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Advances in Research on Synthetic Microbial Communities

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Abstract

The synthetic microbial community is a synthetic microbial system co-cultured with multiple species, which has the characteristics of clear composition and strong controllability. Compared with a single colony, it can achieve more complex functions and adapt to the changing environment more easily, so as to meet a wide range of needs. In this paper, the contents and concepts of microbial community and synthetic microbial community are briefly introduced, the principles that should be followed in the construction of microbial community are expounded, the methods and mathematical models used in the construction of synthetic microbial community are introduced, and the applications of synthetic microbial community in various fields are summarized. Finally, the challenges in the research of synthetic microbial communities are briefly described.

Keywords

Microorganism, Synthetic Microbial Community, Model Building

1. Introduction

Microorganisms refer to all tiny organisms that are difficult to observe with the naked eye, including bacteria, fungi, viruses, and some small protozoa and single-celled algae. Microorganisms are the most abundant and diverse group of organisms on Earth, and they are ubiquitous in our daily lives [1]. Beyond their presence in natural environments, microorganisms are also found in agriculture, industry, medicine, and food production [2]. Microorganisms typically exist within various communities, and these structural units formed by the combination of different microorganisms are known as microbial communities. The interactions among microorganisms within these communities maintain their stability and functional diversity, and to some extent, drive their evolution and development [3]. In microbial communities, the interactions between different types of microorganisms allow them to better adapt to environmental changes compared to single strains. These microorganisms can interact to form network regulatory patterns and participate in complex reactions. Thus, microbial communities are not merely simple assemblies of independent microorganisms but are complex ecosystems formed through mechanisms such as resource competition, nutritional symbiosis, quorum sensing, and horizontal gene transfer [4]. Microbial communities are present in all ecosystems on Earth and play a crucial role in the Earth's material cycles. For instance, the metabolism of microbial communities significantly impacts the global cycles of elements like carbon, nitrogen, and sulfur. In Kazemi, K. [5] research that microbial communities in compost are closely related to enzyme activity. Berrier, D. [5] studied The decomposition of plant litter is also related to the composition of microbial community. Moreover, microbial communities have significant implications for human health, agriculture, and industry. Particularly in health, numerous medical studies focus on the microbial communities within and on the human body, investigating their roles in physiology, immunity, development, and nutrition, and further exploring disease treatment [6].

In the early stages of microbial research, scientists primarily focused on studying single microbial populations. By isolating and modifying key functional groups, they aimed to enhance specific target functions [7]. However, this approach neglected the interactions among microorganisms. In nature, microorganisms do not exist in isolation but rather in dynamic communities where they interact and interconnect to ensure overall stability and functionality [8] [9]. Moreover, traditional microbial research methods, which involve extracting and isolating microorganisms from natural environments, are inefficient, capturing less than 10% of the microorganisms present in nature [10], thus failing to meet the demands of extensive microbial research. To better study microbial communities, scientists have turned to synthetic microbial communities. These are artificially constructed systems where two or more species (including wild-type and genetically modified microorganisms) are co-cultivated based on an understanding of community composition and components [11] [12]. Within synthetic microbial communities, microorganisms interact and divide labor to perform various functions, enabling them to adapt to complex environments. Researchers utilize mixed systems composed of different strains to divide and cooperate on the functions of complex gene networks, employing synthetic microbial communities to study intricate interactions. Artificially constructed synthetic microbial communities can simplify natural microbial communities, allowing the establishment of simplified model systems tailored to specific research needs, thereby facilitating a deeper understanding of microbial communities. The study of synthetic microbial communities not only aids in understanding the performance and stability of microbial communities but also serves to investigate inter-population interactions, community structure, function, material metabolism, and the impact of interactions between communities and their environments. In community research, microbial communities consist of interconnected and interacting microbial groups. Studying a specific group in isolation is an incomplete approach. Only by examining the complex interaction networks among groups can we gain a comprehensive understanding of microbial community behavior in nature, better grasp the transformation of substances in the environment, and advance the development and utilization of microbial resources. Natural microbial communities are often complex, with many species of unknown functions and limited controllability. Synthetic microbial communities address these challenges effectively.

The composition of synthetic microbial communities is simpler compared to environmental microbial communities. The internal structure of these communities is known and highly controllable. Compared with a single strain, it has stronger adaptability and robustness in the face of external environmental changes. Compared with a single strain, synthetic microbial communities can achieve more complex functions, and the microorganisms in the community can practice division of labor to reduce the metabolic burden of microorganisms. In the study of synthetic microbial communities, different communities can be designed and constructed according to specific objectives to meet various research and application needs. Synthetic microbial communities are classified based on the functions of the strains, and interactions between different strains are established as needed to achieve dynamic balance, providing greater adaptability and stability to environmental changes. Additionally, different strains can be constructed to avoid cross-interference during research [13] [14]. Synthetic microbial communities have advantages such as low complexity, high controllability, good stability, and strong robustness [15], and they are widely used in daily life and production. For example, the two-step fermentation process for vitamin C; Increasing the yield of industrial production (ethanol, methane, soy sauce, etc.) through synthetic microbial communities; Enhancing the degradation rate of substances (petroleum, fibers, etc.); And producing high-value-added products (including sugar nucleotides, oligosaccharides, and other biopharmaceuticals) [2]. In recent years, microbial fuel cells, as a new energy solution, have received extensive research and attention. Microbial fuel cells are a practical application of synthetic microbial communities, utilizing microbes to mediate electricity generation with a wide range of substrates, offering significant utilization prospects. The study of synthetic microbial communities provides a holistic research approach, designing communities as a whole rather than being limited to the communities themselves. This method is more flexible and minimizes disturbances to existing communities. Current research on synthetic microbial communities is often associated with synthetic biology, systems biology, and microbial ecology, incorporating mathematical models and computational methods to design and predict community behavior, making the mechanisms between communities clearer.

