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A Review of Application and Development of Omics Technology in Environmental Field

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Abstract： The development of omics technology will accelerate the application of bioremediation technology in the environmental field, showing great potential. This article reviews the application of bionomics technology in environmental fields such as heavy metal pollution remediation, soil salinization, oil pollution remediation and plant growth promotion and discusses the role played by omics technology in helping people understand the pathways and mechanisms of microorganisms in the process of removing pollutants from the molecular level.

Keywords： Omics Technology; Microorganism; Bioremediation;

1. Introduction

With the development of molecular biology, bioinformatics and various detection technologies, major breakthroughs have been made in bionomics research including genomics, transcriptomics, proteomics and metabolomics, and gradually applied to the various fields of environmental science and engineering, especially the bioremediation of polluted environment. This article will focus on the application and development of omics research in the environmental field.

2. Microbial bioremediation and omics technology

Microorganisms play an important role in natural circulation. Therefore, they are widely considered as a reliable choice for biotechnology innovation and improvement to solve the accumulation and pollution of various organic and inorganic pollutants in the environment [1]. Microbial bioremediation is a sustainable method that can effectively remove and degrade various pollutants in the environment under the mediation of microorganisms [2]. Because of its low cost, high efficiency, simple management, and environmentally friendly (especially it will not cause secondary pollution), so it has become a popular method of environmental pollution remediation and has received more and more attention [3]. Catalytic reactions (such as biotransformation, precipitation, sequestration and dissolution) caused by products metabolized by microorganisms are the main processes involved in bioremediation. Therefore, the rational use of the metabolic capacity of microorganisms to catalytically decompose environmental pollutants is the key to perform successful bioremediation [2].

3. Application of omics technology

Due to the lack of microbiology understanding of bioremediation, the metabolic potential and pollutant degradation ability of microorganisms are limited in the process of environmental governance [4]. The important significance of the development and research of bionomics lies in exploring and comprehensively understanding the interaction of microorganisms and their metabolism with pollutants at the single species and community level, which provides attractive ideas for environmental restoration and governance[1]. The following content mainly introduces the application of omics technology to specific environmental problems.

4. Application of omics technology to heavy metal pollution

The development of industrial technology is bringing progress to mankind, but at the same time it produces a lot of toxic and harmful pollution. Among them, heavy metal pollution has become a huge threat on a global scale, seriously threatening the health and survival of human beings and other organisms. Figure 1 briefly illustrates the adverse effects of some heavy metals [4,5].

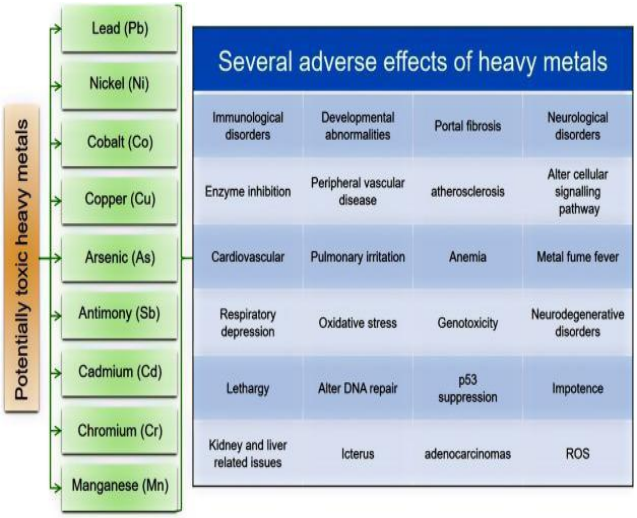

Figure 1. Several adverse effects of heavy metal.

Microorganisms can remove or degrade pollutants through special and inherent biological characteristics (the secretion of enzymes, changes in cell morphology, etc.), so as to achieve the purpose of purifying the polluted environment [5]. Therefore, the use of microorganisms or their metabolites to control heavy metal pollution is considered an attractive environmentally friendly bioremediation technology [6]. The application of omics technology to study the survival, metabolism, function and interaction of microorganisms with heavy metal ions provides a very important theoretical and practical basis for the effective use of biotechnology to deal with heavy metal pollution problems [6].

