Application of Synthetic Microbiome to Bioaugmentation of Pesticide Pollution

Chenxiao Li

College of life science, Nanjing Agricultural University, Nanjing, 210014, China

Abstract. The issue of soil contamination from chemical pesticides is a pressing concern in today's world. The excessive use of these chemicals can lead to their accumulation in soil over time, posing risks to both the environment and human health. And synthetic biology is known as the third biotechnology revolution. So from the perspective of bioaugmentation, this paper elaborated on the use of synthetic microbiome bioaugmentation of organic pesticides to provide an environmentally friendly and effective new solution for pesticide pollution. This paper first reviews the development of synthetic microbiome on the basis of synthetic biology, and then lists the current applications of synthetic microbiome in various fields. By comparing with the characteristics of physical degradation and chemical degradation, the advantages of choosing biodegradable organic pesticides were analyzed, and the examples of synthetic microbiome on bioaugmentation herbicide pollution and insecticide pollution were introduced. Through comprehensive and objective analysis, the problems that need to be solved in the field of bioaugmentation and the future optimization direction of synthetic microbiome are shown in the end.

1 Introduction

With the ongoing advancements in industry and agriculture, the issue of soil contamination from chemical pesticides has escalated worldwide. Chemical pesticides can have long-lasting effects on soil fertility and nutrient cycling processes. These pesticides can contaminate groundwater and nearby surface water bodies, potentially entering the food chain and posing risks to aquatic ecosystems. Furthermore, soil contamination from chemical pesticides can have adverse effects on human health. Exposure to these toxic substances through direct contact with contaminated soil, inhalation of pesticide residues, or consumption of crops grown in polluted soil can lead to various health issues. Atrazine, as one of the largest herbicides used in the world, interferes with the endocrine, reproductive and nervous systems of humans and animals [1]. Paraquat, a highly utilized broad-spectrum cationic contact herbicide, is employed in over 100 countries [2]. However, it poses significant health risks to both humans and animals. Exposure to Paraquat can lead to nerve damage, as well as kidney and liver dysfunction. In severe cases, it can even result in irreversible pulmonary fibrosis, inflammation, and respiratory failure, potentially leading to fatality [3,4]. Consequently, this form of pollution poses significant risks to both the ecological balance and human well-being. Moreover, it directly impacts global food security and hampers economic progress.

Within the realm of available technologies, bioaugmentation stands out as an excellent approach to address the issue of pesticide-contaminated soils [5]. Bioaugmentation has the advantages of environmental friendliness, economic benefits, lasting effect and wide application range in the treatment of soil contaminated by pesticides, which is conducive to the realization of sustainable agricultural development and environmental health protection.

With advances in the fields of metabolomics and synthetic biology in the past few decades, the creation of synthetic microbiomes has gained research attention. Synthetic microbiome is to extend the single-cell system strategy of traditional synthetic biotechnology to multi-cell system, design and manipulate the microbiome from scratch, realize the allocation of different metabolic pathways to multiple strains and build the best combination. Synthetic microbiome has many potential and application prospects. By precisely regulating the composition and function of microbial communities, it is possible to achieve positive impacts in areas such as the environment, agriculture, medicine and energy. And it has a broad application prospect in the direction of bioaugmentation.

2 Developments and applications of synthetic microbiome

2.1 Developments of synthetic microbiome

Microorganism with rich diversity is an important part of soil ecosystem and is the dominant player in the metabolism of organic pollutants in the environment. In

* Corresponding author: 10121211@stu.njau.edu.cn

the past few decades, there have been many reports of pure culture strains that can degrade organic pollutants [6]. However, in practical applications, the traditional way of bioaugmentation based on a single degrading strain is not ideal [7]. There are several reasons that affect the non-ideal bioaugmentation effect of single degrading strains that have been discovered. First, because the single degrading strains extracted from the laboratory are usually difficult to adapt to the real soil environment affected by temperature, humidity, wind and other factors, the survival rate of the degrading strains is generally low after being applied to the contaminated soil. The exogenous nutrients or nutrient limiting factors required by these newly discovered single degrading strains are difficult to be distinguished or these degraded strains have not been analyzed in depth using metabolomics, which makes it difficult to implement the biostimulation strategy of improving the efficiency of pollutant degradation by applying exogenous nutrients to stimulate the emergence and consumption of these degrading strains. In addition, with the continuous development of modern industry and agriculture, compared with the pollution of the environment by a single pollutant, organic composite pollution caused by multiple pollutants co-existing in the environment has become a more and more common phenomenon. The interaction of these multiple pollutants co-existing in the environment greatly increases the difficulty of bioaugmentation[8].However, the single degrading strains found at present have a limited ability to metabolize pollutants, and even they are difficult to achieve complete degradation of various kinds of complex organic pollutants, and the single degrading strain is not suitable for repairing the long-term and widespread composite pollution environment of multiple pollutants.