2. Principles of Synthetic Microbial Communities

In the research of synthetic microbial communities, three primary aspects are emphasized: interactions among microorganisms, spatial coordination among microorganisms, and the stability of the microbial community. The selection of suitable microbial species for synthetic microbial community studies requires careful consideration of microbial interactions. Utilizing these interactions effectively to achieve experimental goals is essential. During the overall construction, attention must be paid to the spatiotemporal structure to ensure spatial coordination, ultimately achieving a stable synthetic microbial community capable of maintaining its structural and functional integrity over the long term.

2.1. Interactions Among Microorganisms

When designing and constructing synthetic microbial community systems, the primary focus is on microbial interactions, the spatiotemporal organizational structure of the synthetic microbial community, and the stability of the community structure. In natural ecosystems, microorganisms interact through various modes, including competition, predation, symbiosis, and mutual exchange. These interactions are extensively studied in ecological research. Multiple interactions may coexist among microorganisms, such as simultaneous resource exchange and competition, forming complex network regulatory effects and fostering interdependence among microorganisms. Stable interactions within the community are essential for achieving desired community behaviors. Metabolic functions and quorum sensing effects can be engineered in synthetic microbial communities to establish interactions among microorganisms.

In numerous studies, synthetic microbial communities are designed by leveraging the exchange of essential metabolites between different microorganisms. This involves artificially designing metabolic nutrient complementation among microorganisms to achieve mutual support or symbiosis within the community. For instance, gene knockout technology can be employed to enable different strains to cross-supply essential metabolic nutrients, allowing strains with different but complementary metabolic capabilities to grow together. These strains form a co-nutritional community, supporting each other's growth. Such approaches can create synthetic microbial communities with 2, 3, or even up to 14 members [16]. Beyond material metabolism, microbial interactions in the environment can also involve sensing the surrounding environment and communicating with neighboring cells through the secretion, diffusion, and exchange of various molecular substances (peptides, hormones, and natural products). Quorum sensing is a communication mechanism among bacteria, where they release molecules to communicate with other bacteria and signal when a critical population density is reached. This mechanism, dependent on cell communication, involves bacteria producing specific signal molecules in a density-dependent manner and responding accordingly. The mechanism is modular and can be engineered to influence group-level behaviors through the design of quorum-sensing effects. For example, Saeidi *et al.* [17] designed an E. coli strain capable of sensing quorum-sensing molecules produced by Pseudomonas aeruginosa and releasing penicillin by activating a self-lysis death switch to detect and kill pathogenic Pseudomonas aeruginosa strains.

2.2. Spatial Coordination Among Microorganisms

In natural environments, microorganisms not only interact with each other but also maintain spatial boundaries. Typically, strains of the same microorganism aggregate to form colonies, with different strains forming distinct colonies that function in their respective niches. Additionally, the diffusion of small molecules such as peptides, antibiotics, and quorum-sensing signals secreted by microbial cells is influenced by spatiotemporal factors. The accumulation of these substances and the distribution differences among various strains create local sub microbial communities, affecting resource utilization rates within the community system and leading to resource heterogeneity. This, in turn, results in varying interaction strengths between local subcommunities. In synthetic microbial community research, it is crucial to simulate the natural microbial communities and consider the impact of spatiotemporal organization on microbial interactions to prevent system collapse. Selecting appropriate microbial species is essential for achieving synthetic microbial communities. Assembling these microorganisms through spatiotemporal structures to produce desired community-level behaviors, while ensuring the manipulability and controllability of the final synthetic system, is of significant importance for constructing synthetic microbial community systems. Spatial coordination of community microorganisms can be designed to create stable synthetic microbial communities through resource partitioning.

The design of resource partitioning can prevent competitive exclusion between strains, with each community member relying on different nutrients or sets of nutrients. These nutrients can be present in the environment, provided by different community members, or isolated using physical barriers. Resource partitioning reduces the degree of resource competition among community members, enhancing strain growth robustness and improving the overall output of the community based on population coexistence. Resource partitioning within the community is typically achieved by altering the spatial structure of the synthetic microbial community. Researchers employ physical separation techniques, including microfluidics, bioprinting, molding/encapsulation, and other manufacturing techniques, to partition resources and study how spatial organization affects interactions among multiple species and the function of the entire community. Microfluidic technology and 3D printing technology are frequently used in this context. Microfluidic technology forms physical isolation barriers between cells, cultivating individual species in separate chambers to simulate environmental spatial constraints. This technology allows for the free exchange of metabolites between microorganisms while restricting direct cell-to-cell contact [18]. In contrast, 3D printing technology positions specific strains within closed chambers formed by cross-linked gelatin, constructing more complex three-dimensional structures of artificial synthetic microbial communities while allowing material transfer [19].

2.3. Stability of Microbial Communities

Synthetic microbial communities are typically studied and constructed using artificial microbiomes. A critical issue in this construction process is ensuring that these synthetic communities maintain long-term stability in complex, dynamic, and open environments. In such environments, synthetic microbial communities encounter challenges such as fluctuating living conditions and competition from other microbial groups. Over time, these communities also face genomic evolution and horizontal gene transfer [20], and even genetically engineered strains can rapidly lose their functionality [21]. The loss of function can disrupt the interaction networks between populations, thereby diminishing overall community performance. To mitigate evolutionary decline during genetic processes, it is crucial to design strategies that minimize mutation rates and avoid designs susceptible to mutations, while optimizing metabolic pathways within the community. Maintaining the robustness and normal functionality of the community during its operational period is a significant challenge for synthetic microbial communities. Designing stable microbial communities with specific functions requires the development of appropriate strategies to observe and enhance cooperation or synergy within the entire community, starting from the level of individual community members (such as single bacteria). Synthetic microbial communities also face challenges from competition and antagonistic interactions with local species, and any random event in environmental changes can cause population fluctuations in mixed communities, thereby disrupting the stability of community structure and composition. Solutions for synthetic communities must be developed during the construction phase to address these issues and minimize the impact of adverse factors on the intended community functions.

