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Review Article

A systematic discussion and comparison of the construction methods of synthetic microbial community

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ABSTRACT

Synthetic microbial community has widely concerned in the fields of agriculture, food and environment over the past few years. However, there is little consensus on the method to synthetic microbial community from construction to functional verification. Here, we review the concept, characteristics, history and applications of synthetic microbial community, summarizing several methods for synthetic microbial community construction, such as isolation culture, core microbiome mining, automated design, and gene editing. In addition, we also systematically summarized the design concepts, technological thresholds, and applicable scenarios of various construction methods, and highlighted their advantages and limitations. Ultimately, this review provides four efficient, detailed, easy-to-understand and -follow steps for synthetic microbial community construction, with major implications for agricultural practices, food production, and environmental governance.

1. Introduction

Microorganisms are ubiquitously found across terrestrial and aquatic environments, including the atmosphere, oceans, soils, plants, animals, and the human body. Their vast populations are essential to biogeochemical cycles and natural ecosystems [1–3]. Early biological studies focused on key functional groups in microbial communities, especially on the isolation and modification of individual microbes to enhance specific functions [4]. However, current research on environmental microorganisms is gradually shifting from analyzing individuals to examining entire community systems [5,6], viewing microorganisms in the environment as a complex symbiotic network of interacting and co-evolving organisms [7,8]. Given current challenges in disease treatment, environmental management, human health and industrial production, assessing a single function of a single strain of bacteria no longer suffices to meet the needs of individual microorganisms. Many complex physiological and biochemical processes cannot be effectively addressed or activated because individual microorganisms are poorly adapted to environmental disturbances [6].

In contrast to single strains, microbial communities display extensive metabolic diversity and enhanced adaptability to complex environments, offering more stable ecological functions through the division of labor [9]. But studying natural microbial communities is a particularly difficult task due to their high complexity, limiting our ability to elucidate their mechanisms of action, to predict their behavior in natural environments [10]. This limitation considerably hinders efforts to understand the transport and transformation of substances in natural systems and the functional role of microorganisms, preventing us from efficiently exploiting microbial resources.

In this context, “synthetic microbial community” provides new opportunities for understanding microbiome-environment interactions, standing out as an emerging research hotspot. Synthetic biologists have recently dissected the components of biological systems to artificially simulate complex microbial ecosystems by reassembling them into synthetic microbial communities [11–15]. Mature synthetic microbial communities have several advantages, such as low complexity, high controllability and good stability, balancing the complexity, relevance and ease of individual microorganisms with the complexity of natural microbial communities [16,17]. In synthetic microbial community, the division of labor among community members also effectively reduces the load of exogenous vectors and the metabolic burden on individual organisms while concurrently mitigating environmental stress, thereby improving their robustness [5,6,18]. Thanks to these properties, synthetic microbial community has a high potential in human health,

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industrial production, pollutant degradation, and food fermentation.

Synthetic microbial community can now be designed with specific functions by leveraging macrogenomics, high-throughput sequencing technology, multi-omics, microfluidics and automation to predict specific metabolic networks and regulatory mechanisms of microbial communities. However, current methods for constructing synthetic microbial community remain confusing and lack systematic summarization and comparison. Therefore, this paper provides an overview of the concept, characteristics, development and application of synthetic microbial community, summarizes in detail various methods for constructing synthetic microbial community, and compares attributes and characteristics of each method, such as their universality, reproducibility, manipulability, precision and control towards fostering research on synthetic microbial community.

2. Synthetic microbial community

2.1. Concepts

In recent years, rapid advances in microbiomics, computational biology, synthetic biology and culturomics have enabled researchers to construct efficient and stable synthetic microbial communities. Synthetic microbial communities are microbial systems with specific functions artificially synthesized by co-culturing different wild-type bacterial species and engineered strains [16,17,19]. Essentially, synthetic microbial community combines multiple coexisting microorganisms with different functional characteristics. Retaining their own characteristics, these microorganisms are simultaneously able to complement or synergize with the functional characteristics of other organisms through artificial selection, thus providing the advantages of a natural microbial community [20,21]. Currently, synthetic microbial community technology has been extensively studied for plant growth, nutrient uptake, and disease control in *Arabidopsis thaliana* [22], maize [23], rice [24] and tomato [25,26]. Moreover, synthetic microbial communities have shown high potential for pollutant degradation [27–29], drug, biofuel and protein complex production [30–33], preparation of functional biomaterials [34,35] and biosensor construction [36,37].

