Pseudomonas stutzeri LH-42* was in the process of degrading phenanthrene. Many related genes involved in cell processes, metabolic processes, and binding processes were differentially expressed, and the differentially expressed genes belonging to the biological processes were the most.

GO enrichment analysis illustrated in the degradation of Phenanthrene by *Pseudomonas stutzeri Lh-42*. Many related genes involved in cell processes, metabolic processes, and binding processes were differentially expressed, and the differentially expressed genes belonging to the biological processes were the most. Among the top five processes ranked in the upward adjustment are single-organism cellular process, organic substance metabolic process, cellular metabolic process, localization of cell, cell motility. The up-regulation of these five processes, moreover, indicated that *Pseudomonas stutzeri Lh-42* had a high activity of various genes associated with organic matter degradation during the metabolic phenanthrene process.

There were 380 differentially expressed genes in the transcriptome analysis, and these genes were matched to 68 KEGG metabolic pathways. The KEGG enrichment analysis of the differentially expressed genes of *Pseudomonas stutzeri LH-42* showed that the differentially expressed genes were mainly concentrated in metabolic pathways (Janbandhu and Fulekar 2011), biodegradation and metabolism, and membrane transport systems in Fig. 9.

We analysed the transcriptome of *Pseudomonas stutzeri LH-42* and analysed the annotation results by KEGG analysis and we identified a number of genes involved in Phenanthrene degradation in *Pseudomonas stutzeri LH-42*. For example, 20 differentially expressed genes in *Pseudomonas*

