BioControl (2025) 70:229–244
https://doi.org/10.1007/s10526-024-10295-w

Recent advances in experimental design of synthetic microbial communities for biocontrol application

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Received: 14 May 2024 / Accepted: 19 November 2024 / Published online: 21 January 2025

Abstract In recent years, synthetic microbial communities (SMC) have garnered significant attention as a promising approach to harness the collective capabilities of multiple microbial species across diverse applications, including plant disease management. Advances in omics technologies have provided deeper insights into the complex interactions between plant microbiomes and their surrounding environments. Notably, significant progress has been made in the design and engineering of SMC that exhibit synergistic interactions, demonstrating great potential in managing phytopathogens. Novel tools, such as automated design and artificial intelligence, are increasingly being integrated to enhance the precision and efficiency of SMC engineering. Given the complexity of natural and agricultural plant-associated systems, along with the multitude of variables that influence SMC performance, developing a universal rationale for engineering SMC for biocontrol application remains challenging. This review discusses the design perspective of SMC for biocontrol application, their underlying design principles, critical considerations, and current research endeavors. Additionally, it briefly contemplates the challenges and prospects of SMC application in plant disease management.

Keywords Synthetic microbial consortia · Design rationale · Biocontrol · Bioinoculants · Agricultural application

Handling Editor: Sotiris Tjamos.

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Introduction

Soil microbial communities are vital in nutrient cycling, promoting plant growth, and suppressing diseases. Research on soil microbial consortia has gained momentum in plant disease management, aiming to understand microbial interactions with host plants to improve crop productivity while reducing reliance on chemical fertilizers and pesticides. Key microbial communities such as nitrogen fixers (*Rhizobium*, *Azospirilli*, *Paenibacilli*), pathogen-suppressors (*Pseudomonas*, *Bacillus*), and poor soil health indicators (*Nitrososphaeraceae*) highlight the crucial role of microbial communities in soil health (Schloter et al. 2018; van Bruggen and Semenov 2000; Wilhelm et al. 2023).

In parallel with investigations into indigenous microbial consortia, there is a growing interest in engineering SMC. This emerging field, at the intersection of microbiology, synthetic biology, and biotechnology, leverages the rational assembly of microbial strains with complementary functions to achieve desired outcomes. The construction of synthetic microbial communities (SMC) entails the guidance of ecological and engineering principles, encompassing the study of microbial interactions in a controlled environment (Jia et al. 2016; Liang et al. 2022). A meticulous selection, testing, and grouping of microbial strains result in consortia with synergistic interactions, with the ability to promote plant growth and disease resilience beyond what individual microbes can achieve. For instance, SMC elicits stronger induced systematic resistance in plants compared to single microbial strains (Berendsen et al. 2018). By harnessing the collective behavior and functional diversity of microbial communities, scientists are unlocking new avenues for sustainable plant disease management.

In plant science, the rhizosphere often serves as one of the sources for selecting consortia members with disease-suppressing and plant growth-promoting traits. This dynamic, complex zone facilitates interactions between plants, soil, and microorganisms, similar to the gut-brain axis, with no definite shape and size. The diverse array of inter- and intra-species interactions within the rhizosphere form complex consortia that play crucial roles in supporting plant health, nutrient cycling, and soil ecosystem functions. Plants actively influence the composition of rhizosphere microbial communities through the secretion of root exudates, which include sugars, amino acids, organic acids, and other compounds (Raza and Shen 2020). These exudates not only serve as a carbon source for microbes but also act as signaling molecules that attract beneficial microorganisms (Pantigoso et al. 2022). In return, these microbes can enhance plant growth, protect against pathogens, and contribute to nutrient acquisition. Microbial consortia in the rhizosphere perform essential functions like nutrient solubilization, nitrogen fixation, production of growth-promoting substances, and disease suppression, fostering a resilient soil ecosystem. Understanding and harnessing these microbial interactions offer great potential for sustainable disease management.

Advances in omics technologies have transformed plant–microbiome studies by rapidly characterizing plant-associated microbes and their roles in plant health. However, the sheer volume of data in publicly available databases makes traditional analysis methods increasingly challenging, necessitating the development of automated approaches (Kang et al. 2022). The automated design of biocontrol for plant disease management employs advanced technologies, including artificial intelligence (AI), to develop effective and sustainable strategies for protecting crops (Karkaria et al. 2021). AI plays a crucial role in this process by analyzing vast datasets to identify patterns and predict disease outbreaks, enabling timely and precise interventions. Machine learning algorithms can sift through genomic data of pathogens and beneficial microbes to select optimal biocontrol agents, enhancing their effectiveness. Additionally, AI-driven systems can simulate various environmental conditions and biotic interactions, allowing researchers to predict plant protection outcomes of biocontrol agents (BCA) and optimize biocontrol formulations (Maraveas 2022; Jing et al. 2024). AI integration into biocontrol design improves the accuracy and efficiency of disease management and reduces reliance on chemical pesticides, promoting ecological balance and sustainable agriculture.

Designing synthetic microbial consortia involves assembling different microorganisms with specific functions to achieve targeted outcomes. The rationale behind designing synthetic microbial consortia requires careful consideration of functional diversity, metabolic interdependencies, and community dynamics. However, there is limited literature focused on the design and construction of SMC for biocontrol application, as well as on key points to consider in this process. Understanding the critical factors and challenges involved in designing SMC can help facilitate the development of more effective construction strategy. This review highlights the important considerations and strategies for engineering SMC aimed at the biological control of phytopathogens. It also discusses recent advancements and applications, while exploring the advantages, challenges, and future perspectives of SMC-based biocontrol.

