CHAPTER 17

Applications of Engineered Synthetic Ecosystems

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INTRODUCTION

A major goal in synthetic biology is to construct biological systems with robust and controllable behavior and functionality.[1] Over recent decades, biologists have tried to deconvolve the complexity of life by elucidating key genetic and regulatory determinants, with the hope of eventually engineering biological systems in more predictable ways. Genetic circuits have now been designed and rewired with relative ease to produce interesting and useful cellular phenotypes.[2] A natural extension of this bioengineering framework is the combination of different cells into groups and artificial consortia of increasing complexity.[3] This approach is important, since heterogeneous populations can often outperform homogeneous populations of genetically identical individuals in many tasks that require more sophisticated divisions of labor.[4,5] Natural microbial consortia, for example, are able to degrade complex substrates more efficiently than any single member can.[6] Furthermore, mixed populations are more robust to environmental variations,[7] and can potentially be reprogrammed in modular ways. However, building higher-order biological systems relies on improving our understanding of the ecology of dynamic multicomponent communities, both natural and synthetic. The area of synthetic ecology is poised to grow in this endeavor.

Just like the age-old tradition of brewing using yeast, we have a history of successes utilizing microbial consortium, albeit crudely, for applications such as composting and waste treatment.[8,9] Coupling biodegradation of complex feedstocks to bioproduction of useful products is also achievable.[10,11] However, attempts to optimize these processes rely on treating natural communities naïvely as black-box operations, because we do not understand much of the underlying design principles and constraints needed to engineer microbial communities. Furthermore, individual cells have their own growth objectives, subject to Darwinian selection, that often do not align with human-designed synthetic objectives such as overproduction of metabolically expensive compounds.[12]

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Methods to engineer microbial communities have thus far relied on natural selection of desirable traits, which is very limiting.[13] Engineering at this next level will require new advances in synthetic biology. Integration of coupled metabolism, directed cell–cell communication, and programmable community structure for real-world applications will require tools in in silico design, large-scale genome engineering, and high-throughput DNA synthesis, among other cutting edge techniques. Here, we highlight important recent developments that have advanced the field of synthetic ecosystems, and outline crucial areas for future innovations.

TARGETING MICROBIAL COMMUNITIES FOR FORWARD ENGINEERING

Genomics has significantly advanced our knowledge of microbial communities, enabling us to have the potential to engineer and control microbes at the genetic level. DNA and RNA sequencing have allowed us to determine genomic diversity and transcriptomic profiles of microbial populations in areas such as bioremediation or bioproduction.[14,15] In silico models of cellular metabolism can be used to assess flow of metabolites through individual cells, and are now being scaled across communities of cells.[16–20] New methods in recombineering,[21] oligo-directed genomic modifications,[22–25] and gene synthesis[26] have revolutionized how we perturb, understand, and improve the interactions between cellular components through strain engineering. Applying these tools to mixed consortia will bring further elucidation of population-level phenomena, such as interspecies metabolic exchange, community stability, and adaptive evolution.[7,27,28] Emerging advances in synthetic ecology require us to control three important features outlined below (Fig. 17.1).

First, engineering ecosystems requires precise understanding and control of metabolism and metabolic exchange. Engineering metabolism has thus far mainly focused on the biosynthetic capabilities of individual cells.[29,30] A key challenge is the elucidation of metabolism at the population level,[31] and the development of techniques to optimally combine different metabolic pathways together in useful ways.[32] One avenue of pursuit may be to physiologically link cells together through metabolic exchange using different metabolite transport systems. By mining metagenomic libraries for transporters,[33] combined with cytosolic exchange strategies,[34] interactions across a metabolic network of cells can be exploited.[35,36] Engineering these metabolic interactions provides a means to control the degree of antagonistic or beneficial relationships between population members. Modifying metabolic exchange will improve our understanding of how metabolism can be partitioned across a heterogeneous mixture of cells, and its effects on the dynamics, functionality, and efficiency of the system.

Second, engineering ecosystems requires directed cell–cell communication. Quorum sensing (QS) molecules, such as acyl-homoserine lactones, enable microbes to communicate with one another by diffusion through the extracellular space.[37] Intercellular signaling may also occur via direct cell-to-cell contact through membrane-bound protein complexes such as the Notch-Delta system[38] and various bacterial intercytosolic transfer systems.[39] Both types of signal exact transcriptional responses that affect various cooperative processes.[40,41] While synthetic gene circuits have been constructed to exploit QS modules,[3,42,43] more sophisticated circuits need to be developed to produce complex population behaviors.