3. Construction of Synthetic Microbial Communities

In synthetic microbial community research, components are typically chosen based on the study's requirements, controlling influencing factors, and constructing a relatively simpler model of the research object in an appropriate environment. This model is characterized by simplicity, stability, definability, predictability, and modularity [22]. Research on synthetic microbial communities elucidates the interactions between populations and establishes metabolic networks among them [23]. The growth and development of individual microbes, microbial interactions, and resource utilization within microbial communities are often parameterized during the research process, with suitable models selected to understand these communities. The core cycle of synthetic biology proposed by Lawson *et al*. [24], which follows the design-build-test-learn process, enhances the foundational theories of microbial ecological communities and drives the development of synthetic microbial communities, thereby enabling precise regulation and modification of microbial community composition and function.

3.1. Methods of Synthetic Microbial Communities

Within the core cycle of synthetic biology, the design-build-test-learn steps facilitate the development of new experimental and data analysis techniques, advancing the study of synthetic microbial communities and enabling artificial modification and precise regulation of their composition and function. Researchers primarily employ "bottom-up" and "top-down" strategies in their exploratory construction research.

3.1.1. Top-Down Approach

The "bottom-up" research strategy primarily centers on the metabolic networks and products of microbial communities, aiming to optimize the metabolic characteristics of specific microbial communities at the molecular level, provided that the microorganisms are culturable. In contrast, the "top-down" approach leverages advancements in multi-omics and automation technologies. This method begins with obtaining the genomes of individual microbes within a community, and reconstructing the metabolic networks through genomic information interpretation and metabolic characteristic analysis. Metabolic reaction models and network analysis tools are then employed to guide the redesign of these networks, ultimately creating synthetic microbial communities with specific functions. This approach emphasizes interaction characteristics within microbial communities, such as synergy and competition. The "bottom-up" strategy provides a framework for better evaluating interaction processes and metabolic networks, facilitating the design of microbial communities with specific functional characteristics and improved rationality. This research strategy focuses on two main areas: the development of tools for synthetic microbial communities and the achievement of target functions within microbial communities using these tools.

Given that microbial communities possess functions such as "internal communication", "sensing environmental changes", and "resource exchange", research on synthetic microbial communities should strive to replicate natural microbial communities as closely as possible. Therefore, researchers have developed three methods—"quorum sensing", "inducible elements", and "co-nutritionalization"—to create tools suitable for synthetic microbial communities. Quorum sensing is a mode of communication among bacteria, functioning as a biological communication system. Bacteria produce and release certain inducer molecules that serve as signals to sense population density, issuing a notification once a critical threshold is reached. As bacterial populations grow, the concentration of these inducer molecules can regulate gene expression, thereby controlling population levels. The Hasty group at the University of California integrated the Rpa and Tra quorum sensing systems with the Lux and Las systems through promoter and protein modifications, constructing a fully orthogonal system [25], thereby proposing the concept of inducible quorum sensing. Inducible elements enable synthetic microbial communities to respond to changes in external environmental conditions and can alter environmental conditions by adding exogenous molecules to control gene expression in community members. For example, the Collins group at MIT [26] constructed circuits in Lactococcus lactis NZ9000 induced by different inducers, adding various inducers to simulate natural community relationships.

They also adjusted environmental conditions by modifying nutrient composition, concentration, and culture environment to induce specific gene expression in synthetic microbial communities. Co-nutritionalization is achieved through the mutual dependence of functional microbial community members. Selecting certain microbial metabolic components can induce dependency among other members of the synthetic microbial community, thereby constructing a more numerous, stable, and interconnected functional microbial community. The Zengler group at the University of California [27] systematically described the principles of constructing complex communities through a nutritional deficiency strategy. Based on these principles, the Wang group at Columbia University [17] constructed 14 different auxotrophic Escherichia coli communities, where community members are mutually dependent and coexistent.

These studies contribute to the establishment of more appropriate and stable synthetic microbial communities. In these communities, highly precise intra-community gene expression and stable functional microbial groups provide reliable tools for achieving community behavior. The "bottom-up" strategy, which leverages species' metabolic information, offers a better understanding of macro-ecological concepts such as community resistance, recovery, and stability. However, the application of this strategy in non-model microbial communities remains limited due to the lack of genomic information and incomplete functional annotations. With advancements in high-throughput sequencing technology and the continuous improvement of genomic databases, it is anticipated that this strategy will play a crucial role in future research on synthetic microbial communities.

3.1.2. Bottom-Up Approach

Traditional microbial community design predominantly employs a "top-down" research strategy, which is the primary focus for microbiologists. This approach typically involves manipulating certain environmental variables (such as optimizing physicochemical parameters in bioreactors) to predict and control the changes, succession, or recovery processes of microorganisms within an ecosystem, ultimately achieving the desired functional outcomes. Compared to the "bottom-up" strategy, the "top-down" approach is more suitable for analyzing and establishing large, complex functional microbial communities. Research on the "top-down" strategy is divided into early and late stages.

In the early stages of "top-down" research, the focus is primarily on the acclimatization of microbial communities. This involves designing and utilizing changes in the physicochemical environment to guide the ecological selection of the microbiome, thereby achieving the desired biological processes. For instance, the van Loosdrecht group at Delft University of Technology [28] developed a multi-species biofilm model to analyze the properties of biofilms formed under various environmental conditions. In the later stages, the research shifts to uncovering the "black box" of environmental microbial communities by seeking methods from nature, such as mining and in situ modification of ecosystems that have evolved over long periods. Researchers collect samples from natural environments and use metagenomic technologies to analyze and extract elements capable of species-specific expression. The key challenge *in situ* modification is to adopt appropriate methods for delivering plasmids into the environment and ensuring the availability of tools for precise gene editing within the community. Early research on microbial community acclimatization using the top-down strategy has addressed many ecological issues, such as the study of ecological sludge [29] [30], which has shown promising results and high application value. However, the later stages require significant experimental efforts for environmental sample collection and element validation. Additionally, the precision of *in situ* engineering needs improvement. This strategy is not highly feasible for industrial production, making it difficult to ensure accuracy and safety. Current top-down research methods have somewhat overlooked the metabolic networks of microbial communities and the interactions between their members, thereby limiting the optimization of synthetic microbial communities.