Construction of synthetic microbial consortia for plant disease management

SMC consists of two or more carefully selected microbial species from the natural environment, based on their complementary functions. The construction of these communities primarily relies on microbial interactions and is guided by design principles, such as interdependence and stable relationships (Che and Men 2019). The two main strategies employed in SMC construction includes function-driven (top-down or complex-to-simple) and interaction-driven (bottom-up or simple-to-complex) approaches (Che and Men 2019; Großkopf and Soyer 2014). Figure 1 illustrates the different steps involved in both approaches.

In the top-down strategy, the first step involves identifying the target environment. Microbial samples are collected using aseptic techniques from environments such as the phyllosphere, rhizosphere. Based on current and previous trends in SMC design for biocontrol (Zhimo et al. 2020; Jing et al. 2023), the strategies commonly applied using the top-down approach can be divided into two categories: (1) the direct isolation of microbes with known function, and (2) processing samples collected from target environments for high-throughput sequencing. While the first approach is relatively straightforward and nearly similar to the bottom-up approach, the second provides deeper insights into community structure and functional characteristics. Although the top-down approach is generally more complex than the bottom-up strategy, it offers advantages by incorporating naturally occurring microbial interactions (Che and Men 2019; Czajkowski et al. 2020). The steps typically involved in the top-down approach (Fig. 1) are: (1) Selection of source environment—the rhizosphere and endosphere are among the most common environments for isolating BCA against phytopathogens (Yin et al. 2022; Zhang et al. 2022a; Tian et al. 2024). (2) Identification, characterization, and selection of microbial community—BCA are selected and isolated from a diverse pool of microbial communities using various selective media and culturing techniques. Methods such as progressive dilution and rhizodepositional attraction can be employed to pinpoint key rhizobacteria with the potential to suppress phytopathogens. In progressive dilution, a 10^{-1} soil suspension is prepared by preparing approximately 10% of fresh soil solution in sterile distilled water. This initial suspension is then serially diluted to form 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} suspensions, effectively removing low-abundance taxa from the microbial community (Xun et al. 2023). Advanced multi-omics techniques, such as culturomics and metagenomics, can further characterize key taxa within microbial communities that possess disease-suppressive potential. For instance, Zhou et al. (2022) identified 205 unique strains through the microbiological culturomics method and developed various SMC. Similarly, Flores-Duarte et al. (2023) found that SMC created using the culturomics approach outperformed those constructed with traditional agar media. (3) Isolation and cultivation of key strains—individual microbial strains that are crucial for the community's function can be isolated and cultured using selective media or specific culturing techniques. The dilution plate method, for example, involves plating diluted soil suspensions onto semi-selective or rich agar media. To isolate BCA, about 5% of the soil solution is suspended in sterile physiological solution. A 100 µl aliquot of this suspension, diluted to 10^{-4}, 10^{-5}, and 10^{-6}, can be plated on lysogeny broth (LB) medium and incubated upside down at 37 °C for 3–7 days. Distinct single colonies, characterized by differences in shape, color, and size, are then transferred to new LB plates for purification (Prigigallo et al. 2022; Tian et al. 2024). (4) Functional analysis—the functional analysis of BCA involves assessing their mechanisms of action, effectiveness, and interactions with pathogens and host plants. In general, functional screening can be performed using dual culture assay, plus (+) streak, and square streak methods (Anith et al. 2021). However, molecular techniques like gene expression profiling can help identify key genes and pathways involved in biocontrol efficacy (Pozo et al. 2004). Additionally, advanced technologies such as metabolomics and proteomics provide insights into the biochemical interactions between BCA and their targets (Massart et al. 2015). (5) Simplification of the community—the complexity of the community is gradually reduced by removing redundant or non-essential species. This step ensures the retention of necessary functions while minimizing the number of species. For instance, Prigigallo et al. (2022) optimized a