Third, engineering ecosystems requires formation of defined spatial structure. Natural biofilms are a source of inspiration for spatially organizing microbial populations as an engineering objective.[44] These structures can define intercellular interactions and improve robustness of the consortia to environmental insults such as antibiotics and predation.[45]

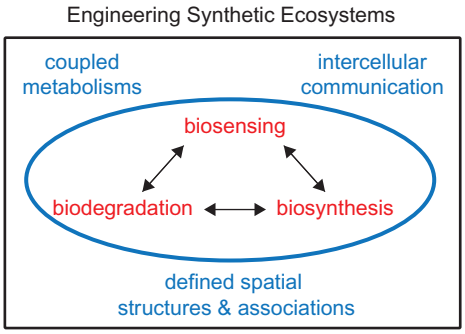

FIGURE 17.1

Engineering ecology requires the synthesis of metabolism, communication, and spatial architecture to generate synthetic ecosystems that can productively sense, degrade, or produce a myriad of biomolecules of industrial, medical or commercial value.

Spatial organization importantly determines the scale of interactions that can occur between given cells as a result of local proximity. Locality has a critical influence over metabolic exchange and signaling in synthetic communities.[46–48] New microfluidic and microdroplet devices have recently been developed to reproduce interactions between spatially defined communities,[47,49,50] formation of structured biofilms,[51] and cell–cell aggregations,[52] and will continue to play an important experimental role.

TOWARDS SYNTHETIC COMMUNITY ENGINEERING

Advances in synthetic biology are beginning to be applied to multispecies systems with direct real-world applications. While most examples of synthetic ecosystems involve only two or three distinct strains, they represent substantial improvement in our capability to engineer complex microbial interactions.[28] We outline different applications of synthetic microbial communities to highlight their potential in improving areas of biosensing, biosynthesis, and biodegradation, where the capabilities of homogeneous populations of genetically identical cells are insufficient (Fig. 17.2).

Biosensing

Abilities to sense diverse environmental signals and actuate appropriate responses are necessary and key features of engineered microbial communities. For example, autodetection of changes among networks of gut microbes in the intestine would allow for real-time monitoring and pinpointed responses to alarming events such as infections or toxins. Such capabilities would present a marked improvement over current monitoring strategies, where symptoms are only recognized once an infection has fully developed and treatment requires indiscriminant depletion of the native community using antibiotics. These population-level behaviors are only now being demonstrated using synthetic communication circuits with quorum sensing modules. Nonpathogenic *Escherichia coli* has been engineered to recognize specific QS molecules diffusing from virulent strains of *Vibrio*[53] or *Pseudomonas*.[54] Upon detection, pathogen-specific antimicrobial proteins or compounds are released, resulting in 99% reduction in the pathogen load.[54]

Synthetic consortia can also be designed to detect and respond to other compounds to regulate programmed behaviors. Consortia growth rate and relative abundances of different members can be tuned in response to the environment.[36,55] These approaches can be used to engineer biofilms to alter its physical architecture and membership composition, to optimize bioprocesses that rely on spatially associated communities.[56] Engineered communities can also be used not only to microscopically sense low-level metabolites, but also to amplify the signal for macroscopic detection. Building on an oscillatory fluorescence-generating