The microbiome is the main form of microorganisms in the natural environment. Bioaugmentation based on the microbiome is an effective method to solve the problem of traditional single-strain bioaugmentation. First, strains in the microbiome can form stable structures through direct or indirect interactions, improving adaptability to the environment and survival [9]. Secondly, the microbiome has rich genetic and metabolic diversity, which can not only achieve complete degradation of complex pollutants through collaborative metabolism, but also metabolize a variety of different pollutants at the same time, which provides the possibility for the restoration of complex pollution environment [10-12]. The composition and structure of the microbiome significantly affect the metabolic efficiency of pollutants and determine the metabolic pathways and final products of pollutants. Therefore, designing and optimizing the composition and structure of the microbiome to obtain a stable and efficient microbiome is an important prerequisite and means for its application in the remediation of composite contaminated soil.

Synthetic biotechnology is the use of engineering design concept of existing organisms to design, transform or new synthetic artificial life, which is hailed as a subversive technology to change the future and an important way to solve major problems of mankind [13,14]. The synthetic microbiome extends the single-cell system strategy of traditional synthetic biotechnology to multi-cell system, designing and manipulating the microbiome from scratch, assigning different metabolic pathways to multiple strains and constructing the optimal combination [15,16]. Synthetic microbiome is an important prerequisite and means for the application of microbiome in the remediation of complex polluted environment.

2.2 Applications of synthetic microbiome

2.2.1 Bioenergy

Bioenergy has the characteristics of renewable and less pollution, and is the key research object of energy development and environmental protection in the 21st century. Many studies have shown that the microbiome is significantly superior to single strain production in terms of fermentation rate, pretreatment, biogas yield, stability. The direct use of microbiome for biopower generation has gradually become a research hotspot. The genetically modified Bacillus subtilis can provide a high concentration of riboflavin for iron-reducing bacteria and other power generation strains, which greatly improves the biopower generation capacity.

2.2.2 Chemical products

Isobutanol, isopropyl alcohol and other chemical products are extracted by traditional physical and chemical methods and produced by microbial fermentation. It has been used more and more widely. A research group has developed a synthetic fungus-bacterial flora that uses lignocellulose as a substrate for biodegradation to produce isobutanol. And the yield is up to 62% of the theoretical maximum. Some people also coupled enzyme producing strains and isopropyl alcohol producing strains, and the coupling between microorganisms greatly reduced the burden of protein expression and other metabolic burdens of each strain, and significantly improved the glycosylation efficiency of cellobiose and the production efficiency of isopropyl alcohol.

2.2.3 Environmental protection

By constructing synthetic microbiome, the degradation and removal of harmful substances, such as heavy metals and organic pollutants in water can be achieved, and the purification efficiency of water quality can be improved. In addition, the use of microorganisms in the synthetic microbiome can degrade pollutants in the soil, such as petroleum hydrocarbons, organic solvents and pesticides, and promote soil repair and restoration.

3 Unique strengths of bioaugmentation compared with physical absorption and chemical degradation

Bioaugmentation technology is to improve the degradation efficiency of pollutants by directly applying degraded strains to the polluted environment [17]. The microorganisms used in bioaugmentation have specific catabolic pathways and enzyme systems, so they can degrade the targeted organic pollutants more effectively and have less impact on other substances in the environment, such as organic nutrients. Some microorganisms carry special plasmids, which gives them the potential to degrade organic pollutants, for example, some microorganisms carry plasmids that degrade pesticides or heavy metals. In addition, there are co-metabolic relationships among some microorganisms in the environment. When a microorganism cannot completely degrade organic pollutants, other microorganisms in the environment may be able to use its intermediate metabolites to further degrade organic pollutants, thus constituting a complete metabolic pathway together. These special functions provide favorable basic conditions for the birth, application and development of bioaugmentation technology.

The effect of chemical and physical degradation of organic pollutants can be easily affected by environmental factors such as temperature, humidity and oxygen concentration and the degradation efficiency may be different under different environmental conditions. The microorganisms used in bioaugmentation are often self-healing, able to adapt to environmental changes and reproduce on their own, thereby sustaining the degradation of organic pollutants.