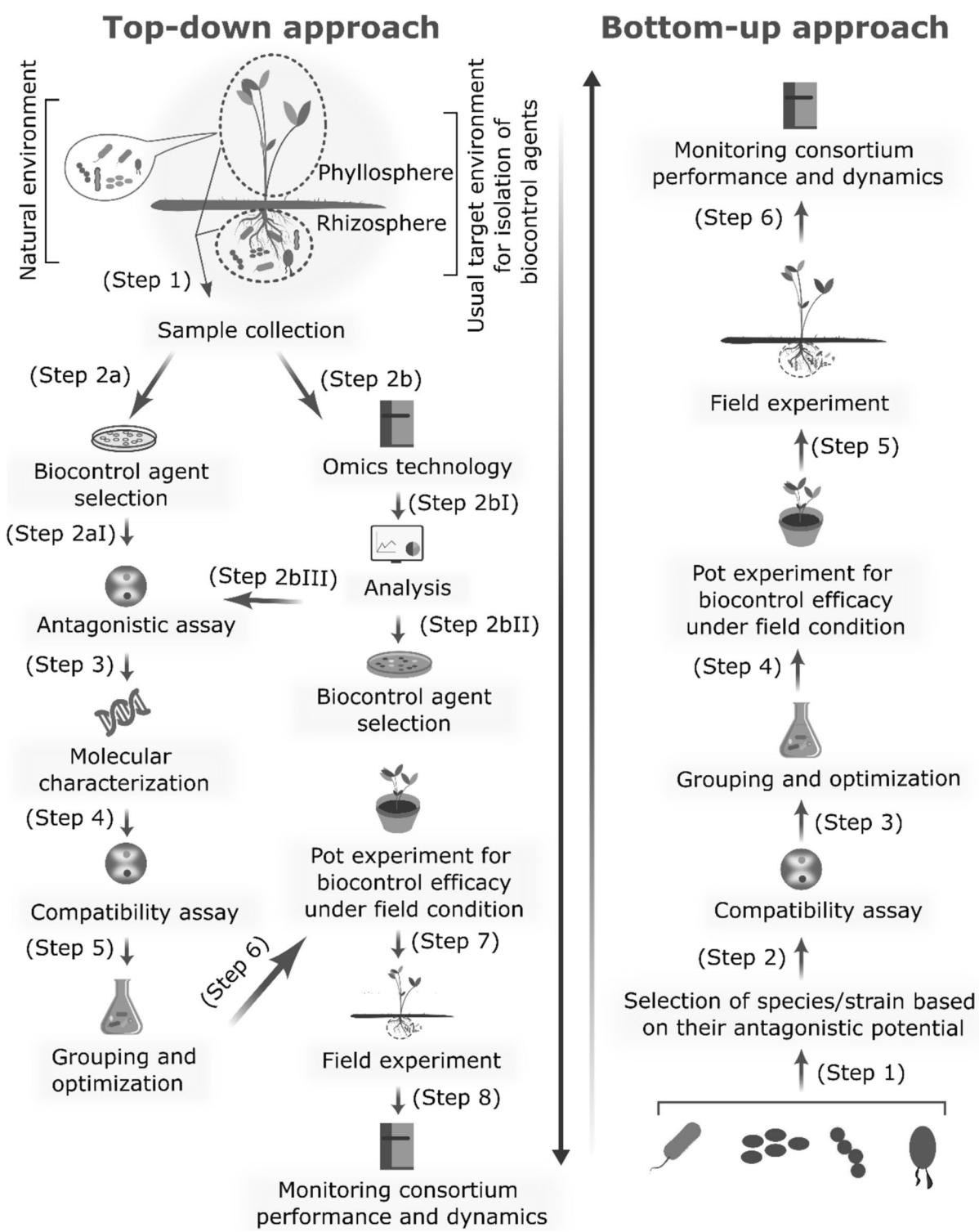

Fig. 1 Illustration of the steps involved in designing and monitoring of synthetic microbial consortia for biocontrol application

biocontrol SMC for banana Fusarium wilt, reducing it from 44 to seven isolates, and ultimately to three isolates, which included *B. velezensis* BN8.2, *P. chlororaphis* PS5, and *Trichoderma virens* T2C1.4. Huang et al. (2023) used 16S rRNA gene sequencing to investigate the rhizosphere bacterial community, isolating culturable microbes and developing a 38-species SMC. This consisted of eight phytopathogen-inducible (which activate defense-related pathways in host plants) and 30 stably-colonizing bacteria, which were later simplified to a three-species SMC. (6) Validation and testing—the simplified consortia are tested under laboratory conditions to ensure they effectively perform the desired function. It is crucial to validate the stability and robustness of SMC under various environmental conditions, including field conditions.

In contrast to the top-down approach, the bottom-up strategy involves the arbitrary assembly of pre-isolated microbial strains, followed by systematic screening of their interaction patterns and growth conditions. For example, Minchev et al. (2021) applied a bottom-up approach to combine bacterial and fungal strains in different combinations for phytopathogen control. In another study, Wang et al. (2024) designed five- and two-strain consortia from a subset of previously isolated bacterial isolates to examine their effects on plant growth. Because of its simplicity, the bottom-up approach is often preferred in plant science. A key advantage of bottom-up consortia is functional redundancy. This means that if one species is affected by a stressor, others can compensate to maintain functionality. This contrasts with the top-down approach, where maintaining community structure can be more challenging (Liang et al. 2022). Despite these differences, both approaches share several common steps, such as optimizing and testing the consortia in practical applications to assess their effectiveness and scalability. The key difference lies in the starting point: the top-down approach usually begins with an existing microbial community and simplifies it by removing unwanted species or functions, while the bottom-up approach starts with individual pure strains with known properties and assembles them by combining specific strains.

It is important to note that the presence of engineered biological systems is not a prerequisite for defining microbial consortia as synthetic (Großkopf and Soyer 2014). Engineered synthetic consortia members are typically explored and employed in bioremediation of pollutants and biosynthesis of natural products (Che and Men 2019). Conversely, due to the potential environmental risks associated with modified consortium members, the use of genetically altered microbes is either uncommon or not practiced in agriculture (Sudheer et al. 2020).