2.2. Goal and advantages

In contrast to individual microorganisms, a sophisticated synthetic microbial community is anticipated to possess superior stability, adaptability, efficiency, and metabolic flexibility: (1) Stability: A mature synthetic microbial community, comprising a diverse range of microorganisms, is defined by human-engineered interactions that predominantly exhibit synergistic effects. These interactions enhance the robustness of the community, which is further strengthened by species diversity that buffers against external perturbations and maintains overall stability [38–40]. (2) Adaptability. A mature synthetic microbial community features multiple interactions and functional synergies among its constituent strains. When environmental changes inhibit or inactivate certain strains, others can fill the void and maintain the overall functional equilibrium. Owing to this adaptability, the microbial community can better adjust to environmental fluctuations and withstand external pressures [41]. (3) Efficiency: A mature synthetic microbial community represents an advanced bioproduction system, meticulously engineered to disaggregate intricate metabolic processes and apportion them among various strains. This strategic distribution alleviates the metabolic load on any single strain, culminating in an elevated overall efficiency in the bioproduction process [42,43]. (4) Metabolic Flexibility. Within a mature synthetic microbial community, different strains leverage their specific substrates and nutrients to form a complementary metabolic network, which significantly enhances the overall resource utilization rate of the community. Moreover, the diverse metabolites produced by the synthetic community can effectively catalyze numerous complex biochemical processes, a feat that would be very challenging, if not impossible, for a single bacterial strain [6,44,45].

Compared to natural microbial communities, synthetic microbial community have the advantage of being less complex, more controllable and reproducible, and can be designed to have targeted functions: (1) Low complexity. synthetic microbial communities have a much lower overall complexity than natural microbial communities as a result of their relatively simple structure and design [16]. (2) High controllability. The diversity of natural microbial communities is affected by the environment and by various complex factors, so they are difficult to control. In contrast, the diversity of synthetic microbial community precisely control by designing and adjusting their microbial composition, abundance, and interactions [46,47]. (3) High reproducibility. Synthetic microbial community is usually constructed under controlled laboratory conditions with well-defined physicochemical properties, thereby enabling us to achieve a high degree of reproducibility through a precise construction process. Conversely, natural microbial communities are affected by various, complex factors, such as environmental factors, competitive relationships, and interactions, so their compositions and functions may change under different conditions, making it difficult to replicate microbial community structures [48]. (4) Directional functions. Synthetic microbial communities can be engineered to degrade pollutants, produce useful metabolites, and promote plant growth, among other specific functions. Selecting appropriate microbial species and optimizing their metabolic pathways and symbiotic networks increases the efficiency and precision of functional expression, opening up more opportunities for practical applications [11,49].

3. Development and applications of synthetic microbial community

3.1. Development history

Synthetic microbial community was first reported in 2007 by Shou et al., who modified *Saccharomyces cerevisiae* by genetic hybridization, obtaining a two-strain *S. cerevisiae* cross-feeding community, laying the foundation for the development of synthetic microbial community [50]. Following that, numerous researchers have delved into the field, leading to rapid advancements in both the theory and practice of synthetic microbial community.

3.1.1. Theory

The development of theories has primarily focused on understanding scientific questions or testing ecological principles. In 2014, Groβkopf et al. defined synthetic microbial community as co-cultures of two or more microorganisms grown in a substrate with well-defined compositions [17]. In the same year, the multidimensional syntrophic system introduced by Mee et al. offered a robust foundation for the study and manipulation of increasingly intricate microbial communities [51]. Furthermore, Goers et al. highlighted the capacity of co-cultivation systems and technologies to advance synthetic biology to unprecedented heights [52]. In 2016, Desai et al. engineered a synthetic microbiota comprising 14 fully sequenced human gut symbionts and successfully established it in germ-free mice, thereby pioneering the elucidation of the complex functional dynamics between dietary fiber, gut microbiota, and the colonic mucus barrier [53]. Thereafter, in 2017, Vorholt et al. delineated the challenges and objectives inherent in the application of reductionist approaches within the realm of synthetic microbial communities [54].

