GENETICALLY ENGINEERED BACTERIA FOR ENVIRONMENTAL REMEDIATION

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ABSTRACT. The presented mini review examines the developed genetically engineered (GE) bacteria for environmental remediation. It also highlights the existed challenges and limitations associated to the releasing of such microorganisms in the nature. The advantage of GE bacteria application in environmental remediation of polluted sites is the eco-friendly approach in comparison to the hazard chemical and physico-chemical methods. A combinatorial strategy including microbiology with multiple biochemical mechanisms, ecological compatibility and engineering design would be a powerful way for successful in situ bioremediation of contaminated regions. Some critical questions exist pertaining to the development and implementation of genetically engineering bacteria for enhanced bioremediation have been identified in the review. As a conclusion, the genetic engineering of indigenous microbiological community, which is well adapted to local environmental conditions, may offer more efficient bioremediation of contaminated sites and making the bioremediation more viable and eco-friendly approach.

Key words: Genetically engineered bacteria, environmental remediation, eco-friendly biotechnology

ГЕНЕТИЧНО ИНЖЕНЕРНИ БАКТЕРИИ ЗА РЕМЕДИАЦИЯ НА ОКОЛНАТА СРЕДА

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РЕЗЮМЕ. Това миниревю изследва биоремедиацията на околната среда, чрез третиране с генетично модифицирани (GE) бактерии, и също така подчертава ограниченията и предизвикателствата, свързани с освобождаването на инженерни бактерии в полеви условия. Прилагането на регенериране на различни замърсители с тежки метали на базата на GE бактерии е на преден план поради екологичните и по-малки рискове за здравето в сравнение с физико-химичните стратегии, които са по-малко екологични и опасни за човешкото здраве. Комбинацията от микробиологични и екологични знания, биохимични механизми и полеви инженерни проекти би била съществен елемент за успешна биомедиация на замърсени с тежки метали региони, използващи инженерни бактерии. Идентифицирани са някои критични изследователски въпроси, свързани с разработването и прилагането на GE бактерии за подобрено биоремедиация. В заключение, генетичното инженерство на местната микрофлора, която е добре адаптирана към местните условия на околната среда, може да предложи по-ефективно биоремедиация на замърсени обекти и да направи биоремедиацията по-приложима и екологична технология.

Ключови думи: Генетично инженерни бактерии, възстановяване на околната среда, екологична биотехнология

Introduction

The problems related to the environmental pollution of soil and water have been escalating in recent decades due to the releasing of numerous toxic contaminants including organic compounds and heavy metals (Fulekar et al., 2009). The primary sources of pollutants are the industrialisation and urbanisation as a result of increased human population. The classical methods for remediation are based on chemical dispersants, which often pose a threat to nature and its ecosystems (Singh et al., 2017). The concept of bioremediation based on the microbial systems for the treatment of contaminants could enable better solution to reduce the pollutants in the environment. The biological mechanism for degradation of organics (mineralisation or biodegradation) is a natural process, without human interference. In general, such a process takes place everywhere and anywhere (Chen et al., 2016). The difference between bioremediation of organic xenobiotics and toxic metals is the existence of metal ions under different oxidation state, which strongly effect their distribution in the soil and atmosphere. To improve the efficiency of bioremediation, some indigenous bacteria might be isolated from a specific region and injected into the contaminated site. This process is known as bio-augmentation. It enables to also apply genetically engineered bacterial strains (GE). One of the existing challenges is the unpredicted end result. The reason is that GE may be affected by numerous environmental factors, which are in general optimised in the laboratory experiments. However, the use of GE micro-organisms in the past decades has been an emerging technology with the potential utilisation as an eco-friendly way for cleaning toxic contamination, including heavy metals (Liu et al., 2011). For example, the cell surface of some GE bacteria (e.g., *Ralstonia eutropha*, *Pseudomonas putida*, *Mycobacterium marinum*, *Escherichia coli*, *Sphingomonas desiccabilis*) contain precise proteins which are able to contribute to the detoxification of toxic metals, as well as of other compounds (Muhammad et al., 2008). These proteins are encoded by specific metal regulatory genes and thus might convert the more toxic form of heavy metals to lesser toxic forms (Hasin et al., 2010). Such bacterial strains might be used as plant-bacterium remediation systems for the removal of heavy metals from plants (e.g. rice, corn, etc.). Such a GE bacterium is *Methylococcus capsulatus*, which is able to bioremediate chromium (VI) pollution over a wide range of concentrations. There are various mechanisms in bioremediation such as bioattenuation, biostimulation, phytoremediation, etc. The applications of these mechanisms are going to be summarised in the current review.

