Advances in genomics analysis for microbial soil remediation

Ruiyi Qu

School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, Sichuan, 610000, China

quruiyi@my.swjtu.edu.cn

Abstract. Soil pollution is a global environmental problem that poses a threat to ecological balance and human health. Microbial soil remediation is an eco-friendly technology that utilizes microorganisms to degrade or transform soil pollutants. Due to some drawbacks of traditional soil remediation methods, microbial remediation method has become a hot research topic nowadays. In recent years, the development of genomics analysis technology has greatly promoted the progress of microbial remediation research, and the use of genomics approach is free from the technical constraints of traditional research and can efficiently obtain the relative abundance of microbial communities and other information. In this paper, we reviewed the application of genomics in microbial soil remediation, and discussed the genomic analysis of several examples of heavy metal contaminated soil remediation, which provides a reference for researchers to quickly screen analytical methods in the field of microbial soil remediation, and reveals the biological significance of genomics analytical techniques.

Keywords: Soil, Microbial remediation, Genomics, Chromium.

1. Introduction

With the rapid development in all aspects of China and the accelerating process of industrialization and urbanization, the soil environment is suffering from the pollution of various types of heavy metals [1]. Microorganisms are widely present in nature with great diversity and adaptability, and the application of microbial remediation technology to heavy metal contaminated soil has great potential in environmental remediation [2]. The process of using microorganisms to break down or change contaminants in the environment into other safe compounds is known as microbial remediation, and it offers significant ecological and financial benefits for cleaning up contaminated soil, water, and other habitats. Microorganisms are widely used in soil remediation, and microorganisms with remediation ability for heavy metals mainly include fungi, bacteria, actinomycetes and so on. The traditional physicochemical remediation methods have the disadvantages of high cost, long cycle time, and easy to produce secondary pollution [3], in contrast, microbial remediation technology has become a hot content in the research field of heavy metal contaminated soil remediation due to the advantages of environmentally friendly, economic and effective, and strong sustainability.

The genome of microorganisms determines their biodegradation properties, and the study of their genomes plays an important role in exploring the mechanisms of microbial remediation of soil. The combination of genomics, transcriptomics, proteomics and metabolomics provides an important way to analyze the mechanisms of microbial remediation [4]. The analytical approach of genomics is a powerful

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tool for selecting and applying microbial strains with high repair efficiency. Microbial-based remediation techniques can be effectively combined with genomics approaches to provide an efficient solution for studying the molecular mechanisms of microbial remediation. This paper analyzes and summarizes the application of genomics in microbial soil remediation, and lists examples of remediation of heavy metal contaminated soil, and finally looks forward to the application of genomics in order to provide a reference for the in-depth study of the mechanism of microbial remediation.

2. Main modalities of microbial remediation

Biodegradation is the process by which microorganisms remove pollutants from the environment by degrading organic pollutants through metabolic pathways and converting them into harmless substances, a process that involves the synergistic action of multiple microbial communities and enzyme systems. Aerobic biodegradation is a process in which microorganisms convert organic pollutants into carbon dioxide and water under aerobic conditions using oxygen as the final electron acceptor. Anaerobic biodegradation is a process in which microorganisms metabolize under anaerobic conditions using alternative electron acceptors. Pesticides may be broken down by a wide range of microorganisms, such as bacteria, fungus, and algae. These organisms can bioremediate pesticides through a number of metabolic pathways, and the enzymes involved are essential to this process.

Biosorption is a process whereby adsorption structures on the surface of microorganisms (e.g., cell walls, extracellular polysaccharides, etc.) can adsorb and enrich pollutants, such as heavy metals and organic matter, in the environment, thereby reducing their concentration in the environment. For example, co-culturing *Enterobacter* sp. A11 and *Comamonas* sp. A23 strains with Cd(II) produces biofilm, hydrogen sulfide and succinic acid, which permits the cells to absorb Cd(II) and produce Cd(II) sulfide precipitates [5].

Biotransformation is the conversion of contaminants into harmless substances or reduction of their toxicity by microorganisms through metabolic pathways, including oxidation, reduction, hydroxylation, methylation and other reaction processes. For instance, the biotransformation of polyfluoroalkyl compounds formed from aqueous film-forming foams depends heavily on dealkylation, oxidation, and hydrolysis processes. These biotransformations may result in the development of several semi-stable intermediates [6].

Bioremediation enzyme activity is facilitated by microbial production of remediation enzymes that can promote the degradation and transformation of organic matter and heavy metals in the environment, including peroxidases, dehydrogenases, and oxidases. For instance, peroxidases are known to be essential enzymes in the biotechnology and pharmaceutical sectors. They are also playing a bigger role in environmental remediation, where they break down a variety of hazardous environmental pollutants, such as peroxides in bleach wastewater, herbicides, pesticides, synthetic textile dyes, phenols and their derivatives, and polycyclic aromatic hydrocarbons. Additionally, they function as indicators of oxidative stress in cells following xenobiotic exposure [7].