3.2. Mathematical Models of Synthetic Microbial Communities

The degree and direction of species interactions within microbial communities are critical factors in analyzing community functions. Relying solely on empirical methods to construct complex biological networks and interactions in synthetic microbial communities, considering temporal and spatial dimensions, is insufficient. Advances in computer technology and mathematical modeling tools can address some of these challenges and reveal aspects of microbial communities that are beyond the reach of experimental methods. The integration of knowledge from microbiology, statistics, and mathematics has given rise to the construction of predictive mathematical models. This approach involves creating mathematical models of the microbiome to predict and describe microbial growth and interactions [31]. Developing predictive mathematical models aids in understanding the properties and dynamic changes of microbial communities, thereby laying the groundwork for research on synthetic microbial communities. The following section introduces some of the most common techniques for modeling microbial communities.

3.2.1. Ecologically Based Modeling

In ecological modeling, the Resource Ratio Theory (RRT) and Maximum Power Principle (MPP) are extensively utilized to analyze ecological issues involving microbial species interactions. Researchers often employ population dynamic models and spatial models to construct theoretical ecology-based microbial community models.

Population dynamic models use coupled differential equations to depict temporal changes in the abundance and composition of microbial species within a community. The Lotka-Volterra (LV) model is the most commonly employed model in this context. Originally developed to study predator-prey dynamics, the LV model has been adapted to explore competitive and cooperative interactions between two species [32] [33]. However, the generalized LV model does not capture indirect interactions such as metabolite exchange or quorum sensing. To overcome this limitation, the LV model can be extended by integrating additional components. For instance, Estrela *et al.* [34] incorporated explicit dynamics of metabolite exchange in unidirectional mutualistic interactions into the generalized LV model. Similarly, Stein *et al.* [35] included the response of microorganisms to environmental disturbances in the generalized LV model to describe changes in gut microbiota under antibiotic perturbation.

In natural environments, microbial communities often exhibit highly complex spatial structures. These complexities arise from variations in living conditions, natural gradients, and the microorganisms' inherent self-organizing properties. Consequently, the interactions and abundance within these communities fluctuate over time and space. Spatial modeling is employed to describe these dynamic changes. Typically, partial differential equations (PDEs) are used in spatial modeling to simulate these processes. These equations determine the density of each species at various points in time and space by accounting for diffusion and population dynamics [36] [37]. Furthermore, spatial models incorporate reaction-diffusion equations to describe how material diffusion and population dynamics influence the population density of each species across different spatial and temporal scales in microbial interactions [38].

3.2.2. Game Theory Models

Interactions among species within microbial communities can be effectively analyzed using game theory and evolutionary game theory approaches. Game theory provides a comprehensive mathematical framework to simulate strategic interactions among multiple agents, where each agent's payoff is influenced by both its own actions and the actions of others [39]. Evolutionary game theory, pioneered by Maynard Smith *et al.* [40], models the dynamic evolution of strategies within a competing population without depending on individual participants. The success of a strategy is determined not only by its inherent quality but also by the frequency of other competing strategies within the population (*i.e.*, relative abundance). This frequency-dependent selection concept implies that as the population structure and member abundance change over time, the fitness landscape also evolves [41]. Furthermore, game theory models have been extended to incorporate the effects of spatial structure.

3.2.3. Individual-Based Modeling

Individual-based models (IBMs) conceptualize each individual unit as a discrete, independent entity that interacts with other individuals and its continuous environment. These models allow for the incorporation of individual variability, such as growth rates, substrate absorption and secretion rates, cell mass, and cell volume, to model the attributes, activities, and interactions of each individual within the population [42]. This modeling approach is bottom-up, where the dynamics and functions of the entire system are governed by the dynamics and functions of individual cells striving for optimal fitness [43]. Introducing randomness and individual variability into the system analysis enhances the realism and specificity of the model descriptions [44] [45].

3.2.4. Genome-Scale Metabolic Network Modeling

The modeling methods discussed thus far are designed to predict the dynamics of microbial communities by describing the abundance of different species and providing a rough outline of how each species influences others. Mathematical modeling of intracellular networks is an evolving research area and a cornerstone of systems biology. A critical challenge is to bridge systems biology and microbial ecology, enabling the study of ecosystem-level functions and dynamics through detailed simulations of intracellular connections. With advancements in science and technology, significant progress has been made in this direction through the rapid development of genome-scale stoichiometric metabolic models. These microbial models are constructed by compiling all biochemical reactions occurring within an organism, based on its genome. Typically, these models also include biomass reactions, where the reactants are precursors essential for cell growth, and their relative contributions to the cell's dry biomass are represented by stoichiometric coefficients. Genome-scale stoichiometric metabolic models are now available for a wide range of organisms, from bacteria and archaea to plants.

Flux Balance Analysis (FBA) is a mathematical modeling approach that utilizes these stoichiometric models to analyze how cells allocate environmental resources to achieve homeostasis and reproduction. FBA can quantitatively predict intracellular reaction fluxes, metabolite output and secretion rates, and cell growth rates under pseudo-steady-state conditions without requiring kinetic parameters. This method has been experimentally validated in several systems and has been successfully applied in model-driven biological discoveries as well as various biomedical and biotechnological applications [45]-[48].

4. Application of Synthetic Microbial Communities

4.1. Application of Synthetic Microbial Communities in the Industrial Sector

Synthetic microbial communities have a wide range of applications in industry, such as fermentation, brewing, and bioproduct production. By utilizing synthetic microbial communities, the target products can be artificially selected, and the production process avoids the impact of cross-reactions and the accumulation of large amounts of by-products, thereby improving productivity and stability to meet the demands of complex production.