4.1.1Cadmium control

According to the research [6], P. chengduensis strain MBR is a kind of bacteria that can control heavy metal pollution, which is able to reduce and transform high concentrations of Cd (II). After the entire genome of MBR was sequenced, the functional genes of the MBR genome were counted, annotated and analyzed through comparison with different databases (NR, GO, COG, KEGG), and the genes related to cadmium resistance, metabolism and transportation were identified[6]. Using the NR database, it was found that the gene numbers related to heavy metals are: PCMBR3309 and PCMBR2993. The former was annotated as "metal binding protein", which indicates that MBR has a specific protein capable of binding Cd (II). The latter one was annotated as "Cd (II)/Pb (II) reactive transcription regulator", indicating that MBR has the ability to regulate transcription in response to Cd (II). It can be inferred that it may have the ability to adsorb Cd (II) from solution [6, 7].

By comparing the genome of P. chengduensis strain MBR with the GO database, it was determined that biological processes, cell components and molecular functions were the three main gene categories in MBR strains, as shown[6] in Figure 2. Among them, "redox process" was the highest value observed in the category of "biological processes", accounting for 8.86% of all annotated genes, indicating that many genes in MBR were involved in the process of Cd (II) redox process. The “whole component of cell membrane” accounted for 20.15% of all annotated genes in the “cell component” category, which indicated that a large number of membrane components in the MBR strain participated in the formation of biofilm under the pressure of Cd (II). "Metal binding" accounted for 3.52% of the items under "Molecular Function", and it can be inferred that MBR has the ability to bind metal ions. In addition, it was also found that the "oxidoreductase activity" accounted for 2.07%, which indicated that MBR has the required enzymes to participate in the redox reaction [6].

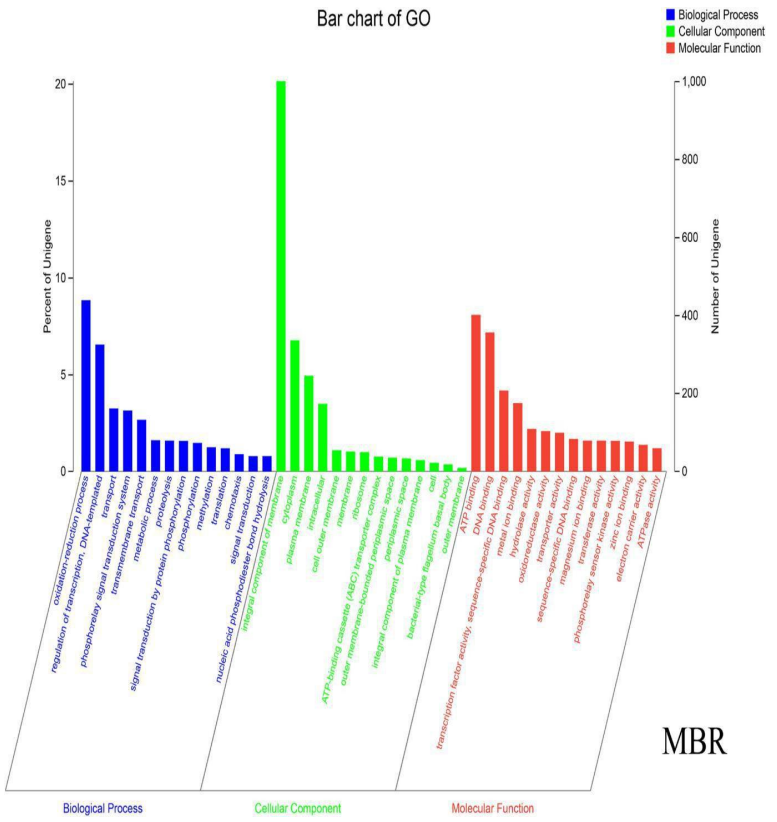

Figure 2. Annotation of the genome P. chengduensis strain MBR based on the GO database.

By comparing with the KEGG gene database, specific pathways related to response genes and heavy metals had been identified. It was found that the annotated genes in this pathway have three typical functions: "aerobic respiration", "electron transfer system" and "regulation of group activity and biofilm formation". It may be guessed that the adsorption of Cd (II) by MBR is closely related to its biofilm. MBR may generate biofilm under Cd (II) stress with aerobic respiration conditions, and redox reaction occurs on the biofilm, thereby reducing Cd (II) to the insoluble Cd (0)[6].