3. Analyzing the mechanism of action of microbial remediation from a genomic perspective

Microbial remediation is based on the metabolic pathways of microorganisms that convert pollutants into harmless or less toxic substances through their natural degradation capacity. This process involves the synergistic action of multiple enzymes, and the genes encoding these enzymes are key to microbial remediation research. Microbial remediation of soil can take advantage of the biological properties of microorganisms to degrade pollutants. Genomics provides an in-depth perspective to understand the mechanism of microbial action in soil remediation, which is mainly revealed by analyzing the expression of microbial functional genes and metabolic pathways.

3.1. Principles of genomics analysis

By whole genome sequencing, all possible gene sequences in microorganisms can be identified. At the same time, using bioinformatics tools such as gene prediction software and database comparisons, it is possible to predict the potential functions of these genes, especially those associated with pollutant degradation. To describe metabolic and regulatory networks in microbial cells, a wealth of information is available, such as annotated genome sequences and the genomic positions of DNA-binding regulatory proteins. High-throughput sequencing studies have been successful in elucidating the composition and diversity of microbial communities in an unknown variety of soil habitats. Utilizing the genome-informed basis of microbial metabolism, METABOLIC allows for consistent and reproducible studies of microbial communities and promotes the integration of uncultured organisms into metabolic models [8].

3.2. Genomics applied to microbial remediation

3.2.1. Functional gene. Analysis of microbial genomes can identify functional genes relevant to soil remediation, such as genes encoding organic pollutant-degrading enzymes and genes related to soil nutrient cycling. Methods including pure culture techniques and macrogenomics can be applied to analyze the genomes of microorganisms to provide information on catabolic genes. All known Cr(VI) remediation genes from culturable microorganisms represent only 1% of environmental microorganisms, while the remaining non-culturable microorganisms offer many potential possibilities for novel and efficient pollution remediation genes [9]. The key genes involved in pollutant degradation were identified by genome sequencing, and the functions of these genes were subsequently investigated by molecular biology techniques such as gene knockout and overexpression.

3.2.2. Metabolic pathway. Genomics can help identify and analyze the metabolic pathways of microorganisms to optimize the screening and design of microbial strains and to improve their efficiency and stability during soil remediation. By comparing the genomes of different microorganisms, the conservation and specificity of metabolic pathways can be identified. Finding the predominant heterotrophic carbon metabolism routes and understanding how microbial physiology affects the relative significance of carbon cycling pathways in response to environmental variables are essential for furthering our understanding of soil microorganisms [10].

3.2.3. Regulation of gene expression. Microbial gene expression in soil is regulated by a variety of factors, including environmental conditions, nutrients and chemical signals. Genomics studies can reveal the gene expression patterns and changing rules of microorganisms under different environmental conditions, and in the presence of pollutants, microorganisms may adjust the expression levels of relevant degradation genes to adapt to the environment and improve degradation efficiency. For example, by immobilizing *Bacillus cereus* WHX-1 on proliferating intestinal-type biochar to establish a microbial aggregation system, the strain's ability to reduce Cr(VI)could be increased, which led to a decrease in the soil redox potential and a significant improvement in the physicochemical properties of Cr(VI)-contaminated soils [11].

4. Genomic analysis of remediation examples of heavy metal contaminated soil

4.1. Removal of Cr(VI) by Sporosarcina saromensis M52

The bacteria *Sporosarcina saromensis* M52 possesses a potent ability to remove Cr(VI) by a process that is most likely related to biotransformation. The results of the investigation, which were obtained using scanning electron microscopy in conjunction with energy dispersive X-ray analysis (SEM-EDX), X-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD) analysis, and FTIR spectroscopy, shown that under Cr(VI) stress, strain M52 remained comparatively stable and that only negligible quantities of Cr(VI) were deposited on the cell surface, indicating that the elimination of Cr(VI) was mostly caused by bioreduction rather than non-biosorption. Numerous investigations have identified genes, such as chromate transporter proteins, reductases, and DNA repair enzymes, that are involved in Cr(VI) tolerance and reduction in strain M52. The findings show that several enzymes are involved in M52's metabolism of Cr(VI), and that a single enzyme is not responsible for Cr(VI) resistance or decrease. Numerous investigations have identified genes, such as chromate transporter proteins, reductases, and DNA repair enzymes, that are involved in Cr(VI) tolerance and reduction in strain M52. The findings show that several enzymes are involved in M52's metabolism of Cr(VI), and that a single enzyme is not responsible for Cr(VI) resistance or decrease.