Bioremediation of heavy metals

Annually, a huge amount of pollutants containing heavy metals are released into the nature (Shukla et al., 2010). Among them, the most dangerous are contaminations with arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb). As mentioned above, the application of GE for bioremediation of metals is developed based on their abilities to transform the toxic oxidation state to such oxidation state, which is less hazardous for nature. In comparison to the conventional physico-chemical methods, the bacterial metal detoxification costs less, possesses higher efficiency, and is environmentally friendly (Rajendran et al., 2003). Recent studies in the field of molecular biology have enabled us to better understand the mechanisms of detoxification machineries of GE. One of the best studied toxic heavy metal is mercury (Hg). Some bacteria have developed powerful resistance mechanisms to overcome the toxicity effect of Hg (Barkay et al., 2003). The detoxification has been achieved thanks to the Hg reductase enzyme which is responsible for the intracellular reduction of Hg^{2+} to less-toxic Hg^0 (Wagner-Dobler, 2003). For example, the strain *Acidithiobacillus ferrooxidans* has been reported to reduce the oxidation state of Hg through the ion transporter gene merC expression (Sasaki et al., 2005). Such a gene has been discovered also in *Mycobacterium marinum*. Thus, genetically engineered *Escherichia coli* bacteria, which simultaneously harboured merT–merP and MT genes, are capable of effectively removing Hg^{2+} ions from electrolytic wastewater (Deng and Wilson, 2001). *D. Radiodurans* are micro-organisms known as some of the most resistant to radiation. They have been developed to remediate radioactive waste sites contaminated by the use of nuclear weapons (Brim et al. 2006). The application of GE bacteria for the removal of cadmium (Cd) with high efficiency has also been demonstrated. The specific reaction of bacteria with Cd is formed due to the metal-binding peptides, such as metallothioneins and phytochelatins (Bae et al., 2001). The mentioned metal-binding proteins might also be successfully used for removing Cd, As, Pb and Hg, because of the high affinity to form thiolate complexes with those heavy metals. The phytochelatins can be cloned from plants and fungi and expressed to *E. coli*. Such genetic engineering bacteria possess both high bioaccumulation capacity and high affinity for the targeted metals (Deng et al., 2007). Recently, *E. coli* expressing arsenite Sadenosylmethionine methyltransferase gene coloned from *Rhodopseudomonas palustris* has been found to methylate the more toxic inorganic As to the less toxic volatile trimethylarsine (Qin et al., 2006; Yuan et al., 2008).

Bioremediation of organic xenobiotics

In general, the xenobiotics are synthetic compounds, which usually have organic structure that is persistent for degradation in nature. Most of them are very toxic and are associated as pollutants with affinity to accumulate in the environment. The genetic engineering techniques are focussed on the construction of bacteria with enhanced degradative abilities to xenobiotics (Hussain et al., 2018). For the bioremediation of organic xenobiotics from the polluted site, the microbes adopt various enzyme mechanisms including oxidation, reduction, hydrolytic cleavage, dehalogenation, etc., (Undugoda et al., 2016). For example, *Mycobacteria sp*. are excellent for remediating sites contaminated with polyaromatic hydrocarbons. These organisms have lipophilic surfaces, which can take up the bound contaminants from the soil and have catabolic efficiency towards benzene derivatives. The bioremediation of organic xenobiotics might be affected by some physical factors, such as temperature, pH, water content, and the presence of nutrition. In some cases, the biosurfactants augment the availability of organic pollutants for remediation purposes. Factors, such as the type of microbial species, the use of fertilizers or inorganic nutrients, and aeration play a vital role in the remediation of sites contaminated with hydrocarbons or their derivatives.

Usage of engineered bacteria in real environment

There are numerous reports in which GE bacteria with potential application for bioremediation are announced to have been developed in laboratory conditions. In addition, the advances of recombinant DNA technologies have paved the way for conceptualising "suicidal genetically engineered micro-organisms". Nevertheless, the release and usage of GE micro-organisms in real environment is still challenge. At the moment, there is successful large-scale demonstration of released *Pseudomonas fluorescens HK44* in the environment and its bioremediation application. However, there are numerous open questions related to the risks associated to the releasing of GE micro-organisms in nature. These questions have genuinely enthused the expansion of research in the field of Environmental Microbiology and molecular biology. The major problem encountered in successful bioremediation technology pertains to hostile field conditions for the engineered microbes. Only few bacteria such as *Escherichia coli*, *Pseudomonas putida*, and *Bacillus subtilis,* are well-characterised, but other bacterial strains need to be tried too. The general concern is to construct GE bacteria for field release in bioremediation, which must always possess an adequate degree of environmental certainty. The major problem is that bacteria designed for bioremediation processes have been designed for specific purposes under the laboratory conditions, ignoring the field requirements and other complex situations. The application in real environment depends on several factors, as the type of bacteria necessary to deteriorate the contaminants, the availability of the pollutants for the microbes to act, and as was mentioned above, the soil characteristics, such as pH, temperature, soil type, presence of nutrition, as well as the level of oxygen. Those factors also include the potential of horizontal gene transfer and bacterial survival, which may affect the present microflora.

Conclusion

The application of GE bacteria for the bioremediation of environmental toxicants is opening a new era in the large-scale purification biotechnology. There are numerous enzymes, genes, and metabolic pathways which can be used for the biodegradation purposes. However, the majority of studies related to bioremediation have been conducted in laboratory conditions and their application in real environment is still a challenge. Therefore, the future research efforts should be focused on identifying the factors that enhance the *in situ* bioremediation by engineered bacteria.

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