Minty *et al.* [49] conducted an experiment where they combined *Trichoderma reesei*, which secretes cellulase, with an engineered Escherichia coli strain that produces isobutanol, forming a synthetic microbial community. In this community, *Trichoderma reesei* hydrolyzes lignocellulose into soluble sugars through cellulase, while the engineered Escherichia coli converts these soluble sugars into isobutanol, achieving the transformation of biomass into bioproducts with high yield and ideal results. Microbial fuel cells have become a primary target of synthetic microbial community engineering, where microbial communities mediate electricity generation. These cells can utilize a wide variety of substrates, making them broadly applicable. The choice of substrate for these microbial fuel cells mainly depends on the specific microbial community. Currently used substrates include glucose [50] [51], acetate [50], lactose [52], cellulose [53] [54], and ammonium [55]. Additionally, the use of microbial fuel cells can simultaneously consume cooperative industrial waste, linking electricity generation with waste degradation to maximize resource utilization. The brewing of Chinese liquor is also closely related to microorganisms. Through the research and design of synthetic microbial communities, more flavor substances can be obtained during the brewing process. For example, Wang Lijuan [56] studied the microorganisms in vinegar mash from distiller's grains and obtained a large number of functional microbial communities, significantly enhancing their ability to produce acetic acid and ethyl acetate.

4.2. Application of Synthetic Microbial Communities in Agriculture

The application of synthetic microbial communities in agriculture primarily targets plant growth and stress resistance, particularly through the influence of root-associated microbial communities. These root microbes assist plants in acquiring essential nutrients, synthesizing plant hormones, and inhibiting pathogenic bacteria. Researchers analyze the metabolic products and synthetic pathways of plant roots to design and construct microbial communities with specific functions. This approach regulates the production of root metabolites, enriches beneficial bacteria, enhances plant growth, increases yield, and reduces pesticide usage. For instance, the microecology and rhizosphere health team at Nanjing Agricultural University has utilized synthetic microbial communities to enhance bacterial interactions, effectively controlling soil-borne diseases caused by Ralstonia solanacearum [57]. Similarly, Zhefei Li and colleagues at Northwest A&F University have employed synthetic microbial communities to activate plant-induced systemic resistance, thereby protecting plants [58]. Current research on synthetic microbial communities in plants predominantly focuses on model plants. In agricultural production, companies like Pivot Bio and Joyn Bio in the United States have used synthetic microbial communities to genetically modify symbiotic nitrogen-fixing microbes, thereby enhancing the nitrogen-fixing capacity of plants. This reduces the need for nitrogen fertilizers and mitigates environmental impact.

4.3. Applications of Synthetic Microbial Communities in the Medical Field

Synthetic microbial communities hold significant promise for applications in human health treatment and drug production. The human gut microbiota comprises numerous microorganisms that influence health, and regulating these microorganisms is a strategy for treating diseases associated with microbiota imbalance. Gut microbes play roles in controlling endocrine functions and neural signals within the gut, digesting food, detoxifying harmful substances, and producing various compounds that impact the host, such as those involved in pathogen resistance and immune responses. The regulation of gut microbiota is thus intimately linked to host health and disease. Fecal metabolomics has been employed to monitor metabolites produced by gut microorganisms and to analyze the composition of the host and diet. Fecal microbiota transplantation (FMT) has been utilized for treating various diseases; however, the microbial communities that can be extracted and transplanted from healthy feces are limited, and the mechanisms of treatment are not fully understood, presenting certain risks. Research on synthetic microbial communities addresses these limitations. Currently, synthetic microbial communities and FMT are being used to treat Clostridium difficile infections [59] and inflammatory bowel disease [60], with significant progress leading to clinical trial stages. Biosynthetic methods for drug production have addressed the issue of low yields in natural drug production and have reduced production costs. For instance, Stephanopoulos *et al*. [61] synthesized the paclitaxel precursor using two modules, E. coli and Saccharomyces cerevisiae. Although neither organism can produce the paclitaxel precursor independently, their interaction and mutual utilization of metabolites ultimately result in the formation of the paclitaxel precursor.

4.4. Applications of Synthetic Microbial Communities in Ecology

For environmental pollutant treatment, physical, chemical, and biological methods are typically employed, with microbial communities being extensively utilized in biological treatments. These microbial communities offer advantages such as ease of operation, significant efficacy, low cost, and the absence of secondary pollution. By introducing microbial communities with specific metabolic capabilities into the environment, corresponding pollutants can be effectively degraded. Mishra *et al*. [62] designed a synthetic microbial community comprising *Aspergillus lentulus*, *Aspergillus terreus*, and *Rhizopus oryzae* to remediate pollution caused by various metals and fuels. Wastewater containing pollutants from various chemical production processes can be degraded and converted into non-toxic substances by microbial communities.

Furthermore, based on microbial community ecology principles, synthetic microbial communities are increasingly important in resisting harmful species invasions. Synthetic microbial communities with high biocompatibility and specific biocontrol functions can be utilized to improve ecological environments, such as enhancing the biocontrol efficacy against soil-borne diseases through synthetic microbial communities [63].

5. Summary and Challenges

In natural environments, microbial communities exhibit greater capabilities than single-cultured microbial communities, inspiring researchers to design synthetic microbial communities to meet diverse research and application needs. Synthetic microbial communities leverage the latest theoretical and experimental advancements in synthetic biology, ecology, and computational biology to rationally design functional microbial communities for various environmental and biotechnological applications. The construction of these communities must adhere to principles that ensure microbial interactions, spatial coordination, and community stability, employing both "top-down" and "bottom-up" approaches. Mathematical models are introduced to predict and describe the growth and interactions within these communities, leveraging the flexibility of model design and the expansion of genomic data to enable faster and more accurate synthesis of desired communities. Recent advancements in synthetic microbial community research and computer simulations have facilitated the widespread application of these communities across various fields.