The gene annotation results of the MBR strain also identified a large number of genes related to carbohydrate metabolism, ensuring that the strain can carry out stable energy metabolism. This may explain why MBR strains can maintain a low concentration of Cd (II) inside the cell in the external environment with a high concentration of Cd (II) to maintain normal metabolic functions [6]. Through this analysis, it may be inferred that the growth and metabolism of MBR strains have a stable energy supply, so that they can adapt to changing environments [8]. In addition, a comparative analysis according to the Transporter Classification Database (TCDB) identified transporters related to heavy metals. Among them, the existence of two heavy metal-dependent phosphate hydrolases were found, indicating that MBR will not lack energy supply when transporting heavy metals [6].

Based on other annotation information of the MBR genome, it was found that the strain also had excellent resistance to other heavy metals such as Pb^{2+}, Ni^{2+}, Zn^{2+}, and can adapt well to various growth environments, which indicated that P. chengduensis strain MBR has great potential for bioremediation of metal-contaminated sites[9].

Through sequencing, annotation, identification and analysis of the genome of MBR strains, the resistance and removal mechanism of MBR to heavy metals (especially Cd (II)) were explored. It speculated that MBR strains may form biofilms under cadmium stress to adsorb Cd (II), then reduce and convert it into Cd (0) nano-scale particles, so as to reduce biological toxicity and reduce heavy metal pollution. This may provide valuable ideas and research directions for the control of cadmium pollution.

4.1.2 Chromium control

Wastewater produced in tanning production activities contains a large amount of heavy metals, especially chromium. Cr (VI) is a highly toxic substance that not only pollutes the terrestrial and aquatic ecosystems, but also poses a serious threat to human health (such as lung cancer) [10]. Many studies have shown that various microorganisms (such as Enterobacter sp. HU38, Cellulosi microbium sp., Escherichia coli, Amphibacillus sp. KSUCr3, Bacillus firmus KUCr1 and Pseudomonas aeruginosa CCTCC AB93066) can reduce hexavalent chromium in wastewater or soil to trivalent chromium, which can effectively reduce chromium pollution [11]. Therefore, the use of microbial ecological restoration to control heavy metal contaminated sites is considered to be a reliable and potential method [10].

M. metallidurans TL13 is a new type of heavy metal-resistant plant growth-promoting bacterium (PGPB) isolated from the wastewater of a tannery. The TL13 strain was sequenced through the Illumina MiSeq platform, the SPAdes algorithm was used for genome assembly, and the RAST server was applied quickly annotation, revealing the mechanism of the TL13 strain with resistance to heavy metals and promoting plant growth [10].

According to RAST's annotation of the TL13 genome, there were as many as 32 genes related to heavy metal resistance, such as copper resistance protein, copper responsive transcriptional regulator, arsenic resistance protein, arsenate reductase, arsenical resistance operon repressor, cobalt–zinc–cadmium resistance protein, multidrug resistance transporters, mercuric resistance operon regulatory protein, organomercurial lyase and mercuric ion reductase[11]. According to the annotation and analysis of the genome of the TL13 strain, several genes related to chromium reduction were also revealed, including chromate transporter, thioredoxin reductase, superoxide dismutase and the glutathione peroxidase. This result may reveal two mechanisms of TL13 resisting chromate from molecular level: one is chromate transporter may be a key factor in the efflux of cytoplasmic chromate, and another mechanism is the bacterial enzymes (such as hioredoxin reductase, superoxide dismutase and the glutathione peroxidase) may play an significant role in participating in resisting oxidative stress response induced by chromate[12, 13].