Bioaugmentation technology is suitable for more organic pollutant scenarios, such as contamination in groundwater and soil. Due to differences in chemical structure and physicochemical properties, the degradation of specific organic pollutants requires different chemicals. And there are many kinds of organic pollutants, which may need to use different treatment techniques to degrade different types of organic pollutants, increasing the complexity and cost of the treatment process. The diversity and metabolic capacity of microorganisms enable them to adapt and degrade various organic pollutants. So bioaugmentation is suitable for different types of organic pollutants, including petroleum hydrocarbons, organic solvents and pesticides, etc.

4 Synthetic microbiome on bioaugmentation of pesticide pollution

The bacteria found in natural bacterial communities, mainly those found in polluted locations, can be exploited to extract the microorganisms for bioaugmentation. For instance, *Pseudomonas putida* is capable of resisting harmful chemicals and may use them as a carbon source, including benzene, toluene, and xylene [18, 19]. *Pseudomonas putida* is therefore a useful substance for repairing harmful chemical molecules.

Numerous research has revealed that certain microbiomes frequently have some kind of enhancing impact on the decomposition of organic contaminants. For example, the synergic degradation efficiency of strains *Klebsiella* sp. A1 and *Comamonas* sp. A2 is significantly higher than that of single strain A2 [20]. However, this enhancement effect by direct combination of degraded strains does not exist in arbitrary combinations of degraded strains. For example, the combination of *Achromobacter* sp. A01 and *Pseudomonas* sp. A02 did not improve the degradation efficiency of atrazine [21].

4.1 Steps for screening synthetic microbiomes that degrade pesticides

It is first necessary to collect samples that may contain degrading microorganisms, which can be soil, water, plant rhizosphere, etc., contaminated by pesticides for a long time. Strains that can survive in these places are often tolerant or resistant to pollution sources, and can even use pollution sources as carbon sources. The microorganisms are then isolated from the sample and purified by gradient dilution and microbial culture. The purpose of this step is to obtain a separate microbial isolate for subsequent screening. A culture medium with a specific pesticide as the sole carbon source is then prepared to serve as the sole energy source for microorganisms and degradation substrates. The isolated microorganisms are inoculated into the medium containing the degradation substrate and cultured for a period of time. By observing the changes of the medium, such as the formation of pesticide degradation products, color changes or solubility changes, the microorganisms with high degradation ability to pesticides are screened.

Through further experimental verification, it is determined whether the selected microorganisms really have the ability to degrade the target pesticides. For example, the growth curve of the strain was measured, the pesticide residues and the degradation rate of the strain after 5 hours,8 hours,11 hours, 14 hours,24 hours and 48 hours of pesticide application were respectively used by HPLC, and the metabolites of the strain were analyzed by metabolomics technology. High-throughput sequencing also may be used to examine changes in the soil microbiome's structure. 16sRNA sequencing technology is used to identify and classify microorganisms with degradability, and determine what kind of microorganisms they belong to and their genus or species level.

Further optimization of the culture conditions and culture methods for the selected microorganisms, such as screening from the pollution source to the cornerstone auxiliary strains and co-culture with degrading strains to improve the efficiency and stability of degrading pesticides.

4.2 Herbicide pollution

In agricultural practices, herbicides are essential for weed control and account for around 48% of the total pesticide market. Nonetheless, incorrect herbicide usage can result in serious health concerns for humans, including birth defects, cancer, and disorders in the endocrine system. Herbicides work by interfering with various plant processes, including photosynthesis, the production of plant hormones, cell division, and the pathways for amino acid and lipid synthesis. The widespread use of these pesticides has raised concerns about potential effects on the health of humans and animals [22].

A microbial consortium made up of three bacterial strains—*Arthrobacter sulfonivorans*, *Variovorax soli*, and *Advenella* sp. JRO—was used to break down diuron in polluted soil. The consortium's effectiveness was assessed in a soil solution with diuron acting as the only carbon source. Surprisingly, within a few days, more than 98.8% of the diuron that was first added had mineralized. However, none of the three strains could successfully mineralize diuron when tested separately on a mineral medium (MSM) supplemented with micronutrient solution (NS). Together, they mineralized 40% of the diuron in the solution when coupled in pairs. Full diuron mineralization was accomplished within just a few days when all three strains were present, which produced the most striking results [23].