Technological advancements in exploration of biological control agents and design of synthetic microbial communities

Omics technologies

Omics technology, including next-generation sequencing (NGS), genomics (pangenomics, metagenomics), transcriptomics, proteomics, and metabolomics, has transformed plant-microbiome studies. This advancement allowed researchers to gain comprehensive insights into BCA-host–pathogen-environment interactions. The genetic, functional, and metabolic profiles of microbial communities facilitate the discovery of new BCA, essential for effective biocontrol formulation. For instance, metagenomic analysis of fungi associated with jojoba plants allowed Elbakary et al. (2024) to identify candidate BCA with biocontrol activities. Additionally, functional analyses and metabolite fingerprints are powerful tools that provide deeper understanding of biocontrol mechanisms. Analyzing biocontrol activities and their association with gene clusters is critical for developing effective biocontrol strategies. For example, Tomita et al. (2023) identified gene sequences related to non-ribosomal peptide synthetase and polyketide synthase in metagenomes derived from activated sludge, pinpointing candidate BCA capable of antagonizing phytopathogenic fungi. Similarly, Zhang et al. (2022b) examined microbial communities in two cultivars of tea plant—one resistant and the other susceptible to gray blight disease—and found a significant difference in the relative abundance of *Penicillium*, identifying it as a potential biomarker.

Omics technologies also enable detailed characterization of microbial interactions and dynamics. By studying microbial transcriptomes and proteomes, researchers can uncover the complex networks of cooperation, competition, and signaling mechanisms

that drive microbial community behavior. For instance, Ma et al. (2021) used transcriptome analysis to investigate how SMC that either suppress or do not suppress root growth inhibition (RGI) affect plant immunity. Their findings revealed that RGI-suppressive activities modify root development and nutrient transport functions while decreasing the expression of certain immune-related genes. They also found that precolonizing plants with RGI-suppressive SMC increases susceptibility to opportunistic *Pseudomonas* pathogens. These studies underscore the potential of omics technologies to harness the diverse functionalities of microbial communities to enhance sustainability, productivity, and resilience in various ecosystems.

Integrating artificial intelligence with omics technologies

Omics technologies often face challenges due to the sheer scale of data that needs to be analyzed. However, advancements in these technologies, along with large-scale consortia projects, are enabling researchers to explore biological systems at an unprecedented level, resulting in heterogenous and often extensive datasets (Haas et al. 2017). By integrating AI with omics technologies, these datasets can be analyzed faster and more efficiently to recognize patterns and uncover insights into the genetic, protein, and metabolic pathways involved in plant-pest interactions. Machine learning models are particularly effective at distinguishing between disease-conducive and -suppressive soils with the ability to pinpoint specific genera that could be potentially beneficial. Furthermore, AI can be used to discover BCA and optimize media for better biocontrol efficacy against target pathogens. For instance, Liao et al. (2020) utilized machine learning technology based on quantitative morphological traits, achieving 100% accuracy in BCA identification. Similarly, Smaoui et al. (2018) applied AI to optimize media conditions, enhancing antimicrobial activity against phytopathogenic fungi. By analyzing extensive datasets from various sources—including genomic, enviromic, and crop performance data—AI can identify patterns and correlations that may be overlooked by human researchers (Xu et al. 2022). Predictive modeling further allows AI to forecast BCA effectiveness under different environmental conditions, optimizing strategies for specific crops and regions (Agboka et al. 2024; Manganiello et al. 2024). Additionally, AI enhances precision agriculture by integrating data from drones, satellites, and ground sensors to monitor crop health in real time. This ensures that BCA are applied precisely where needed, reducing waste and improving efficacy.

Automated design of synthetic microbial consortia

The automated design of SMC is a cutting-edge field that integrates computational methodologies with synthetic biology to screen, engineer, and optimize SMC for specific functions. For instance, Kehe et al. (2019) introduced the kChip platform, which utilizes droplet microfluidics for high-throughput screening and the rapid construction of SMC. They demonstrated that the kChip platform can screen and discover multispecies consortia with various optically measurable functions, such as enhancing pathogen suppression by BCA. Karkaria et al. (2021) employed Bayesian methods to automate the design of two- and three-strain consortia, identifying resilient candidates suitable for stable steady-state communities, with 69 unique combinations for two-strain and 4182 for three-strain system. Mathematical models and simulation software, such as COMETS, BacArena, and COBRA, are often used to predict the interactions and dynamics within these consortia (Heinken et al. 2021). However, predicting the behavior of SMC in real-world environments continues to pose significant challenges. Despite these difficulties, the field is advancing rapidly.

Key factors for consideration in designing synthetic microbial consortia

Designing SMC involves several critical considerations to ensure functionality, stability, effectiveness in real-world scenarios, scalability, and ethical compliance. Some of these key considerations and rationales are outlined in Fig. 2.

Compatibility

One of the essential criteria for designing SMC is the meticulous selection of microbial species and compatibility among the selected microbial strains to ensure stable and functional interactions. It is also