Based on the analysis of its genome, enzymatic and non-enzymatic mechanisms were prosed to explain the path and mechanism of Cr (VI) degradation by this strain, as shown [11] in Figure 3

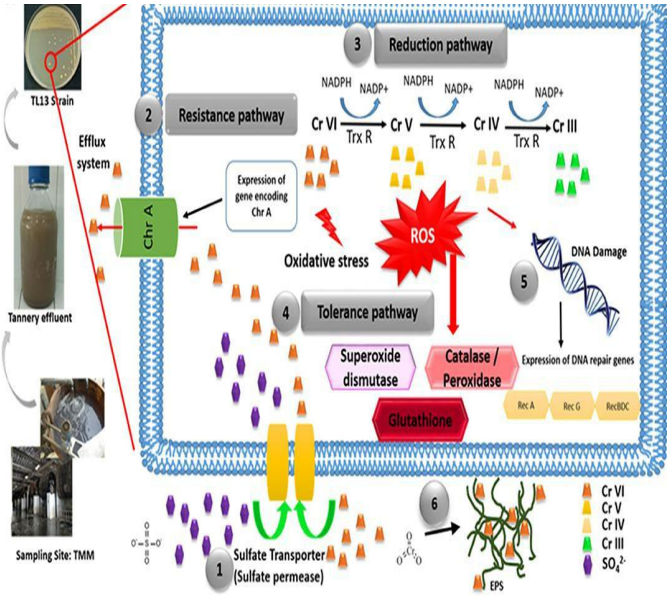

Figure 3. Hypothetical pathways and mechanisms related to chromium resistance and reduction of M. metallidurans TL13.

In addition, according to the annotation analysis and experimental research on the genome of the TL13 strain, a large number of genes for environmental adaptability and plant growth promotion (such as glycine-betaine production, cold and heat shock tolerance, siderophores, ammonia assimilation and mineral phosphate solubilization) had also been discovered. It may reveal that TL13 can synthesize siderophore by dissolving inorganic phosphate, and then produce hydrolase, EPS and

IAA to promote the path and mechanism of plant growth [10].

Through the application of genomics technology, it is helpful to deeply explore and understand the mechanisms of the resistance of M. metallidurans TL13 to heavy metals and plant growth promotation, and provide new insights and potential methods for heavy metal pollution control [10].

5. Application of omics technology to soil salinization

In the agricultural ecosystem, the large accumulation of nitrate in the soil is a major cause of soil salinization. It will not only reduce soil productivity, but also accumulate in vegetables, enter the human body through the food chain, and cause adverse effects on human health. Low-cost bioremediation methods are considered to be an attractive option for removing nitrate from soil [14].

Bacillus megaterium NCT-2 is a kind of bacteria with high salt adaptability and nitrate reduction ability isolated from secondary salinization soil and it is considered to have a good bioremediation function. The complete gene sequence of NCT-2 was obtained through Illumina Hiseq 4000 short-read sequencing platform and PacBio RSII long-read sequencing platform, the prediction and analysis were performed after genome assembly and annotation [14].

Having a large amount of rRNA is a typical feature of soil microorganisms, which helps them to grow and develop quickly, form spores and respond quickly to changes in external conditions (such as changes in nutrient utilization)[14] . It was compared the general genome features of Bacillus megaterium NCT-2 with those of Bacillus megaterium QM B1551, Bacillus cereus Q1, Bacillus megaterium DSM 319, Bacillus subtilis subsp. subtilis str. 168 and Bacillus licheniformis DSM 13. and the results are shown in Table 1[14]. According to the results of comparative analysis, it was found that the NCT-2 strain had the most coding sequences and RNA, and the largest genome size. For example, NCT-2 had 53 rRNA and 142 tRNA (Table 1). This may show that they have strong environmental adaptability [14].

Strain	*B. megaterium* NCT-2	*B. megaterium* QM B1551	*B. megaterium* DSM 319	*B. subtilis* 168	*B. cereus* Q1	*B. licheniformis* DSM 13
Genome size (Mb)	5.88	5.52	5.10	4.22	5.51	4.22
Chromosome size (Mb)	5.19	5.10	5.10	4.22	5.21	4.22
G+C content (%)	37.8	37.97	38.1	43.5	35.5	46.2
Chromosomal G+C content (%)	38.2	38.3	38.1	43.5	35.6	46.2
Gene number	6039	5674	5245	4536	5856	4382
Coding sequence number	5606	5379	4941	4237	5513	4219
RNA gene number	203	182	153	116	137	98
rRNA genes (5S, 16S, and 23S)	53 (19, 17, 17)	37 (13, 12, 12)	33 (11, 11, 11)	30 (10, 10, 10)	39 (13, 13, 13)	21 (7, 7, 7)
tRNA gene number	142	137	114	86	93	72
Plasmid number	10	7	0	0	2	0

Table 1. General features of B. megaterium NCT-2 compared with other five genomesof Bacillus strains

It used GO, KEGG, COG database and RAST Web server to annotate and analyze the 5606 coding sequences of B. megaterium NCT-2 strain to study its function and the mechanism of nitrate removal[14] .