New breakthrough occurred in 2019, Lawson et al. proposed the "design-build-test-learn" (DBTL) cycle, which has now become a widely used general guideline for microbiome engineering [55]. Furthermore, McCarty et al. have discussed how relatively simple approaches in synthetic biology can be utilized to design complex communities [56]. Research by Carlström et al. indicates that community assembly is

influenced by historical contingencies and characterized by priority effects, with initially established communities exhibiting greater stability and resilience [57]. In the subsequent years, the innovative concept "DefenseBiome" was proposed [58]. In addition, Hu et al. integrated prevailing theories and empirical outcomes to formulate an innovative strategy that integrates bottom-up and top-down microbiome engineering within natural environments [59]. Vaccaro et al. suggested an expansion of research to encompass a diverse array of non-model crops [60]. Concurrently, the latest findings from Schäfer et al.'s research on the leaf microbiota of *Arabidopsis thaliana* underscored the pivotal role of carbon utilization and the significant contributions of niche differentiation and cross-feeding to the microbiome assembly [61]. Furthermore, Emmenegger et al. accentuated the potential of integrating microbiota screening methods with machine learning algorithms, broadening their utility across host-microbiota systems [62].

3.1.2. Practice

The development in practice tends to be more inclined towards specific practical applications. In 2011, Goyal et al. used synthetic microbial community for industrial ethanol production [63] , and in the same year, Faith et al. applied synthetic microbial community in human gut flora studies [64]. A few years later, in 2017, Niu et al. constructed synthetic microbial community to suppress the pathogenic fungus *Fusarium verticillioides* [23]. More recently, synthetic microbial community development has shifted from individual microbial strains to complex microbial communities designed to improve plant growth. Case in point, seven complex synthetic microbial communities acting on *Arabidopsis thaliana* were assembled in 2018, demonstrating that multi-kingdom microbial symbiotic communities promote plant growth [65]. A year later, after isolating a total of 1079 pure bacterial isolates and constructing synthetic microbial community from indica and japonica rice roots, Zhang et al. found that synthetic communities enriched in indica rice roots were more effective in improving rice growth under organic nitrogen conditions than synthetic communities enriched in japonica rice roots [24].

Thanks to advances in biology, ecology and engineering, researchers currently focus on constructing controllable and predictable microbial communities with disease prevention effects. For example, Salas et al. constructed synthetic microbial community specifically to control endodermal suberization and to regulate nutrient balance in *Arabidopsis thaliana*, in 2020 [66]. And as reported in 2022, transboundary synthesized microbial communities can prevent *Fusarium* wilt disease in tomato [25].Synthetic microbial communities can also be designed to degrade pollutants and biomass, as well as enhance the flavors of food. For instance, stable, synthetic microbial community constructed using PAH-degrading *Paracoccus aminovorans* HPD-2 and autotrophic nitrogen-fixing *Azotobacter chroococcum* HN bacteria, isolated from PAH-contaminated soils, can promote the degradation of the pollutant pyrene in a nitrogen-deficient environment [67].A core synthetic microbial community have also been constructed to efficiently degrade lignocellulose [68]. Similarly, Wang et al. constructed a core synthetic microbial community that significantly enhances the flavor of white wine [69].

The above examples are but a glimpse into the diverse practical applications of synthetic microbial communities, underscoring the swift expansion of this domain over the past decade, with an accentuated surge in the past five years. Synthetic microbial community has become a leading research area in biotechnology. Related research results have spurred the development of synthetic microbial community technology, laying strong foundations for the use of synthetic microbial community technology.

3.2. Applications of synthetic microbial communities

With the rapid development of multi-omics technologies, positive and negative interactions with members of the microbial community have been highlighted in medicine, agriculture, and the food industry. These interactions are important for targeting and regulating the microbiome towards maximizing its function. To achieve this goal, synthetic microbial communities is widely used in human health, disease treatment, biotechnology processing, environmental treatment, fuel production, pollutant degradation, food fermentation, and plant cultivation (Table 1).