The extensive usage of the herbicide chlorimuron-ethyl (CE) has significant adverse effects on subsequent crops and poses serious environmental risks. Through enrichment culture, researchers have successfully isolated a new microbial community known as L1. This L1 microbial community has exhibited remarkable abilities in degrading CE, surpassing all previously reported communities. Within a span of 6 days, the L1 community achieved a degradation rate of 98.04% for a concentration of 100 mg L^{-1} of CE. Analysis of the community's taxon-functional contributions has revealed the crucial roles of enzymes such as glutathione transferase [EC 2.5.1.18], urease [EC 3.5.1.5], and allophanate hydrolase [EC 3.5.1.54] in CE degradation [24].

A diverse bacterial consortium, comprising 21 distinct species, has demonstrated an enhanced capability to degrade a range of pesticides including atrazine, 2,4-dichlorophenoxyacetic acid (2,4-D), carbofuran, diazinon, and glyphosate. This consortium showcases the promising potential for efficient degradation of multiple pesticides with diverse chemical properties [25].

4.3 Insecticide pollution

Within the environment, different microbial strains collaborate through crossfeeding, dividing metabolic tasks among the group, and assigning distinct functions to each member of the community. Some members contribute to pesticide bioaugmentation, while others are involved in maintaining cellular biochemical production. The degradation of the insecticide fenitrothion is facilitated by synergistic interactions between microbial strains. In particular, two bacterial isolates, *Sphingomonas* sp. TFEE and *Burkholderia* sp. MN1, have been identified for their remarkable ability to degrade fenitrothion. This cooperative relationship among the microbes allows for efficient breakdown of the insecticide [26].

In a Costa Rican study, soil previously exposed to imidacloprid was used to isolate a microbial community capable of degrading imidacloprid. Through co-metabolic enrichment, a consortium consisting of eight bacterial strains and one yeast strain was obtained. This consortium not only demonstrated the ability to degrade imidacloprid but also thiamethoxam and acetamidine, as confirmed in cross-degradation experiments. The effectiveness of this biological removal process was further tested in a batch stirred tank bioreactor (STBR), where simultaneous removal of imidacloprid+thiamethoxam or imidacloprid+thiamethoxam+acetamidine mixtures was achieved. The process resulted in impressive rates of 95.8% and 94.4% removal of total neonicotinoid insecticides, respectively.

A large synthetic pyrethroid insecticide called cypermethrin and its metabolite, 3-PBA, are known to significantly pollute the environment and agricultural products. Two strains, JQ-L (*Rhodococcus* sp.) and A-3 (*Comamonas* sp.), have been discovered in soil that has been exposed to pyrethroids for a long length of time. Within a 60-hour culture period, JQ-L effectively transforms 100mg/L of cypermethrin into 3-PBA, but it does not further deteriorate 3-PBA. In contrast, strain A-3 fully destroys 100mg/L of 3-PBA in 15 hours while using 3-PBA as its only carbon source. When co-cultured, JQ-L and A-3 show that they are capable of thoroughly metabolizing 100mg/L of cypermethrin in under 24 hours. A strategy to successfully remove cypermethrin residues from the environment and commercial crops is presented in this study as a promising one.

5 Conclusion

This paper demonstrates that synthetic microbiome is a promising technology that has matured under the development of various omics and genome editing techniques. Combined with metabolomics, the metabolic flux and growth changes of synthetic microbiome can be analyzed to better design synthetic microbiome. In combination with CRISPR/Cas and other technologies, non-model microorganisms can be modified to obtain microbial elements with targeted metabolic pathways. In this paper, it is found that synthetic microbiomes can be used for the production of biofuels and chemical products, and can also be used for bioaugmentation of agricultural soil pollution caused by organic herbicides and pesticides. Many examples are used to prove the irreplaceable advantages and feasibility of synthetic microbiomes in bioremediation.

At the same time, there are many non-negligible problems that need to be solved to truly achieve the application from the synthetic microbiome to

bioaugmentation. Improvements can be divided into two directions: analyzing and optimizing existing synthetic microbiomes and creating more powerful microbiomes. Measures that can be improved in the direction of optimization are: Further in-depth analysis of nutrient and metabolic communication between the chassis strain and the target strain was carried out by using metabolomics analysis technology, prediction of microbial succession and clarification of interaction models within the microbiome were carried out by computational biology, new chassis strains suitable for orthogonal genetic circuit operation were developed, and survival ability of synthetic microbiome was improved by finding local auxiliary strains. In the direction of creating a new microbiome, it is possible to study and design a synthetic microbiome that can repair the pollution of multiple organic compounds and pesticides at the same time, and transform chassis strains or degraded strains that can metabolize harmful organic compounds or their derivatives rapidly and in large quantities through directed evolution technologies such as PACE (Phage-Assisted Continuous Evolution), base editing technology and error-prone PCR.

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