According to the annotation results of the GO database, biological processes, cellular components and molecular functions are identified as the three main gene categories in the NCT-2 strain (Figure 4) [14]. Among them, catalytic activity (1,822), metabolic process (1,786), cellular process (1,567), single-organism process (1,400), and binding (1,214) are the top five categories.

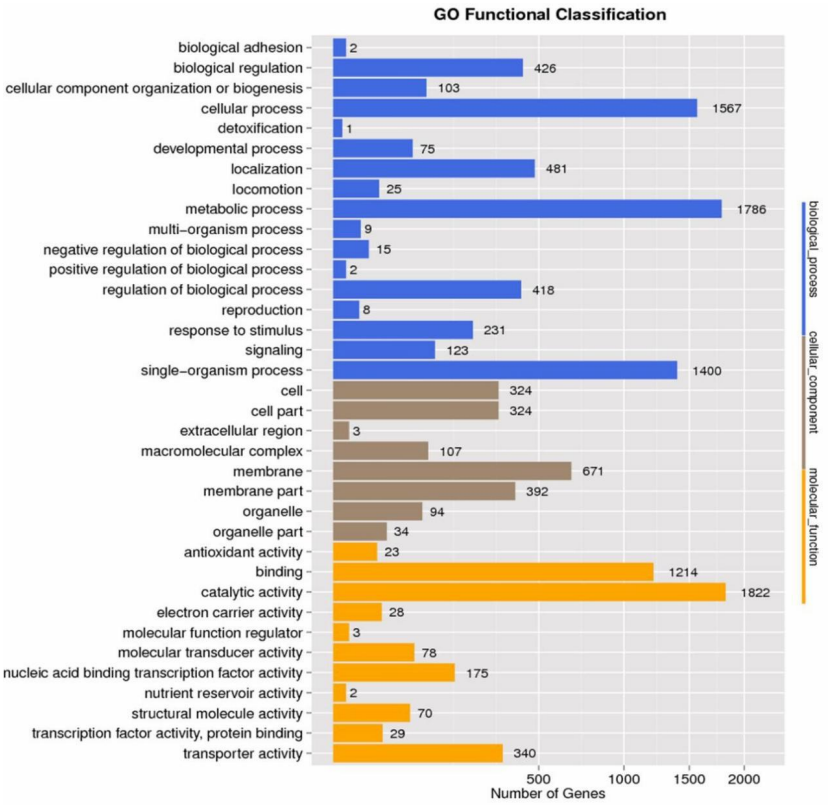

Figure 4. GO classifications (biological process is in Blue, cellular component is in Brown and molecular function is in Orange).

By submitting the translated protein sequence of Bacillus megaterium NCT-2 downloaded from RAST to the FusionDB Web server, it was found that the NCT-2 strain had the highest functional similarity with B. megaterium DSM 319 and B. megaterium QM B1551, with 90% and 89% respectively [14, 15]. The annotations related to nitrogen metabolism of NCT-2 strains included nitrate/nitrite sensor protein, encoding nitrate transport protein, nitrite transporter protein, nitrite reductase [NAD(P)H] large subunit, nitrite reductase [NAD(P)H] small subunit, nitrogen-fixing NifU domain protein, and nitroreductase family protein, etc. Genomic analysis showed that the Bacillus megaterium NCT-2 strain may be able to convert nitrate in the soil into biomass (see Figure 5), which provided a theoretical and practical basis and effective methods for understanding the use of Bacillus megaterium NCT-2 strain for bioremediation of secondary salinized soil[14].