4. Methods for synthetic microbial community construction

4.1. Isolation culture

Description: Microbial species were widely isolated and cultivated based on high-throughput cultivation and separation techniques. Subsequently, a screening and antagonism testing of microbial strains were conducted based on the required functions, with the aim of obtaining a synthetic community constructed from symbiotic candidate strains.

Steps: (1) Isolation and culture. Conventional microbial separation techniques or high-throughput culture methods are employed to systematically isolate and cultivate microbial strains present in the samples, thereby establishing a comprehensive strain resource library. (2) Screening. Isolated strains are functionally tested for the desired functions of the synthetic community, such as nitrogen fixation, phosphorus solubilization, pollutant degradation, IAA production, and disease resistance, to select candidate strains. (3) Co-cultivation. Candidate strains are co-cultivated in various combinations and proportions (which can be determined by microbial functional metabolic networks or real-time quantitative q-PCR) to assess the growth of the co-cultured strains. The absence of growth inhibition indicates no antagonistic effects between the strains, allowing for the establishment of a synthetic community. (4) Construction of synthetic microbial communities. Under sterile conditions, the selected strain combinations after co-cultivation are evaluated based on specific functional responses (traits, physiology, metabolism, etc.) to assess the functionality of the synthetic community, and one or several most effective artificial communities are selected. (5) Functional verification. After strain combination

Table 1
Applications of synthetic microbial communities in humans, the environment, food and agriculture fields.

Field	Summarize
Human	◆ Synthetic microbial communities offer significant potential for increasing the production efficiency of biosynthesized pharmaceuticals [30,70]. ◆ Engineering and refining synthetic microbial communities prevent and treat gastrointestinal diseases by regulating the balance of the gut microbiota [71–73].
Environment	◆ Synthetic microbial communities can produce bioethanol, biodiesel and other biofuels efficiently through fermentation, and can effectively reduce the emission of pollutants [74–76]. ◆ Synthetic microbial communities can clear contaminants from soil and water bodies in an environmentally friendly and efficient manner, significantly advancing the field of environmental remediation [67,77,78].
Food	◆ Synthetic microbial communities can abridge the fermentation process of food and ameliorate the flavor profiles of fermented products, effectively satisfying consumer expectations regarding the quality and taste of fermented foods [69,79–81]. ◆ Synthetic microbial communities can modulate the nutritional structure of fermented foods, increasing the production of beneficial compounds while reducing the formation of harmful substances [80,82–84].
Agriculture	◆ Synthetic microbial communities can effectively enhance plant growth and their adaptive capacity to abiotic stress [24,48,85–89]. ◆ The interaction between synthetic microbial communities and plants can enhance the host's ability to resist pathogen invasion, which is crucial for plant protection and biological control [65,90–95].

optimization, the functionality of the synthetic community is experimentally tested on the host in a natural environment. (Fig. 1).

Case: Durán et al. screened 148 bacterial, 34 fungal, and 8 oomycete species as candidate strains from microbial strains isolated from *Arabidopsis thaliana* intergrowth in 2862 antagonism experiments (binary bacterial-fungal interactions *ex situ* combined with community perturbation experiments in planta) and assembled seven complex synthetic microbial communities. Experimental validation revealed that synthetic microbial community consisting of bacteria-fungi and oomycetes significantly promoted plant growth [65]. Similarly, Li et al. isolated 423 bacterial strains from the inter-root of *Astragalus mongholicus* using various media and screened 10 high- and three low-abundance strains based on their growth-promoting and disease-resistant properties. When combining 13 bacterial strains into three different synthetic microbial communities, they experimentally verified that the synthetic community composed of *Stenotrophomonas* spp., *Rhizobium* spp. and *Advenella* spp. with *Ochrobactrum* spp. had good pro- and anti-root rot functions on *Astragalus* [96]. Shi et al. isolated a total of 113 bacterial strains from potato samples, and subsequently selected 61 strains to construct 18 synthetic microbial communities. Preliminary assessments for inhibitory activity against synthetic microbial communities were performed ex vitro using potato tissues. Ultimately, the most effective synthetic community was identified, which directly suppressed the occurrence of potato dry rot disease by inhibiting the mycelium and conidia of *Fusarium* species, thereby enhancing the biocontrol activity against potato dry rot [94]. Bai et al. isolated and cultured bacteria from healthy *Arabidopsis thaliana* inter-roots and leaves by cell sorting and colony selection in liquid medium, in 96-well microtiter plates. Subsequently, after genome sequencing, identification, and further screening for symbiosis and function, they obtained two synthetic communities, one consisting of 218 leaf bacteria and the other consisting of 158 root bacteria with 30 soil bacteria. Validation experiments showed that both synthetic communities were able to colonize their hosts and that root bacteria were more conducive to ecological niche colonization of a homologous host than leaf bacteria [97].