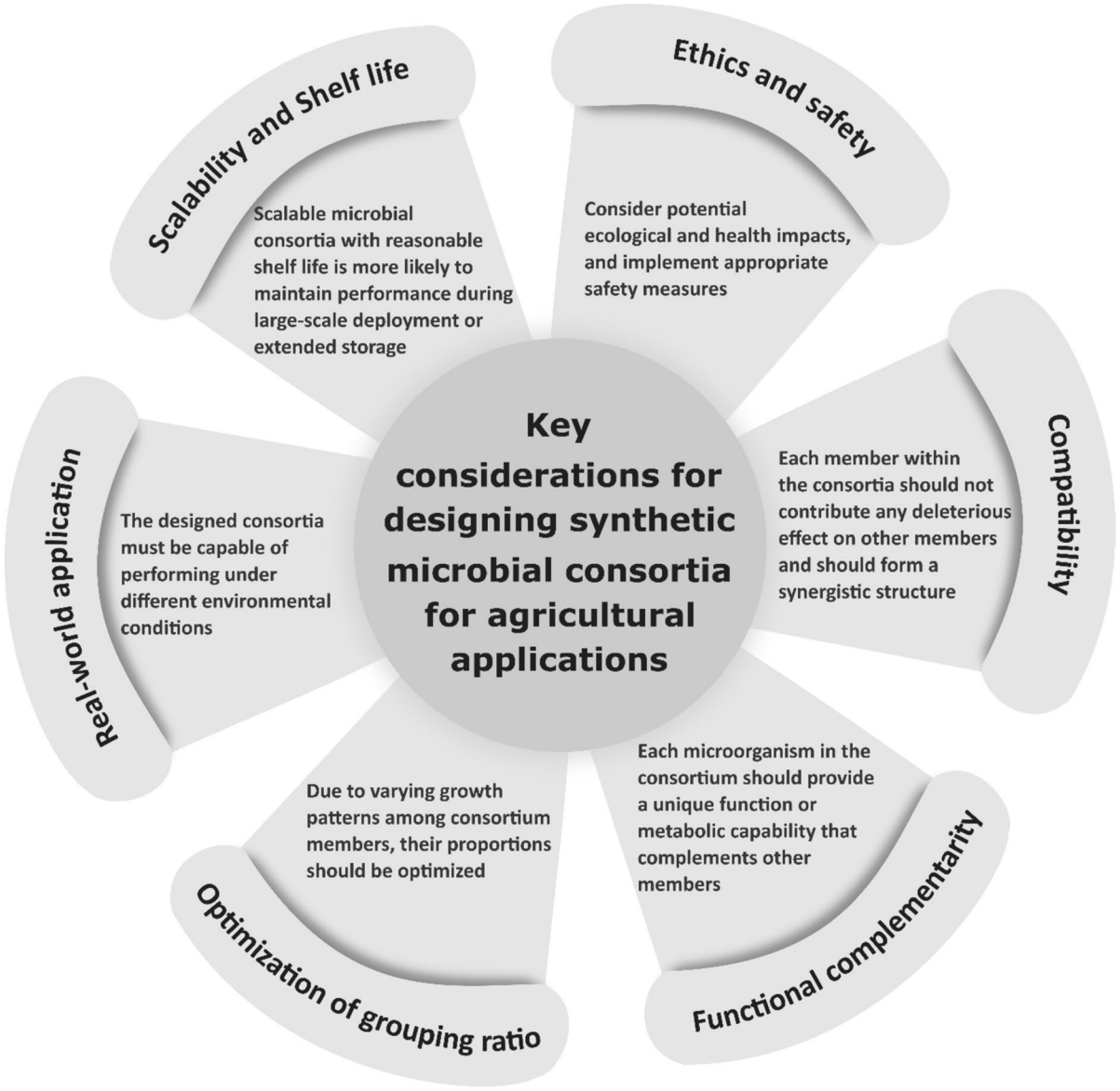

Fig. 2 Key considerations for designing synthetic microbial consortia for biocontrol and biofertilizer applications

important to consider additional selection criteria, such as ensuring that the strains are non-pathogenic to humans, animals, and plants, resilience to adverse environmental conditions, and exhibit synergistic and fast-acting behavior (Czajkowski et al. 2020). Metabolically, the selected microbes should possess complementary pathways that support mutualistic relationships. One key mechanism for this is cross-feeding, where microbes exchange metabolic byproducts, thereby helping to stabilize cooperative interactions within the consortium (Kemen 2014). Ensuring compatibility in these aspects fosters robust and resilient consortia capable of maintaining functionality under varying environmental conditions for extended periods (Santoyo et al. 2021).

In addition to environmental conditions and edaphic factors, the diverse range of antimicrobial secondary metabolites (iturin, fengycin, hydrogen cyanide, cellulase, etc.) produced by microbial species (Naz et al. 2022) plays a central role in

antagonizing phytopathogens. The production of these metabolites is influenced by the types of microbial interactions that occur within the consortia. Typically, inter-species interactions tend to decrease secondary metabolite production, while intra-genus interactions often increase it (Wei et al. 2021; Hansen et al. 2022). However, inter-kingdom interactions can enhance plant resilience by, for example, suppressing Fusarium wilt and producing beneficial metabolites (Zhou et al. 2022). While these metabolites target pathogens, they may also have a negatively impact on beneficial members of the consortia. For instance, Prigigallo et al. (2022) demonstrated that metabolites produced by beneficial strains could also exhibit proclivity for targeting other advantageous microorganisms. This finding highlights the importance of careful strain selection and synergistic pairing when designing effective SMC.

Functional complementarity

Microbial consortia, which are communities of microorganisms working together, hold significant promise for biocontrol applications in agriculture. Their functional complementarity—where different microbes contribute unique capabilities to the consortium—can greatly enhance the efficiency and resilience of biocontrol strategies. For instance, Hao et al. (2022) found that the success of *Trichoderma* consortia relied more on functional complementarity than on the number of strains present. In a well-designed microbial consortium, the diverse metabolic activities and ecological roles of various microorganisms work synergistically to suppress plant pathogens, promote plant growth, and improve soil health. For example, certain bacteria and fungi produce antimicrobial compounds that inhibit pathogenic organisms, while others compete with these pathogens for resources or space, effectively reducing pathogen loads (Moënne-Loccoz et al. 2015; Santoyo et al. 2021). Additionally, microbes in a consortium can act together to induce systemic resistance in plants, making them more resilient to future infections (Berendsen et al. 2018).