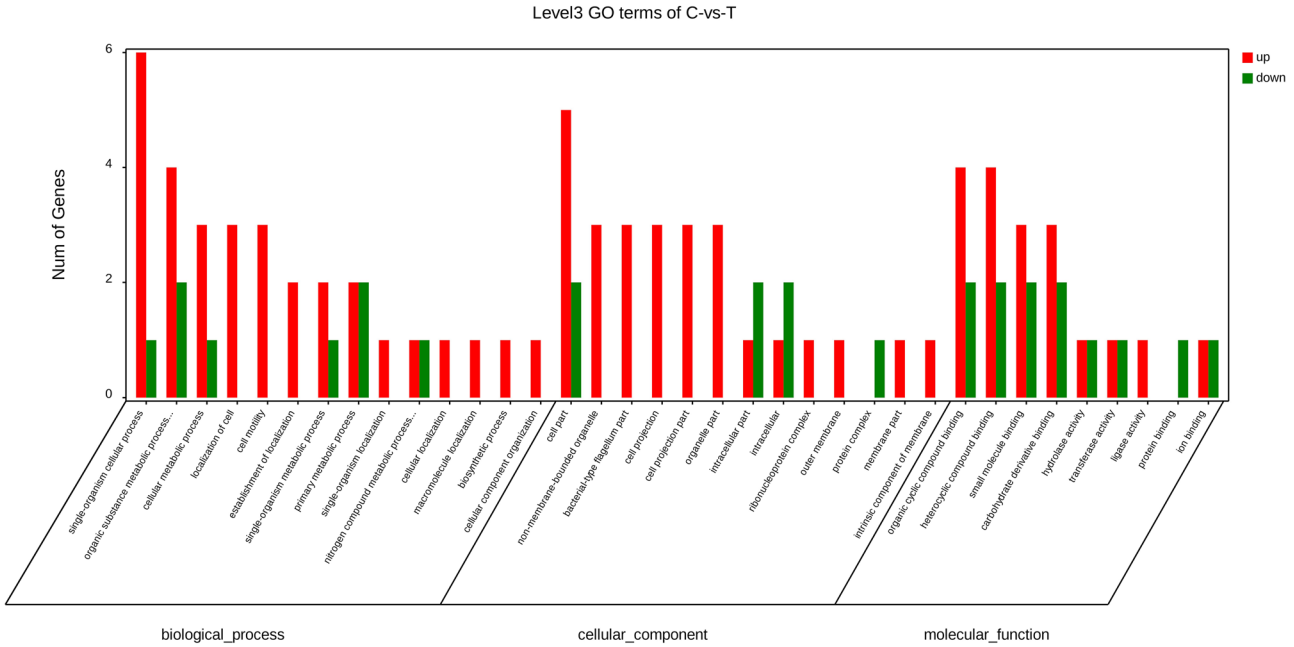

Fig. 8 Functional classification of differentially expressed genes GO

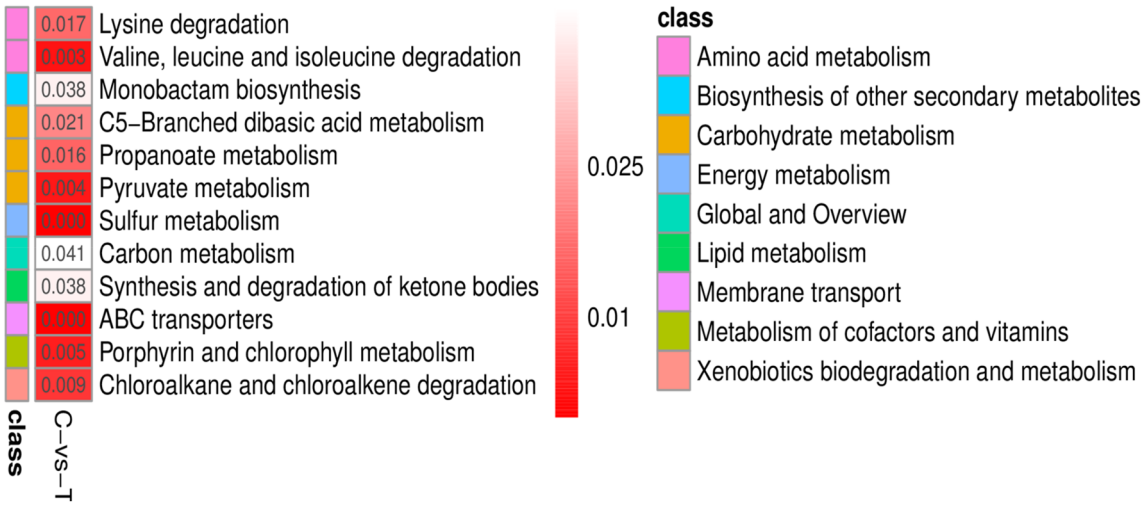

Fig. 9 Major metabolic pathways in which differentially expressed genes are involved

stutzeri LH-42 were involved in carbon metabolism under Phenanthrene-induced culture conditions (ko01200); 15 differentially expressed genes were associated with PAH degradation (ko00624); 8 differentially expressed genes were involved in the glyoxylate and dicarboxylic acid metabolic pathways (ko00630); and in naphthalene degradation (ko00626) Three differentially expressed genes were found to be involved in the phenylalanine metabolism (ko00360) process and two differentially expressed genes were found to be associated with benzoate degradation (ko00362).

In addition, analysis of the transcriptome of *Pseudomonas stutzeri LH-42* identified 28 differentially expressed genes matching to those associated with ABC transporters that may be involved in substrate transport during bacterial Phenanthrene degradation; 12 bacterial chemotaxis (ko2030)-related differentially expressed genes were also identified that may be associated with In addition, 22 differentially expressed (significantly overexpressed) genes were matched to the two-component system, which may be involved in the regulation of expression of key genes regulating the degradation of Phenanthrene in the strain and in the regulation of microbial avoidance of unfavourable environments.

We also found it interesting that 13 significantly differentially overexpressed genes were associated with microbial flagellar assembly. For most motile bacteria, the flagellum is both a motor organ and a protein transport and assembly apparatus, and is a reversible and regenerative motility organ, and because the synthesis and operation of the flagellum is very energy intensive for bacteria, the entire flagellar system is tightly regulated. In the present study, several genes associated with flagellar assembly and motility were significantly up-regulated more than twofold in the presence of the PAH Phenanthrene as the sole carbon source, a phenomenon also found by others studying transcriptional analyses of PAH degradation in micro-other microorganisms. For microorganisms flagellar motility may act as a tropism for PAHs, and to increase exposure to PAHs in an oligotrophic environment and enhance uptake of PAHs for degradation, bacteria increase exposure to substrates through accelerated motility and thus gain more energy.