The study of synthetic microbial communities holds significant potential in microbial ecology, yet their future development faces numerous challenges and opportunities. Despite the proof-of-concept results discussed in this review, designing and constructing synthetic microbial communities with three or more species remains challenging due to the need for a detailed understanding of complex species interactions and functions. Additionally, the dynamic nature of microbial communities, such as genetic instability and changes in microbial composition, necessitates maintaining long-term stability in challenging environments. Current research on synthetic microbial community construction is still in its infancy, requiring continuous measurement and adjustment to adapt to environmental changes. There is a need for ongoing optimization of ecological models to better integrate empirical methods and model-driven analyses. The advancement of computer technology also significantly aids the study of synthetic communities, providing better descriptions and predictions of dynamic changes within these communities.

Synthetic microbial communities are emerging as a new research focus in microbiology and synthetic biology. Research on synthetic microbial communities should adhere to the principles of "constructing to understand" and "constructing to apply." As research deepens, efforts should be directed towards leveraging synthetic microbial communities to address significant challenges in industrial and agricultural production, healthcare, ecology, and other domains.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Thompson, L.R., Sanders, J.G., McDonald, D., Amir, A., Ladau, J., Locey, K.J., *et al.*

(2017) A Communal Catalogue Reveals Earth's Multiscale Microbial Diversity. *Nature*, **551**, 457-463. https://doi.org/10.1038/nature24621

[2] Fang, Y. (2020) The Influence of Random Processes on Community Succession and Construction Based on Artificial Microbial Communities. Master's Thesis, Hefei University of Technology.

[3] Gorter, F.A., Manhart, M. and Ackermann, M. (2020) Understanding the Evolution of Interspecies Interactions in Microbial Communities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **375**, Article ID: 20190256. https://doi.org/10.1098/rstb.2019.0256

[4] Cai, Y.F. and Liao, Z.W. (2002) Advances in Research Methods of Soil Microbial Ecology. *Soil and Environment*, No. 2, 167-171.

[5] Berrier, D.J., Rawls, M.S., McCallister, S.L. and Franklin, R.B. (2014) Influence of Substrate Quality and Moisture Availability on Microbial Communities and Litter Decomposition. *Open Journal of Ecology*, **4**, 421-433. https://doi.org/10.4236/oje.2014.48037

[6] Eng, A. and Borenstein, E. (2019) Microbial Community Design: Methods, Applications, and Opportunities. *Current Opinion in Biotechnology*, **58**, 117-128. https://doi.org/10.1016/j.copbio.2019.03.002

[7] Bartley, B.A., Kim, K., Medley, J.K. and Sauro, H.M. (2017) Synthetic Biology: Engineering Living Systems from Biophysical Principles. *Biophysical Journal*, **112**, 1050-1058. https://doi.org/10.1016/j.bpj.2017.02.013

[8] Song, H., Ding, M., Jia, X., Ma, Q. and Yuan, Y. (2014) Synthetic Microbial Consortia: From Systematic Analysis to Construction and Applications. *Chemical Society Reviews*, **43**, 6954-6981. https://doi.org/10.1039/c4cs00114a

[9] Ren, G., Ma, A., Zhang, Y., Deng, Y., Zheng, G., Zhuang, X., *et al.* (2018) Electron Acceptors for Anaerobic Oxidation of Methane Drive Microbial Community Structure and Diversity in Mud Volcanoes. *Environmental Microbiology*, **20**, 2370-2385. https://doi.org/10.1111/1462-2920.14128

[10] Li, G.J., Liu, J.S., Li, B.Y. and Tian, Y.Q. (2009) New Methods and Technologies for Microbial Isolation and Cultivation. *Animal Husbandry and Veterinary Science Information*, No. 11, 10-11.

[11] Großkopf, T. and Soyer, O.S. (2014) Synthetic Microbial Communities. *Current Opinion in Microbiology*, **18**, 72-77. https://doi.org/10.1016/j.mib.2014.02.002

[12] De Roy, K., Marzorati, M., Van den Abbeele, P., Van de Wiele, T. and Boon, N. (2013) Synthetic Microbial Ecosystems: An Exciting Tool to Understand and Apply Microbial Communities. *Environmental Microbiology*, **16**, 1472-1481. https://doi.org/10.1111/1462-2920.12343

[13] Gerchman, Y. and Weiss, R. (2004) Teaching Bacteria a New Language. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 2221-2222. https://doi.org/10.1073/pnas.0400473101

[14] Shong, J., Jimenez Diaz, M.R. and Collins, C.H. (2012) Towards Synthetic Microbial Consortia for Bioprocessing. *Current Opinion in Biotechnology*, **23**, 798-802. https://doi.org/10.1016/j.copbio.2012.02.001

[15] Duan, Y.L. (2021) Research on the Application of Synthetic Microbial Communities in Fermented Foods. *Food Safety Guide*, No. 20, 191-192.

[16] Mee, M.T., Collins, J.J., Church, G.M. and Wang, H.H. (2014) Syntrophic Exchange in Synthetic Microbial Communities. *Proceedings of the National Academy of Sciences of the United States of America*, **111**, E2149-E2156.

https://doi.org/10.1073/pnas.1405641111

[17] Saeidi, N., Wong, C.K., Lo, T., Nguyen, H.X., Ling, H., Leong, S.S.J., *et al.* (2011) Engineering Microbes to Sense and Eradicate *Pseudomonas aeruginosa*, a Human Pathogen. *Molecular Systems Biology*, **7**, Article 521. https://doi.org/10.1038/msb.2011.55

[18] Kim, H.J., Boedicker, J.Q., Choi, J.W. and Ismagilov, R.F. (2008) Defined Spatial Structure Stabilizes a Synthetic Multispecies Bacterial Community. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 18188-18193. https://doi.org/10.1073/pnas.0807935105

[19] Connell, J.L., Ritschdorff, E.T., Whiteley, M. and Shear, J.B. (2013) 3D Printing of Microscopic Bacterial Communities. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 18380-18385. https://doi.org/10.1073/pnas.1309729110

[20] Niehus, R., Mitri, S., Fletcher, A.G. and Foster, K.R. (2015) Migration and Horizontal Gene Transfer Divide Microbial Genomes into Multiple Niches. *Nature Communications*, **6**, Article No. 8924. https://doi.org/10.1038/ncomms9924