Another vital aspect of functional complementarity is the ability of microbial consortia to adapt to varying environmental conditions. Different microbes possess varying environmental tolerances and metabolic capabilities, enabling the consortium to remain effective under conditions that may be challenging for a single microbial species. This adaptability ensures consistent biocontrol performance even in the face of fluctuating temperatures, moisture levels, and soil pH. For instance, some microbes may be more active in colder temperatures, while others thrive in warmer conditions, allowing the consortium to remain functional throughout the growing season (Smith et al. 2022). This redundancy ensures that the consortium's functionality remains effective even when environmental conditions or edaphic factors impact certain members of the consortium. Such resilience is crucial for sustainable agriculture, as biocontrol agents must consistently perform over time to reduce reliance on chemical pesticides.

Optimization of grouping ratio

In general, the aim of grouping strains is to establish a well-optimized consortium that can efficiently perform the desired task. The composition and grouping ratio may vary depending on the intended application and target environment. Typically, consortium members are grouped in a 1:1 ratio (Jain et al. 2020; Zheng et al. 2024). However, a notable observation from a comprehensive review of literature on SMC for plant disease management shows a lack of defined grouping criteria. An imbalance in the ratio of fast- to slow-growing strains can lead to decreased efficacy and functionality. For example, Wang et al. (2023) explored different ratios in a two-strain consortium and discovered that an 8:2 ratio was the most effective in breaking down mycotoxins produced by phytopathogens. Moreover, previous studies have shown that certain species, such as *Sinorhizobium meliloti* PP3, *Bacillus licheniformis*, and *Acinetobacter calcoaceticus*, exhibit faster growth rates when grown in mixed cultures compared to monocultures (Pandey et al. 2005; Jha and Saraf 2012). These findings highlight the importance of considering strain dynamics when grouping consortia members and the impact these dynamics can have on consortia performance in a more nuanced view.

Real-world application

Conducting field studies is crucial for translating the promising findings of developed microbial consortia from controlled laboratory and greenhouse environments to practical agricultural applications. Field

trials also provide valuable insights into how these consortia function under the dynamic conditions of actual farms, and also help assess their economic viability. Unlike laboratory settings, field trials capture the complexity of microbe-microbe interactions that occur in natural conditions (Sergaki et al. 2018). Additionally, soil ecosystems influence the composition of the root microbiome, meaning that the survival of an inoculant within the soil community does not necessarily ensure its successful colonization of the plant host. To develop effective strategies that leverage plant–microbe interactions in real-world conditions, it is essential to thoroughly understand these dynamic interactions and to advance innovative techniques that take the complexities of natural environments into account. One promising strategy for assessing abiotic factors, such as temperature and pH, which can help simulate natural environmental conditions, is the use of microcosm studies. For example, Perazzolli et al. (2016) analyzed soil microcosm and identified complex metabolic adaptations and interactions of BCA in the soil environment. Chen et al. (2021) demonstrated through a microcosm study that temperature significantly influences the efficacy of BCA, but they also noted that such effects are unlikely to be accurately predicted by laboratory studies.

Scalability and shelf life

Designing SMC for practical applications requires a comprehensive approach that balances scalability and shelf life. Scalability can be achieved by selecting strains with compatible growth rates to ensure community stability, as well as by optimizing culture conditions such as temperature, pH, and nutrient availability (Gu et al. 2022). It is essential to develop cost-effective media that can be produced in large quantities for industrial scalability. This might involve using agricultural by-products, organic wastes, or other inexpensive substrates to help reduce production costs. For example, Namasivayam et al. (2024) demonstrated the bioconversion of agro-food waste into a low-cost medium for the mass multiplication of BCA. In the bioconversion of agro-waste, solid state fermentation (SSF) is often preferred due to its availability, low cost, and eco-friendly nature (Sala et al. 2020).

To extend shelf life, preservation methods such as lyophilization, cryopreservation, and encapsulation can help maintain microbial viability and functionality upon reactivation (De Corato et al. 2018; Teixidó et al. 2022). These methods must be tailored to the specific requirements of the microbial strains involved, as different species may exhibit varying tolerances to desiccation or freezing. Additionally, optimal storage conditions—including controlled temperature, humidity, and light protection—are vital for preserving the consortia (Khan et al. 2023).

Ethics and safety

As shown in Fig. 2, ethics and safety are critical factors to consider when designing SMC. Current biocontrol practices are guided by frameworks that prioritize environmental sustainability and minimal risks to human health, focusing on preservation of ecological balance and biodiversity (Ferron and Deguine 2009). Safety protocols for biocontrol agents are comprehensive and multifaceted. Before these agents are deployed, they undergo extensive testing to evaluate their specificity, efficacy, and potential non-target effects. Regulatory agencies, such as the Environmental Protection Agency (EPA) and similar organizations, ensure that only those agents meeting stringent safety criteria are released into the environment (Ehlers et al. 2020; Romeis et al. 2020).