4.2. Core microbiome mining

Description: Based on high-throughput sequencing data analysis, definition of core microbial taxa and targeted isolation of core microbial strains, synthetic microbial communities are constructed through further functional screening, symbiosis testing, and experimental validation steps.

Steps: (1) Sample collection. Selection of study subjects and collection of microbial samples. (2) High-throughput sequencing. Sample macro-genomic DNA is extracted, sequenced and characterized by high-throughput sequencing. (3) Core microbiome definition. Based on high-throughput sequencing data, relevant core functional microorganisms are mined by symbiotic network analysis and predictive functional profiling of microbial communities. (4) Isolation and screening. Targeted isolation and culture of core microbial strains based on the results. (5) Symbiotic testing. Core microbial strains are subjected to functional optimization and symbiotic and antagonistic tests, such as antagonistic activity against pathogenic microorganisms and plant growth-promoting traits. (6) Construction. The best-performing microorganisms are mixed and cultured in specific proportions under experimental conditions to construct a synthetic microbial community. (7) Functional verification. Functional validation of constructed synthetic microbial community under natural or artificially controlled conditions is assessed by determining their activity in specific metabolic pathways, among other indicators (Fig. 1).

Case: Wu et al. screened five core microbial genera, namely *Lactobacillus*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, and *Diplococcus*, by analyzing the relative abundance, flavor-enhancing ability, and symbiotic performance of the dominant genera in sausage fermentation. Then, the species with the highest relative abundance in each genus were screened out, and the most representative strains were used in the mixed cultures to construct synthetic microbial community. The validation experiments showed that synthetic microbial community improved the aroma of sausages [98]. In turn, combining PacBio full-length diversity sequencing for metagenomics with traditional culture methods, Li et al. identified 11 core functional microorganisms, namely *Brettanomyces bruxellensis*, *Pichia Kudriavzevi*, *Lactobacillus plantarum*, *Lactobacillus amylolyticu*, *Lactobacillus fermentum*, *Lactobacillus acetotolerans*, *Acetobacter pasteurianus*, *Lactobacillus amylovorus*, *Acetobacter pomorums*, *Clostridium beijerinckii* and *Lichtheimia ramose*. These microorganisms affected the fermentation flavor of Sichuan sun vinegar, as shown by metabolic network analysis, symbiosis analysis and functional screening. Synthetic microbial community constructed from these 11 strains provided Sichuan sun vinegar with key flavors and facilitated the production of amino acid [99]. Qiao et al. isolated 394 rhizosphere bacteria from field-grown grafted watermelon plants and, through statistical analysis, selected 16 core strains to construct a synthetic microbial community. This community was found to enhance the growth and disease resistance of ungrafted watermelons grown in non-sterile soil. Subsequent research further identified a streamlined synthetic community composed of eight bacterial strains, which also possesses plant growth-promoting and disease-resistant functions [93].