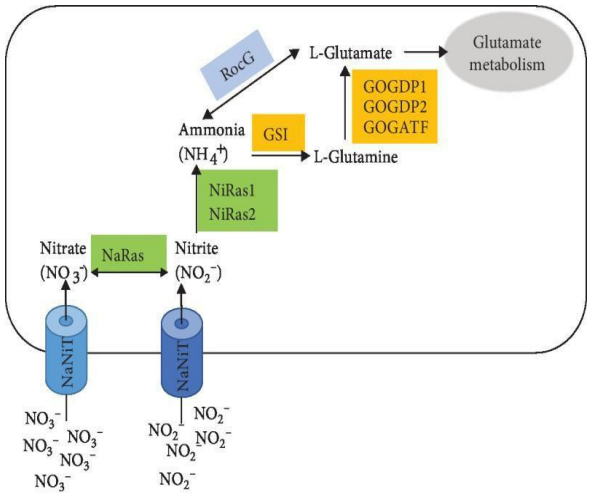

Figure 5. The proposed nitrate conversion process in secondary salinized soil by B. megaterium NCT-2

Through sequencing, annotation, identification and analysis of the genome of the MBR strain, it explored the clues related to the bioremediation characteristics of B. megaterium NCT-2 for secondary salinized soil, and speculated the possible nitrogen metabolism pathways and mechanism in the NCT-2 strain, which provided valuable genomic resources and development direction for the further research of applying B. megaterium NCT-2 to the bioremediation of secondary salinized soil[14].

5.1 Application of omics technology to petroleum pollution

Diesel is a light petroleum product, which is composed of a mixture of different hydrocarbons and has strong toxicity. Diesel is in high demand worldwide, however, accidental leakage often occurs during storage, transportation, and use, causing great harm to the environment and ecosystem [16]. Microbial bioremediation is considered to be one of the most cost-effective, labor-saving, effective and environmentally friendly methods to remove hydrocarbons from polluted sites [17].

Isolated the Acinetobacter calcoaceticus CA16 strain from Canadian soil, sequenced the whole genome, and annotated the genome characteristics. Through the sequencing of the whole genome, it was determined that CA16 had a total of 3798 gene codes, 6 rRNA operons and 65 tRNAs. The closest bacterial type to it is the strain A. calcoaceticus DSM30006. According to sequence similarity and protein homology, 11 genes related to the diesel metabolism pathways of other microorganisms were found in CA16 (Table 2) [16]. The proposed CA16 genome will help clarify the pathways and mechanisms related to the degradation of hydrocarbons by the bacteria, as well as the future application of this strain in the use of bioremediation to treat oil-contaminated sites.

Gene	Genome Position	Accession #	Predicted function	Most closely related RefSeq Protein	%ID
Alkane hydroxylase (*AlkM*)	1203709–1204902	AQZ81143	Alkane Monooxygenase Complex, Alk gene cluster	alkane 1-monooxygenase [Acinetobacter guillouiae] WP_004720613.1	99.45
Transcriptional Regulator (*AlkR*)	1813655–1812696	AQZ81718	Regulation of hydrocarbon degradation Alkane Monooxygenase Complex, Alk gene cluster	AraC family transcriptional regulator [Acinetobacter calcoaceticus] WP_080026973.1	100
Esterase (*EstB*)	363212–361917	AQZ80440	Esterase; Alkane Monooxygenase Complex, Alk gene cluster	MULTISPECIES: serine hydrolase [Acinetobacter] WP_075431253.1	99.77
Rubredoxin A (*RubA*)	1004293–1004457	AQZ80981	Alkane degradation	MULTISPECIES: rubredoxin [Bacteria] WP_000760495.1	100
Rubredoxin B (*RubB*)	1004198–1003023	AQZ80980	Alkane degradation; NAD(P)H-dependent rubredoxin reductase	MULTISPECIES: FAD-dependentoxidoreductase [Acinetobacter] WP_003653048.1	99.75
Lipoyl Synthase (LipA)	1866491–1865511	AQZ81763	Protein lipoylation	lipoyl synthase [Acinetobacter calcoaceticus] WP_080026996.1	100
Lipoyl Synthase (LipB)	3254410–3255063	AQZ82924	LipA Chaperone Protein	lipoyl (octanoyl) transferase LipB [Acinetobacter calcoaceticus] WP_005036485.1	100
Outer Membrane Protein A (*OmpA*)	861883–862536	AQZ80868	Type IV Secretion	MULTISPECIES: OmpA family protein [Acinetobacter] WP_005042643.1	100
Outer membrane lipoprotein (*wza*)	36420–35323	AQZ80175	*wee* Gene Cluster (polymer export: potential channel)	polysaccharide biosynthesis/ export family protein [Acinetobacter calcoaceticus] WP_080026070.1	100
Protein tyrosine kinase (*wzc*)	34868–32688	AQZ80173	*wee* Gene Cluster (polymer export: autophosphorylation)	polysaccharide biosynthesis tyrosine autokinase [Acinetobacter calcoaceticus] WP_080026068.1	100
General secretion pathway protein E (*xcpR*)	634244–635575	AQZ80682	Secretory Protein, associated with regulating dodecane degradation	MULTISPECIES: type II secretion system ATPase GspE [Acinetobacter] WP_004641278.1	100