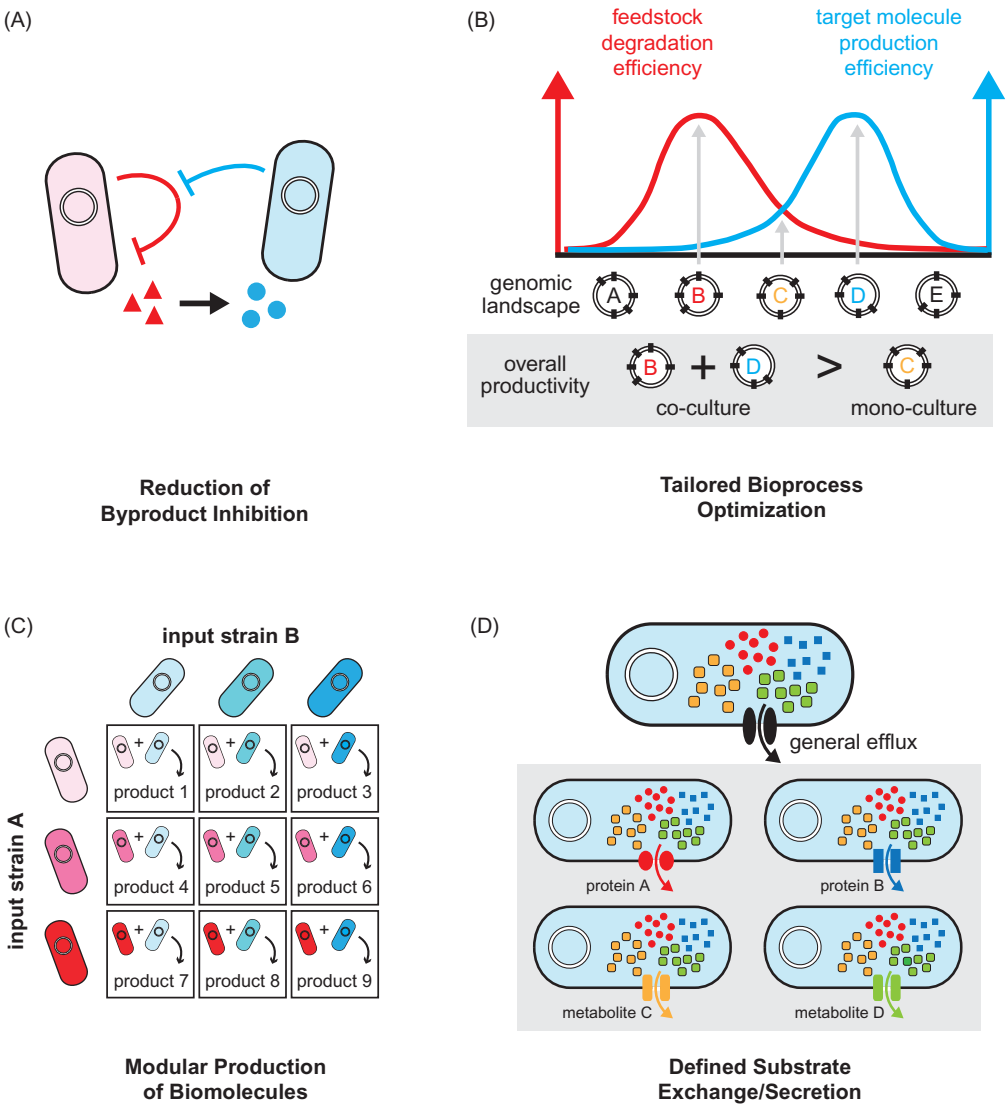

FIGURE 17.2

Engineering improvements for synthetic consortia. (A) A synthetic consortium can be designed to reduce byproduct inhibition that accompanies overaccumulation of a toxic intermediate. (B) A coculture bioprocessing strategy can be used where multiple steps are separately optimized in different cells to maximize overall productivity. (C) Modular assembly of synthetic consortia using common intermediate metabolites enables reprogrammable bioprospecting. (D) Production and secretion of multiple metabolites may saturate general cellular machinery. Specific metabolite export across different cell-types may increase productivity.

circuit, Prindle et al. synchronized local and global sensing mechanisms to generate periodically synchronized signals that changed in response to arsenic concentration, thereby generating a macroscopic biosensor using populations of cells.[57] Further demonstrations of synthetic consortia for biosensing applications are needed.