Phenanthrene degradation gene analysis

The cyclic hydroxylated dioxygenase and its degradation gene family for the Phenanthrene degradation process were inferred from the transcriptome sequence analysis of the genome of *Pseudomonas stutzeri LH-42*. In general, PAH degradation genes are arranged in the genome in a relatively compact manner, thus allowing the functions of neighbouring genes to be inferred from the gene arrangement. Possible degradation pathways are inferred from gene clusters. The degradation of PAHs in aerobic bacteria is generally initiated by the action of dioxygenases and the double hydroxylation of the substrate. Among them, by analyzing the transcriptome sequence of *Pseudomonas stutzeri LH-42* at Phenanthrene inducibility, we obtained a more complete list of enzyme genes related to the catechol degradation pathway (underlined genes in Table S2), mainly concentrated in gene cluster II, and the degradation pathway and functional gene arrangement of this bacterium is shown in Fig. 10.

Pseudomonas stutzeri LH-42 is now clearer for the degradative enzymes downstream of the degradation process of phenanthrene, as is the gene annotation. In the transcriptome of this strain, we analysed a more complete up-regulation of enzymes associated with the salicylic acid–catechol degradation pathway (Fig. 10). Several studies have shown that the microbial degradation of phenanthrene generally begins with an oxygen addition catalytic reaction catalysed by a

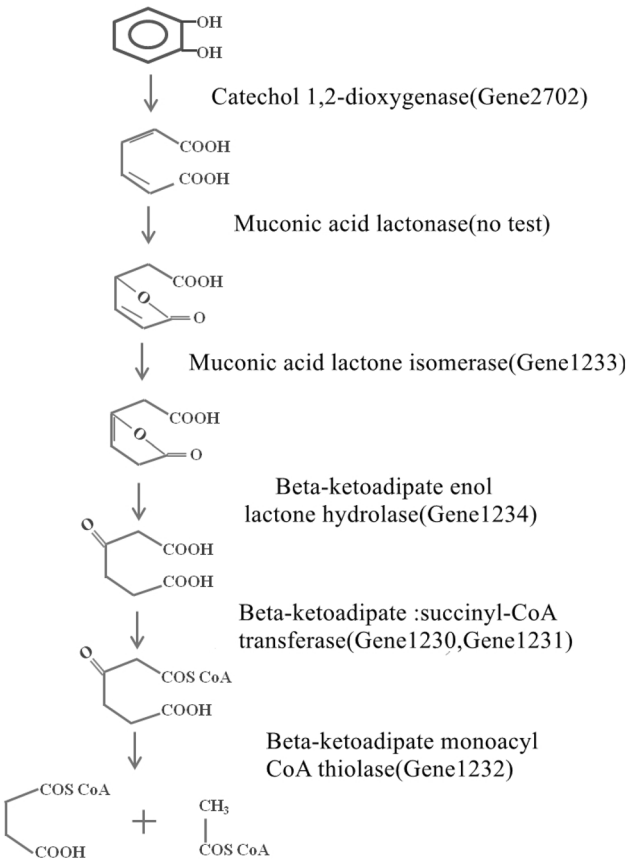

Fig. 10 Catechol degradation pathway

bioxygenase enzyme. The process involves the addition of two oxygen atoms at the C3 and C4 positions of phenanthrene to form 3,4-dihydroxyphenanthrene, followed by the further cleavage of 3,4-dihydroxyphenanthrene to form 1-hydroxy-2-naphthoic acid, and finally the oxidative cleavage of the benzene ring to form catechic acid and salicylic acid, which enter the tricarboxylic acid cycle and participate in microbial growth and metabolism, thus achieving green degradation of phenanthrene. The result is a green breakdown of phenanthrene. There are two classical pathways for the biodegradation of phenanthrene, one is the phthalic acid pathway and the other is the salicylic acid–catechol pathway, and some of the genes corresponding to some of the dioxygenases related to the degradation of PAHs listed in Table 1, such as catechol 1,2-dioxygenase, benzoate 1,2-dioxygenase large subunit, protocatechuate 3,4-dioxygenase subunit beta, protocatechuate 3,4-dioxygenase subunit alpha, dioxygenase, and the genes for these enzymes in *Pseudomonas stutzeri LH-42* showed increased expression at the transcriptional level for the degradation of phenanthrene. In particular, catechol 1,2-dioxygenase, an enzyme that indicates that phenanthrene is catabolised during biodegradation by *Pseudomonas stutzeri LH-42* to produce catechols, which are only produced in the salicylic acid–catechol pathway, demonstrates that the biodegradation of phenanthrene by *Pseudomonas stutzeri LH-42* does occur via the salicylic acid–catechol pathway. *Pseudomonas stutzeri LH-42* is indeed biodegraded by the salicylic acid–catechol pathway (Gupta et al. 2015). We, therefore, infer that the bacterium degrades phenanthrene via the salicylic acid pathway and that the mechanism of Phenanthrene metabolism in this bacterium needs to be further validated by intermediate metabolite (Haritash and Kaushik 2009) detection techniques.