Recent advancements in the application of synthetic microbial consortia as biocontrol agents

Synthetic microbial consortia can be engineered to specifically target plant pathogens by grouping a variety of microbial species selected based on their ability to produce antimicrobial compounds, compete for resources, or induce systemic resistance in plants (Maciag et al. 2023). These consortia can colonize the rhizosphere or phyllosphere, forming protective barriers that reduce disease incidence (Li et al. 2021). Numerous studies have demonstrated the effectiveness of SMC in controlling phytopathogens (Table 1). For instance, some earlier studies revealed that combinations of different microbial strains can effectively manage diseases in host plants. *Trichoderma virens* and *Bacillus velezensis* were shown to suppress Fusarium wilt in cape gooseberry, *Trichoderma* and *Streptomyces* managed *Pyrrhoderma noxium*

Table 1 Summary of recent studies on synthetic microbial consortia with biocontrol potential, listed in chronological order by year of publication, followedlisted chronologically and by alphabetical order of the authors' names

Microbial consortium	Target pathogens	Experimental conditions	References
Metarhizium robertsii formerly known as *Metarhizium anisopliae* + *Trichoderma viride*	*Pseudopestalotiopsis curvatispora*	Field condition	Bora et al. (2022)
Trichoderma asperellum (GDSF1009) + *Trichoderma asperelloides* (Z4-1) + *Trichoderma harzianum* (10569) + *Trichoderma asperellum* (10264)	*Fusarium oxysporum*	Greenhouse condition	Hao et al. (2022)
Trichoderma strains (5029 and 5001) + *Streptomycetes* (USC − 6914 and USC − 595-B)	*Pyrrhoderma noxium*	Greenhouse condition	Panchalingam et al. (2022)
Pseudomonas chlororaphis subsp. *piscium* PS5 + *Bacillus velezensis* (BN8.2) + *Trichoderma virens* (T2C1.4)	*Fusarium oxysporum* f. sp. *cubense* tropical race 4	Greenhouse condition	Prigigallo et al. (2022)
Azotobacter chroococcum + *Azospirillum lipoferum* + *Pseudomonas putida*	*Neocosmospora rubicola*	Pot condition	Riaz et al. (2022)
Pseudomonas fuorescens (Pf-2) + *P. fuorescens* (Pf-3) + *Trichoderma asperellum* (T-11) + *T. asperellum* (T-14)	*Phytophthora infestans*	Field condition	Singh et al. (2022)
Pseudomonas chlororaphis strains (PCL1601 + PCL1606 + PCL1607)	*Rosellinia necatrix*	Greenhouse condition	Villar-Moreno et al. (2022)
Cupriavidus campinensis B20 + *Rhodococcus erythropolis* B43 + *Janthinobacterium lividum* BJ + *Chryseobacterium soldanellicola* P38	*Rhizoctonia solani* AG8	Greenhouse condition	Yin et al. (2022)
Lysobacter antibioticus (13–6) + *Lysobacter capsici* (ZST1-2) or *Lysobacter antibioticus* (13–6) + *Bacillus. cereus* (BT-23) or *Bacillus cereus* (BT-23) + *Lysobacter antibioticus* (13–6) + *Lysobacter capsici* (ZST1-2)	*Plasmodiophora brassicae*	Field condition	Zhang et al. (2022a)
Paenibacillus peoriae + *Bacillus subtilis* + *Lysinibacillus* sp. + *Bacillus simplex* + *Pseudomonas chlororaphis* + *Beauveria bassiana* + *Scopulariopsis brevicaulis* + *Trichoderma gamsii* + *Trichoderma ghanense* + *Trichoderma lignicola*	*Fusarium oxysporum* f. sp. *physali*	Greenhouse condition	García et al. (2023)
Acinetobacter lwoffii + *Bacillus amyloliquefaciens* + *Curtobacterium luteum* + *Curtobacterium* sp. NG1.12 + *Methylobacterium platani* + *Pantoea* sp. PRS8-3 + Pseudomonas *parafulva* + *Pseudomonas* sp. OBYHD3-2	*Penicillium digitatum* and *Geotrichum candidum*	Laboratory condition	Jing et al. (2023)
Lysobacter enzymogenes (OH11W) + *Bacillus safensis* (ZK-1)	*Pseudomonas syringae* pv. *Actinidiae, Diaporthe actinidiae*	Laboratory condition	Lin et al. (2023)

Table 1 (continued)

Microbial consortium	Target pathogens	Experimental conditions	References
Bacillus spp. + *Pseudomonas dimunita*	*Meloidogyne incognita*	Pot condition	Pradana et al. (2023)
Bacillus subtilis (IS1) + *Bacillus amyloliquificiens* (IS6) + *Bacillus fortis* (IS7)	*Fusarium oxysporum*	Pot condition	Raza et al. (2024)
Ralstonia solanacearum FJAT-1458 (avirulent strain) + *Bacillus tequilensis* FJAT-51047 + *Streptomyces fradiae* FJAT-31535	*Ralstonia solanacearum* (virulent strain)	Pot condition	Zheng et al. (2024)

pathogen, *Bacillus*, *Pseudomonas*, and *Trichoderma* spp., controlled *Fusarium oxysporum* and *Botrytis cinerea* in tomato (Izquierdo-García et al. 2020, 2021; Minchev et al. 2021; Panchalingam et al. 2022; García et al. 2023). A study by Win et al. (2021) demonstrated that a synthetic consortium consisting of *Bacillus velezensis*, *Bacillus subtilis*, and *Penicillium* sp., significantly reduced disease incidence in bananas caused by *Fusarium* and *Alternaria* sp. pathogens. Furthermore, Singh et al. (2022) showed that combinations of *Trichoderma* and *Pseudomonas* inhibited the late blight pathogen in tomato plants, resulting in improved plant health and yield. These findings highlight the immense potential of SMC as a biocontrol tool, paving the way for more sustainable and eco-friendly agricultural practices.