[21] Sleight, S.C., Bartley, B.A., Lieviant, J.A. and Sauro, H.M. (2010) Designing and Engineering Evolutionary Robust Genetic Circuits. *Journal of Biological Engineering*, **4**, Article No. 12. https://doi.org/10.1186/1754-1611-4-12

[22] Goers, L., Freemont, P. and Polizzi, K.M. (2014) Co-Culture Systems and Technologies: Taking Synthetic Biology to the Next Level. *Journal of The Royal Society Interface*, **11**, Article ID: 20140065. https://doi.org/10.1098/rsif.2014.0065

[23] Dolinšek, J., Goldschmidt, F. and Johnson, D.R. (2016) Synthetic Microbial Ecology and the Dynamic Interplay between Microbial Genotypes. *FEMS Microbiology Reviews*, **40**, 961-979. https://doi.org/10.1093/femsre/fuw024

[24] Lawson, C.E., Harcombe, W.R., Hatzenpichler, R., Lindemann, S.R., Löffler, F.E., O'Malley, M.A., *et al.* (2019) Common Principles and Best Practices for Engineering Microbiomes. *Nature Reviews Microbiology*, **17**, 725-741. https://doi.org/10.1038/s41579-019-0255-9

[25] Scott, S.R. and Hasty, J. (2016) Quorum Sensing Communication Modules for Microbial Consortia. *ACS Synthetic Biology*, **5**, 969-977. https://doi.org/10.1021/acssynbio.5b00286

[26] Kong, W., Meldgin, D.R., Collins, J.J. and Lu, T. (2018) Designing Microbial Consortia with Defined Social Interactions. *Nature Chemical Biology*, **14**, 821-829. https://doi.org/10.1038/s41589-018-0091-7

[27] Zengler, K. and Zaramela, L.S. (2018) The Social Network of Microorganisms—How Auxotrophies Shape Complex Communities. *Nature Reviews Microbiology*, **16**, 383-390. https://doi.org/10.1038/s41579-018-0004-5

[28] Picioreanu, C., Kreft, J. and van Loosdrecht, M.C.M. (2004) Particle-Based Multidimensional Multispecies Biofilm Model. *Applied and Environmental Microbiology*, **70**, 3024-3040. https://doi.org/10.1128/aem.70.5.3024-3040.2004

[29] Arne Alphenaar, P., Visser, A. and Lettinga, G. (1993) The Effect of Liquid Upward Velocity and Hydraulic Retention Time on Granulation in UASB Reactors Treating Wastewater with a High Sulphate Content. *Bioresource Technology*, **43**, 249-258. https://doi.org/10.1016/0960-8524(93)90038-d

[30] Liu, Y. and Tay, J. (2002) The Essential Role of Hydrodynamic Shear Force in the Formation of Biofilm and Granular Sludge. *Water Research*, **36**, 1653-1665. https://doi.org/10.1016/s0043-1354(01)00379-7

[31] Li, B.L., Guo, J.F. and Ou, J. (2004) Methods for Constructing Mathematical Models

in Predictive Microbiology. *Food Science*, No. 11, 52-57.

[32] Hofbauer, J. and Sigmund, K. (1998) Evolutionary Games and Population Dynamics. Cambridge University Press. https://doi.org/10.1017/cbo9781139173179

[33] Wangersky, P.J. (1978) Lotka-Volterra Population Models. *Annual Review of Ecology and Systematics*, **9**, 189-218. https://doi.org/10.1146/annurev.es.09.110178.001201

[34] Estrela, S., Trisos, C.H. and Brown, S.P. (2012) From Metabolism to Ecology: Cross-Feeding Interactions Shape the Balance between Polymicrobial Conflict and Mutualism. *The American Naturalist*, **180**, 566-576. https://doi.org/10.1086/667887

[35] Stein, R.R., Bucci, V., Toussaint, N.C., Buffie, C.G., Rätsch, G., Pamer, E.G., *et al.* (2013) Ecological Modeling from Time-Series Inference: Insight into Dynamics and Stability of Intestinal Microbiota. *PLOS Computational Biology*, **9**, e1003388. https://doi.org/10.1371/journal.pcbi.1003388

[36] Cantrell, R.S. and Cosner, C. (2004) Spatial Ecology via Reaction-diffusion Equations. Wiley. https://doi.org/10.1002/0470871296

[37] Cosner, C. (2008) Reaction-Diffusion Equations and Ecological Modeling. In: Friedman, A., Ed., *Tutorials in Mathematical Biosciences IV*, Springer, 77-115. https://doi.org/10.1007/978-3-540-74331-6_3

[38] Datta, M.S., Korolev, K.S., Cvijovic, I., Dudley, C. and Gore, J. (2013) Range Expansion Promotes Cooperation in an Experimental Microbial Metapopulation. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 7354-7359. https://doi.org/10.1073/pnas.1217517110

[39] Zomorrodi, A.R. and Segrè, D. (2016) Synthetic Ecology of Microbes: Mathematical Models and Applications. *Journal of Molecular Biology*, **428**, 837-861. https://doi.org/10.1016/j.jmb.2015.10.019

[40] Nowak, M.A. (2006) Evolutionary Dynamics. Harvard University Press. https://doi.org/10.2307/j.ctvjghw98

[41] Nowak, M.A. and Sigmund, K. (2004) Evolutionary Dynamics of Biological Games. *Science*, **303**, 793-799. https://doi.org/10.1126/science.1093411

[42] Hellweger, F.L., Clegg, R.J., Clark, J.R., Plugge, C.M. and Kreft, J. (2016) Advancing Microbial Sciences by Individual-Based Modelling. *Nature Reviews Microbiology*, **14**, 461-471. https://doi.org/10.1038/nrmicro.2016.62

[43] Ferrer, J., Prats, C. and López, D. (2008) Individual-based Modelling: An Essential Tool for Microbiology. *Journal of Biological Physics*, **34**, 19-37. https://doi.org/10.1007/s10867-008-9082-3