Table 2. Genes related to the diesel metabolic pathway of other microorganisms inCA16.

GC/MS was used to analyze the metabolites produced by CA16 in the process of diesel degradation. It was found that the CA16 metabolites after diesel treatment were very complex, including aromatic hydrocarbons, aliphatic and alcohols, but it was determined that the total composition of the final metabolites changed significantly due to the degradation of diesel by CA16[16]. Through the analysis of metabolites, it is found that CA16 can at least degrade part of the hydrocarbons present in diesel. Due to the large overall difference in the final products produced by the process of CA16 degrading diesel fuel, it focused on the degradation effect of CA16 on n-alkanes, and it was detected by metabolomics that this strain has a very significant removal effect on n-alkanes, and the results can be seen from Figure 6[16]. This confirmed the removal effect of CA16 on aliphatic hydrocarbons in diesel.

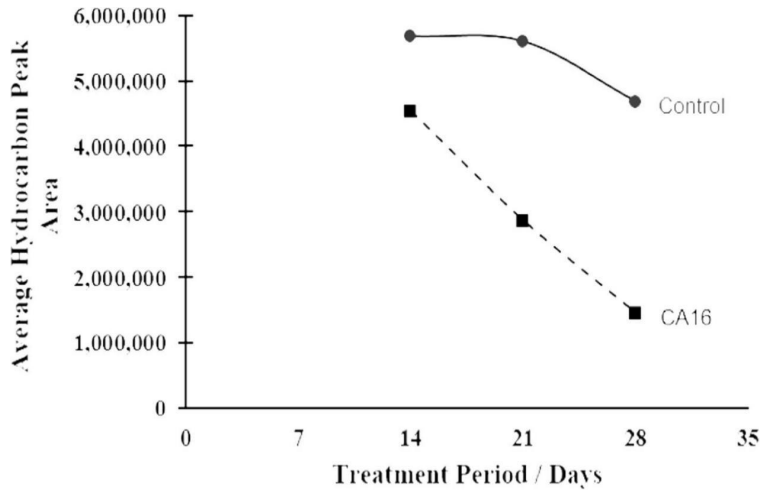

Figure 6.Total n-alkane hydrocarbons decreased by treating with CA16.

Generally, bacteria used to degrade hydrocarbons can only remove a limited number of hydrocarbon components (such as n-hexadecane (C16) and n-icosane (C20)) in the bioremediation process [16, 18]. However, Acinetobacter calcoaceticus CA16 strain isolated can degrade n-aliphatic alkanes (C12-C18) in a wide range, which shows that this strain has very specific potential for bioremediation of diesel-contaminated sites [16]

6. Conclusion and discussion

The research and application of omics technologies (genomics, transcriptomics, proteomics and metabolomics) in bioremediation are still in the development stage, but they have shown great potential. The development of bionomics technology will help us to explore and understand the relevant pathways and mechanisms of microorganisms in the process of removing pollutants from the molecular level. This will improve our understanding of bioremediation and promote its application and development in environmental science and engineering related fields (such as heavy metal pollution remediation, soil salinization, petroleum pollution remediation and plant growth promotion). How to perfect and combine the research of all the omics techniques, and analysis and storage the huge amount of information and data brought by omics technology will be the problems and challenges in future development. With the development of bionomics and related technologies, it is believed that low-cost and environmentally friendly microbial remediation methods will be the first choice to solve the increasingly serious environmental pollution problems.

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