Advantages

Synthetic microbial consortia can outperform individual microorganisms by combining species with complementary abilities, such as unique metabolic pathways or enzymatic activities. This synergy enables them to perform complex tasks like waste degradation, biofuel production, and biocontrol of phytopathogens more efficiently (Liang et al. 2022; Wu et al. 2023). Compared to single species, these consortia also offer increased stability and robustness due to functional redundancy, ensuring they remain effective even during environmental fluctuations or disturbances (Mahmud et al. 2021; Minchev et al. 2021). This resilience is especially beneficial in real-world applications where conditions can vary significantly. Microbial consortia facilitate division of labor, with each species specializing in a specific task, thereby enhancing overall efficiency and optimizing resource utilization. For example, one species might break down a complex substrate into simpler compounds, which are then utilized by another species for further processing (Roell et al. 2019; Liang et al. 2022). These capabilities expand their applicability in various industries, including agricultural application, bioremediation, waste treatment, and biosynthesis.

Limitations

The development and application of SMC for biocontrol face several limitations. One major challenge is the complexity involved in their design and optimization. Selecting and combining suitable microbial species requires careful consideration of their interactions, growth rates, and compatibility (Roell et al. 2019; Czajkowski et al. 2020). Establishing a stable and functional consortium often demands iterative experimentation, which can be both time-consuming and resource-intensive. Moreover, our limited understanding of microbial interactions within these consortia adds to the complexity. Such interactions can be cooperative, competitive, or influenced by environmental factors, making it challenging to accurately predict the behavior of consortia (Jia et al. 2016; Widder et al. 2016). Given these challenges, designing consortia with desired functionalities typically involves a significant amount of trial and error. Additionally, implementing SMC on a large scale poses technical and engineering challenges. Maintaining optimal

conditions, ensuring uniform distribution of microorganisms, and managing dynamic interactions within the system can be quite difficult. Moreover, scaling up synthetic consortia for biocontrol applications also requires overcoming challenges related to cost, regulatory compliance, and operational logistics (Jia et al. 2016; Mahmud et al. 2021).

Challenges and future perspective

To create efficient and reliable marketable SMC, several challenges must be addressed, including regulatory frameworks, efficacy, slow acting, unreliability, inconsistency, shelf life, production and logistic costs, and commercialization. For example, due to regulatory constraints, Santhanam et al. (2015) limited their consortium members to five. Despite technological advancements, the successful formulation of reliable, stable, and fast-acting consortia still needs to tackle issues related to shelf life and the cost of large-scale production.

Recent advancements in high-throughput sequencing technologies and computational modeling have provided researchers with valuable tools to study the structure and dynamics of SMC. Omics technologies have enabled a deeper understanding of the fundamental principles governing microbial ecology in the rhizosphere environment. For instance, techniques such as culturomics combined with metagenomics, have allowed researchers to explore new avenues for designing SMC (Flores-Duarte et al. 2023; Lian et al. 2023). These methods enable the characterization of microbial community composition, metabolic interactions, and the identification of key factors that influence consortium behavior. By integrating experimental and computational approaches, researchers gain deeper insights into the complex dynamics of SMC, which can lead to the development of efficient SMC that supports sustainable agricultural practices, reduces dependence on chemical fertilizers and pesticides, enhances crop productivity, and improves plant resilience to environmental stress. The future of synthetic microbial consortia holds tremendous potential, offering a promising path toward sustainable agriculture, with applications that extend beyond the management of phytopathogens. Ongoing research and technological advancements will help unlock their full potential in the coming years.

Concluding remarks

The application of SMC shows great promise in addressing global challenges and has attracted significant interest and investment for both academia and industry. As research in this field continues to expand, further advancements are expected in the design and engineering of SMC, leading to innovative solutions and practical application for managing plant diseases. Technologies such as culturomics and metagenomics will significantly enhance our understanding of unculturable microbes and help identify genetic sequences not currently available in reference databases. With continued efforts, SMC has the potential to revolutionize various industries and contribute to more sustainable and efficient agricultural practices. However, several factors must be considered in the construction of synthetic consortia. These include strain dynamics, grouping ratios, and the type of grouping (inter- and intra-species, inter- and intra-genus, or cross-kingdom). Additionally, the designed consortia should have a long shelf life, be easily manageable, and be economically viable for production. Moreover, regulatory frameworks need to evolve more quickly to keep pace with technological advancement, ensuring a smooth transition from laboratory-commercialization interfaces to field applications. While SMC offers several advantages, such as enhanced functionality, stability, division of labor, and increased substrate utilization, challenges related to design complexity, limited knowledge of microbial interactions, and technical limitations must be addressed to fully harness their potential for managing plant diseases under varying environmental stressors.

Acknowledgements BK is grateful to the Indian Council of Agriculture for the North-Eastern Hill Region, Meghalaya, and to Assam University, Silchar.

Declarations

Conflict of 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.

Research involving human and animal participants This article does not contain any studies involving human participants or animals performed by any of the authors.

Informed consent Not applicable.

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