[44] Hellweger, F.L. and Bucci, V. (2009) A Bunch of Tiny Individuals—Individual-Based Modeling for Microbes. *Ecological Modelling*, **220**, 8-22. https://doi.org/10.1016/j.ecolmodel.2008.09.004

[45] Bordbar, A., Monk, J.M., King, Z.A. and Palsson, B.O. (2014) Constraint-Based Models Predict Metabolic and Associated Cellular Functions. *Nature Reviews Genetics*, **15**, 107-120. https://doi.org/10.1038/nrg3643

[46] Zomorrodi, A.R., Suthers, P.F., Ranganathan, S. and Maranas, C.D. (2012) Mathematical Optimization Applications in Metabolic Networks. *Metabolic Engineering*, **14**, 672-686. https://doi.org/10.1016/j.ymben.2012.09.005

[47] McCloskey, D., Palsson, B.Ø. and Feist, A.M. (2013) Basic and Applied Uses of Genome-Scale Metabolic Network Reconstructions of *Escherichia coli*. *Molecular Systems Biology*, **9**, Article 661. https://doi.org/10.1038/msb.2013.18

[48] O'Brien, E.J., Monk, J.M. and Palsson, B.O. (2015) Using Genome-Scale Models to

Predict Biological Capabilities. *Cell*, **161**, 971-987.
https://doi.org/10.1016/j.cell.2015.05.019

[49] Minty, J.J., Singer, M.E., Scholz, S.A., Bae, C., Ahn, J., Foster, C.E., *et al.* (2013) Design and Characterization of Synthetic Fungal-Bacterial Consortia for Direct Production of Isobutanol from Cellulosic Biomass. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 14592-14597.
https://doi.org/10.1073/pnas.1218447110

[50] Zhang, E., Xu, W., Diao, G. and Shuang, C. (2006) Electricity Generation from Acetate and Glucose by Sedimentary Bacterium Attached to Electrode in Microbial-Anode Fuel Cells. *Journal of Power Sources*, **161**, 820-825.
https://doi.org/10.1016/j.jpowsour.2006.05.004

[51] Chaudhuri, S.K. and Lovley, D.R. (2003) Electricity Generation by Direct Oxidation of Glucose in Mediatorless Microbial Fuel Cells. *Nature Biotechnology*, **21**, 1229-1232. https://doi.org/10.1038/nbt867

[52] Kim, B.H., Kim, H.J., Hyun, M.S. and Park, D.H. (1999) Direct Electrode Reaction of Fe (III)-Reducing Bacterium, Shewanella Putrefaciens. *Journal of microbiology and biotechnology*, **9**, 127-131.

[53] Ren, Z., Ward, T.E. and Regan, J.M. (2007) Electricity Production from Cellulose in a Microbial Fuel Cell Using a Defined Binary Culture. *Environmental Science & Technology*, **41**, 4781-4786. https://doi.org/10.1021/es070577h

[54] Seto, A., Saito, Y., Matsushige, M., Kobayashi, H., Sasaki, Y., Tonouchi, N., *et al.* (2006) Effective Cellulose Production by a Coculture of Gluconacetobacter Xylinus and Lactobacillus Mali. *Applied Microbiology and Biotechnology*, **73**, 915-921.
https://doi.org/10.1007/s00253-006-0515-2

[55] He, Z., Kan, J., Wang, Y., Huang, Y., Mansfeld, F. and Nealson, K.H. (2009) Electricity Production Coupled to Ammonium in a Microbial Fuel Cell. *Environmental Science & Technology*, **43**, 3391-3397. https://doi.org/10.1021/es803492c

[56] Wang, L.J. (2018) Isolation of Functional Microbial Communities Producing Ethyl Acetate and Their Application in Vinegar Brewing. Master's Thesis, Jiangnan University.

[57] Li, M., Wei, Z., Wang, J., Jousset, A., Friman, V., Xu, Y., *et al.* (2018) Facilitation Promotes Invasions in Plant‐Associated Microbial Communities. *Ecology Letters*, **22**, 149-158. https://doi.org/10.1111/ele.13177

[58] Li, Z., Bai, X., Jiao, S., Li, Y., Li, P., Yang, Y., *et al.* (2021) A Simplified Synthetic Community Rescues Astragalus Mongholicus from Root Rot Disease by Activating Plant-Induced Systemic Resistance. *Microbiome*, **9**, Article No. 217.
https://doi.org/10.1186/s40168-021-01169-9

[59] Gianotti, R.J. and Moss, A.C. (2017) Fecal Microbiota Transplantation: From Clostridium Difficile to Inflammatory Bowel Disease. *Gastroenterology & Hepatology*, **13**, 209-213.

[60] Anderson, J.L., Edney, R.J. and Whelan, K. (2012) Systematic Review: Faecal Microbiota Transplantation in the Management of Inflammatory Bowel Disease. *Alimentary Pharmacology & Therapeutics*, **36**, 503-516.
https://doi.org/10.1111/j.1365-2036.2012.05220.x

[61] Zhou, K., Qiao, K., Edgar, S. and Stephanopoulos, G. (2015) Distributing a Metabolic Pathway among a Microbial Consortium Enhances Production of Natural Products. *Nature Biotechnology*, **33**, 377-383. https://doi.org/10.1038/nbt.3095

[62] Mishra, A. and Malik, A. (2014) Novel Fungal Consortium for Bioremediation of

Metals and Dyes from Mixed Waste Stream. *Bioresource Technology*, **171**, 217-226. https://doi.org/10.1016/j.biortech.2014.08.047

[63] Ji, Y., Wu, Y.P., Wang, Yi., Wang, X.Q., Liu, X.Z., Liu, J.B. and Xiang, M.C. (2016) Research on the Control of Cucumber Root-Knot Nematode Using Functional Composite Microbial Agents. *Chinese Journal of Biological Control*, **32**, 493-502.