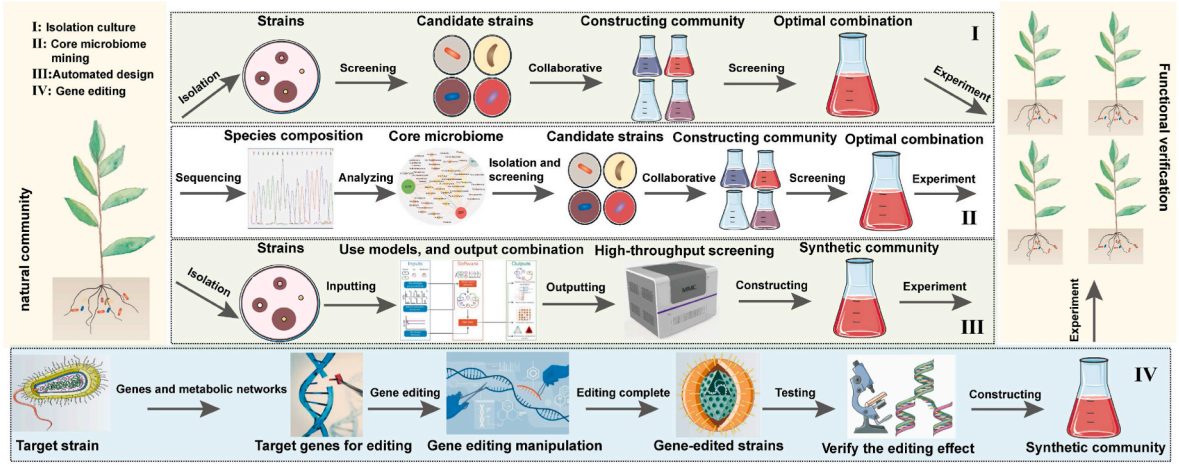

Fig. 1. Operation steps of four synthetic microbial community construction methods (including isolation culture, core microbiome mining, automated design, and gene editing).

4.3. Automated design

Description: Once numerous microbial strains are isolated and identified, microbe-microbe and microbe-metabolism interactions are modeled based on their molecular data to determine the optimal combination of synthetic microbial community.

Steps: (1) Isolation and identification. Conventional microbial culture techniques are used to isolate and culture sample-associated microbial strains on a large scale and to molecularly characterize them to prepare strain libraries. (2) Metabolic network model. These microbial strains are input into a metabolic network model to predict interactions between different microorganisms, metabolite flow, and optimal microbial combinations. (3) Algorithm Optimization. Using methods such as evolutionary, genetic and optimization algorithms, the best microbial combination is identified among a high number of possible combinations. (4) High-throughput screening. High-throughput technologies, such as microfluidic microarrays and multi-sample analysis platforms, enable us to rapidly asses the performance of large microbial assemblages. (5) Construction. Synthetic microbial community is constructed based on microbial assemblages and proportions presented in model predictions and algorithm results. (6) Experimental verification. The accuracy of the models and algorithms is ascertained by testing the synthetic microbial community in the laboratory and assessing whether them perform the expected function based on metrics (Fig. 1).

Case: By Approximate Bayesian Computation with Sequential Monte Carlo Sampling (ABC SMC) for model selection and parameterization, Karkaria et al. designed stable genetic oscillators and multi-stable genetic switches. These authors identified candidate systems with the highest probability of generating stable communities in bioreactors before determining optimal candidate strains for generating stable two- and three-strain communities by automated design [100]. Harcombe et al. introduced a multiscale modeling framework termed Computation of Microbial Ecosystems in Time and Space (COMETS) to calculate ecosystem spatiotemporal dynamics on a detailed intracellular metabolic stoichiometry level and to implement dynamic flux equilibrium analysis algorithms on grids for tracking spatiotemporal dynamics of multiple microbial species in complex environments at full genome-scale resolution. From these COMETS calculations, they constructed a two-strain synthetic community consisting of *Escherichia coli* and *Salmonella enterica* and a stable three-strain synthetic community additionally with *Methylobacterium extorquens* [101].

4.4. Gene editing

Description: Individual strains can be targeted and genetically modified for specific traits or functions by gene editing and subsequently combined with other strains to construct synthetic microbial community with specific functions and interactions.