The upregulation of five degradation genes was further verified by Real time PCR. The results showed that four of the genes were significantly up-regulated, which is consistent with the transcriptome result (Wang et al. 2009a). The expression level of another gene was slightly lower than that of the internal reference gene, indicating that these genes are in large associated with the degradation of phenanthrene. The results are shown in Fig. 11.

Conclusions

Pseudomonas stutzeri LH-42 is a strain screened from the soil near oil wells in the Liaohe oilfield in our laboratory in the previous phase. In the previous stage, we conducted a series of analyses on the morphological and physiological characteristics of *Pseudomonas stutzeri LH-42* and the sequence of 16sr RNA. It was found that the optimum growth temperature of the strain was 30 °C and the optimum growth pH was 6. It was also confirmed that *Pseudomonas stutzeri LH-42* has a genetic basis for degradation of phenanthrene. In conclusion, *Pseudomonas stutzeri LH-42* has a good application in the degradation of PAHs.

In this study, the Phenanthrene degradation mechanism of *Pseudomonas stutzeri LH-42* was investigated using transcriptome analysis. The transcriptome results of *Pseudomonas stutzeri LH-42* showed that 380 genes in the experimental group were more than twice as differentially expressed as the control group, of which 197 genes were significantly up-regulated by Phenanthrene induction. These genes associated with PAH degradation, cellular chemotaxis, substrate transport, bacterial regulation, signalling and transduction were significantly up-regulated during Phenanthrene degradation. All enzymes associated with the catechol degradation pathway were also identified. Therefore, based on the results of transcriptome analysis, we hypothesize that the bacteria may degrade Phenanthrene via the salicylic acid–catechol pathway. The results of this study provide technical support and theoretical basis for future in situ remediation of *Pseudomonas stutzeri LH-42* in PAH-contaminated environments.

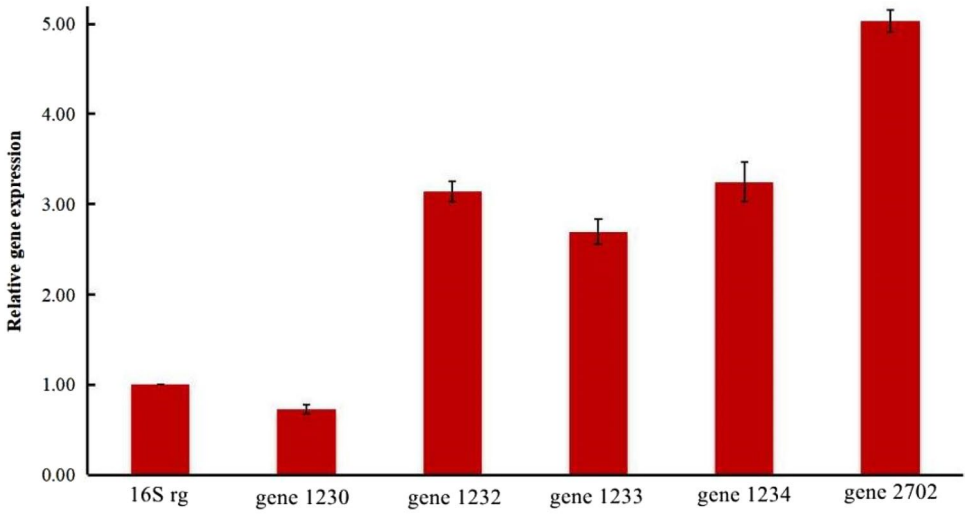

Fig. 11 Transcriptional level of target gene of *Pseudomonas stutzeri LH-42*. Gene1230, gene1232, gene1233, gene1234 and gene2702 are key genes of the metabolic pathway shown in Fig. 7. *Rg* reference gene

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Data Availability Authors can confirm that all relevant data are included in the articleand/or its supplementary information files.

Declarations

Conflict of interest The authors declare that they have no conflict of interest in the publication. As a graduating student, I hereby declare that all the experimental contents and materials in the research were carried out under the supervision and guidance of the school and the tutor. The other authors are my tutor and several classmates, there were no legal or moral violations of any kind throughout the study.

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