The genomic analysis of strain M52 reveals potential mechanisms of bioreduction, which suggests that the mechanisms by which strain M52 responds to Cr(VI) stress are similar. For example, the ChrA gene-encoded chromate transporter proteins could aggressively transport Cr(VI) outside the cell to reduce the intracellular concentration of Cr(VI), consequently lowering the direct damage; multiple genes encoding nitrate reductases may work together to drive enzymatic redox reactions to achieve Cr(VI) bioreduction; reactive oxygen species-induced oxidative damage to genetic material may be minimized by genetically encoded proteases possessing DNA repair capabilities; and the development of particular cellular components to reduce the damage that Cr(VI) causes to biofilm structures [12].

4.2. Biosorption and biotransformation of pseudomonas chengduensis strain mbr under cd(ii) stress

Under aerobic conditions, *Pseudomonas chengduensis* strain MBR may biomineralize Ni(II) to Ni(0) and decrease heavy metals like Cu(II), and Mn(II). It was discovered that in the first 48 hours following static cell treatment with Cd(II), near-complete adsorption of Cd(II) can occur by examining the biosorption and biotransformation mechanisms of MBR strains under Cd(II) stress. MBR strains have the ability to reduce the biotoxicity of Cd(II) by adsorbing the metal and converting it into Cd(0) nanoparticles through the formation of biofilms on the cell surface.

Through genome sequencing, annotation, and analysis, Wang et al. found many functional genes associated with resistance to heavy metals. These genes may be associated with transport, metabolism, and resistance to Cd(II). Numerous genes in MBR are implicated in the Cd(II) redox reaction, according to the GO database. This suggests that a sizable portion of the strain's membrane components might have a role in the formation of biofilm under stress from Cd(II) and have a steady source of energy in their metabolism. This implies that MBR has a molecular method of attaching to metal ions [13].

4.3. Metabolic processes of chromium degradation by serratia sp.S2

Serratia sp. S2 is very capable of degrading Cr(VI). Chromium metabolism-related genes, including flavoprotein reductase, NAD(P)H-dependent azoreductase, nitroreductase, and chromium tolerance-associated proteins, are present in *Serratia* sp. S2. The membrane transporter proteins encoded by the genes chrA and chrB may use energy from the membrane potential to pump Cr(VI) out of the cytoplasm, reducing the cytotoxicity of Cr(VI) ions to cells. The chrA gene is associated to transporters relevant to Cr resistance. By using soluble enzymes that are encoded by chromosomal genes, bacteria can decrease Cr(VI) in aerobic environments.

By using the BLAST software with Glimmer 3.02, Dong et al. were able to successfully predict the entire *Serratia* sp. S2 coding sequence. They next achieved homology annotation and classification of these protein coding sequences by comparing them with the COG database. This procedure showed that several genes, particularly those involved in inorganic ion transport, might be crucial for *Serratia* sp. S2's absorption and excretion of Cr(VI). Moreover, there may be a substantial correlation between the expression of proteins that regulate Cr(VI) metabolism and genes related to the synthesis of cell membranes and amino acid transporters. On the other hand, *Serratia* sp. S2 growth and propagation may be strongly associated with genes related to DNA replication, recombination, and repair, indicating that this strain has the capacity to repair damaged DNA in order to mitigate Cr(VI) toxicity.*Serratia* sp. S2's predicted protein coding sequences were compared to the KEGG database in order to produce a thorough annotation of the strain's metabolic pathways. The regulation of intracellular transport and enzyme-catalyzed reactions, in particular, includes the secretion of soluble Cr(VI) reductases in the cytoplasm and the transport of these enzymes to extracellular membrane vesicles or extracellular media, thereby reducing Cr(VI) toxicity. In this regard, membrane transport and energy metabolism processes may be particularly important. Ultimately, GO annotation was achieved for *Serratia* sp. S2's CDS coding sequence through comparison with the Interpro and GO databases. This demonstrated the strain's capacity for substance metabolism and reproduction, suggesting that it is likely to make use of the energy supplied by the membrane [14]. These findings could demonstrate that *Serratia* sp. S2 may have the potential to treat chromium-contaminated environments.

5. Conclusion

Genomics analysis provides new breakthroughs and tools for studying microbial remediation mechanisms, and the use of various genomics technologies enables a comprehensive understanding of microbial genome structure and functional characteristics. By sequencing and analyzing microbial genomes, genes and metabolic pathways related to remediation mechanisms can be identified, thus revealing the potential mechanisms of microorganisms in responding to environmental stresses and pollutant degradation. The complexity and high diversity of soil microbial communities make data interpretation and functional prediction from genomics analysis complex and challenging. At the same time, genomics techniques typically focus on the presence of genes rather than their expression or activity, which may limit the assessment of actual microbial remediation capacity. With future technological advances, genomics technology can play a role in microbial community function analysis, targeted design of microbial agents, environmental adaptation improvement, ecosystem function restoration and so on. With the continuous development and improvement of genomics technology, the research in the field of microbial remediation will achieve richer and deeper results.

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