Steps: (1) Select the target strain. Microbial strains are selected as candidates for constructing microbial community. These strains can be either naturally occurring or genetically edited strains. (2) Design Editorial Objectives. Technical tools, such as genomics and metabolomics, are used to analyze genetic and metabolic networks of the target strains to identify target genes or metabolic pathways for editing. (3) Gene editing. Gene knock-in, knock-out, or modifications at selected loci are performed using methods such as CRISPR-Cas9. (4) Editing effect assessment. Edited strains undergo PCR, sequencing, and metabolite analysis, among other techniques, to confirm the expected editing effect. (5) Construction. Edited strains are co-cultured, adjusting culture conditions and interactions between strains to construct a synthetic community. (6) Functional verification. The stability and function of the constructed microbial community are assessed in cultures by metabolite analysis and functional identification (Fig. 1).

Case: Losoi et al. co-cultured wild-type strains *Acinetobacter baylyi* and knockout strains AbΔgntT and EcΔptsI of *Escherichia coli*. because AbΔgntT can metabolize glucose to produce gluconate, but not gluconate, whereas EcΔptsI can only metabolize gluconate to produce acetate. As such, these two strains can form a cooperative relationship by exchanging gluconate and acetate, thus maintaining each other's growth while forming stable synthetic microbial community [102]. Pande et al. used the KEGG pathway database to identify gene targets for *Escherichia coli* and *Acinetobacter baylyi* gene editing, deleting genes associated with histidine (His) and tryptophan (Trp) biosynthesis pathway. As a result, both organisms were unable to grow without an external supply of His or Trp. When the two nutrient-deficient strains were co-cultured and exchanged amino acids required for their growth, they formed a synthetic community with a cooperative relationship [103]. Wen et al. constructed a synthetic microbial community using an engineered strain of *Clostridium cellulovorans* that overexpresses the heterologous adhE1 gene, in conjunction with *Clostridium beijerinckii* NCIMB 8052. Within 83 h, the engineered consortium was able to ferment 30.1 g per liter of alkali-extracted defatted corn cob, yielding 3.94 g per liter of butanol without the need for pH adjustment. This output was more than five times greater than that of the wild-type strain consortium [104].

5. Advantages and limitations

In this review, we comparatively analyzed the characteristics of isolation culture, core microbiome mining, automated design, and gene editing (Table 2). These four methods are all designed based on "bottom-up design" [55,59]. Compared to core microbiome mining, automated design, and gene editing, the technical barriers and experimental operational requirements (professional skills and knowledge) for isolation culture are relatively lower. Moreover, automated design and gene editing also rely on specialized equipment.

Each method has specific characteristics. (1) Isolation culture is a highly reproducible method and has a low technical threshold, enabling gradual improvement in microbiota construction through a trial-and-error approach. (2) Core microbiome mining can reduce the workload by targeting the isolation culture of candidate core strains. The microbial communities synthesized using this method are highly diverse, ecologically well adapted, and do not disrupt the diversity of environmentally native microorganisms. (3) Automated synthesis is a highly efficient and accurate method, which can quickly screen suitable microbial strains and optimize the combination ratio. (4) Gene editing technology enables us to precisely modify the genome of microbial strains to introduce the required metabolic and product synthesis functions, rapidly yielding microbial communities with specific functions.

Besides the advantages, each method also has its inherent limitations. Given the complexity of microbial interactions and competition in natural environments [105], automatically synthesized microbial communities must undergo extensive experimental validation to assess their effectiveness. Constructing synthetic microbial communities through isolation culture is a time-consuming method that requires the processing of vast amounts of microbial data, while the ratio of microbial species during the construction process is challenging to control. Core microbiome mining is a time-consuming method that requires substantial resources to define, screen, and optimize core functional strains. Gene editing necessitates the selection of appropriate gene-editing technologies and maintaining the stability and genetic safety of the microbial strains.

6. Challenges and perspectives

Notwithstanding our efforts, synthetic microbial community construction still faces major issues. 1) Based on high-throughput sequencing, taxonomic data on synthetic microbial community members can be determined using various algorithms, statistics and models, but microbial resources are still the key to successful synthetic microbial community construction. And while advances in cultigenomics,

Table 2
Comparison of the five construction methods by required instrumentation, technical threshold, advantages, limitations and application scenarios.

Methods	Instrument	Technology	Advantages	Limitations	Applicable scenarios
Isolation culture	No special requirements	low	High reproducibility Low technical thresholds	Heavy workload High time cost	Longitudinal and exploratory research
Core microbiome mining	No special requirements	high	High efficiency Good reducibility	High technical requirements High economic cost	Sufficient sample size and biological replication
Automated design	No special requirements	high	High efficiency Highly targeted	High technical requirements Long experiment period	Ability to apply relevant models and algorithms
Gene editing	Gene editing tools	high	Good controllability High accuracy	High technical requirements Uncontrollable stability and security	Mastering gene-editing technology

amplification sequencing of microbial marker genes and other new technologies have greatly improved our understanding of the diversity of culturable microorganisms, synthetic microbial community construction remains in the initial stages of development [25]. In other words, synthetic microbial community construction depends on microbial resources. 2) Currently, the majority of studies focus on pairwise interactions, overlooking the potentially significant impact of higher-order microbial interactions. Higher-order interactions refer to the relationships between two or more species within an ecosystem. By exploring these higher-order interactions, we can gain a more comprehensive understanding of the structure and function of ecosystems, as well as the interdependencies among different species. Furthermore, co-existence theory and stability and function theory discuss how different species coexist in environments with limited resources and potential competition, and how they maintain their functionality and stability in the face of disturbances and changes. Therefore, in the process of constructing synthetic microbial communities, exploring higher-order interactions, co-existence theory, and stability and function theory is particularly important. They contribute to expanding our understanding of ecosystem functions and aid in the construction of synthetic microbial communities that are more aligned with ecological theory [106–110]. 3) Currently, the functional validation of synthetic microbial community is a long, expensive and difficult operation, so current research is focused on precisely customizing the function of synthetic microbial communities by leveraging advances in molecular technology. For example, specific functions, such environmental pollutant degradation, specific compound production, and plant growth promotion can be optimizing by selecting appropriate microbial species, adjusting their compositional ratios, and editing genes for specific functions. Therefore, synthetic microbial community construction aims at developing precise functions.

Overall, synthetic microbial community is a field full of potential. However, fulfilling its promise through broad applications in agricultural production, environmental remediation, biomedicine and other fields requires further progress. Here, we propose a set of strategies for achieving this goal. 1) Gene editing technology can be used to acquire key microbial resources. Based on the functional genes of key microorganisms defined by microbiomics, gene editing improves recipient strains by providing them with the same functions as the key strains, thus yielding efficient and stable synthetic microbial colonies. 2) Acquiring a diverse array of microbial candidates with specific functionalities is crucial for the refined design of synthetic microbial community. However, the sheer magnitude of microbial data poses a considerable challenge. Thus, computational tools, including machine learning and artificial intelligence (AI), are indispensable for the identification of potential microbial strains from extensive datasets and culture collections. While these tools have demonstrated efficacy in the biomedical domain, particularly in the discovery of new antibiotics, their application in the assembly of synthetic microbial community remains underexplored. Machine learning and AI are critical for forecasting the construction of microbiome-driven synthetic community, offering the promise of advancing the field to unprecedented level [62, 111–113]. 3) Shifting from a microbial portfolio to a synbiotic portfolio, composed of core functional microorganisms (probiotics) and their beneficial host metabolites (prebiotics) [114], may enable synthetic microbial community to overcome fluctuations in seasonal environments and soil microbial communities. Plants, for example, are not isolated organisms but a symbiotic system functioning with numerous microorganisms. The plant metabolism is important for sustaining interactions between microorganisms [115]. Thus, synbiotic assemblages may be a new approach to attain high efficiency, persistence and stability in synthetic microbial communities.

7. Concluding remarks

This review concisely summarizes five prevalent methods to constructing synthetic microbial community, comparing their features and limitations, to demystify the process and enhance the application of microbial potential in agriculture, medicine, environmental science, and biology.

CRediT authorship contribution statement

Chenglong Li: Conceptualization, Writing – original draft, Data curation, Validation, Writing – review & editing. **Yanfeng Han:** Conceptualization, Data curation, Validation, Writing – review & editing. **Xiao Zou:** Visualization, Writing – review & editing. **Xueqian Zhang:** Writing – review & editing. **Qingsong Ran:** Writing – review & editing. **Chunbo Dong:** Conceptualization, Funding acquisition, Project administration, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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