Biodegradation

Microbial communities naturally degrade various compounds into nutrients to sustain metabolism. Synthetic communities are increasingly being used to degrade xenobiotic and recalcitrant compounds. Similarly to the process of synthesis, degradation can be improved through careful engineering of organisms with desired functionalities that may be modular and complementary in physiology, resulting in overall improvement in performance of the

community. The sequestration of undesirable compounds or pollutants can be augmented by reducing inhibition of cell growth that results from accumulation of inhibitory intermediates. For example, Li et al. demonstrated that an engineered *E. coli* and *Ochrobactrum* consortia can enhance the degradation of methyl parathion (MP), an insecticide and toxin, through removal of the growth-inhibitory intermediate p-nitrophenol (PNP), resulting in 98% MP removal.[58] Degradation profiles can further be improved using engineered microbes that supplement a consortium with limiting metabolites, such as biotin, thiamine, cobalamine, and siderophores that help facilitate growth and bioconversion. Examples of these communities of cyanobacteria or microalgae with bacteria have been documented to greatly improve degradation of hydrocarbons in oil spills.[59]

Microbial communities play a significant role in digestion and metabolism of foods in the mammalian gut, and its dysfunction may lead to diseases of maldigestion.[60] Perturbation of synthetic communities of gut microbes in gnotobiotic mice using altered diets demonstrated that digestive capabilities may be a viable avenue of forward engineering through synthetic biology.[61] For example, gut communities that additionally carry methanotrophic Archaea can lead to an overall increase in microbial metabolism though removal of inhibitory levels of hydrogen that are otherwise generated. This has the direct effect of increased degradation of nutrient into absorbable nutrition, leading to elevated nutritional uptake by the host which when in excess can cause obesity.[62] Engineering and altering the degradation capacity of gut-associated microbial communities will likely be an important avenue to develop for synthetic microbial ecosystems.

Biosynthesis

Synthesis of new compounds, or existing ones using safer and better approaches, is critically needed – a task well-suited for engineered consortia. More efficient use of otherwise waste feedstocks as input materials into fermentation bioreactors is a highly desirable objective. Many of these materials, such as cellulosic biomass, are complex feedstocks that are not well-suited for current bioproduction pipelines. Use of engineered communities[63] presents a better solution than current monoculture production approaches, as excretion of different cellulases from different strains can improve degradation of complex cellulose polymers into smaller monomers. Additionally, cells that optimally excrete these cellulases may not be well-suited for bioproduction due to inherent metabolic costs. Shin et al. demonstrated the advantage of using a synthetic consortium with a divided labor structure for ethanol production from hemicellulosic feedstock.[64] Two *E. coli* strains were cocultured; one genetically optimized for cellulase production and excretion, and the other for utilization of the digested substrate for conversion to ethanol. Ethanol production reached 70% of theoretical yield in the coculture compared to 26–28% with single strains. The use of orthologous secretion systems can further improve specificity of secretion, and improve efficiency of secretion by limiting saturation through dividing different processes across multiple strains.[65]

Medical applications of engineered microbes include in situ biosynthesis and excretion of therapeutic compounds such as cytokines and immunomodulating proteins at the site of injury.[66] Introduction of nonpathogenic engineered *Lactococcus lactis* that can produce interleukin 10 in the mouse gut ameliorated autoinflammatory diseases such as colitis, Crohn's, and inflammatory bowel disease.[67] Improving the engineering of complex microbial ecosystems to stably maintain desired strains in challenging environments such as the human gastrointestinal tract will increase the longevity and effectiveness of these therapies.

Microbial consortia with modular architecture may enable more programmable reconfiguration of biosynthesis objectives and optimization conditions. Degradation strains and production strains can be combined modularly using shared common intermediate

metabolites to generate useful products such as biofuels or biomaterials. Metabolic interactions, inhibitory or beneficial, across the microbial networks must be carefully engineered.[6,27,68,69] For example, byproduct inhibition occurs when growth or productivity of a strain is impaired by the compound it produces.[70] For example, *Actinotalea fermentan* can efficiently process cellobiose feedstocks (switchgrass, corn stover, bagasse, etc.) into acetate, but its growth rate is significantly impaired by even moderate levels of acetate. Bayer et al. demonstrate that acetate byproduct inhibition of *A. fermentan* can be removed by addition of an engineered *Saccharomyces cerevisiae* strain which utilizes acetate for growth.[71] The yeast is then engineered to produce methyl halides, which is a useful biofuel precursor. A 12 000-fold improvement was achieved using this approach compared to levels from single-culture bioreactors.

Synthetic consortia additionally enable membrane-bound enzyme complexes, such as those for engineering H_2 production in *E. coli*, to be maximally utilized.[72] Integration of strain into an engineered consortium through metabolic cross-feeding is a more modular approach that allows optimization of partitioned functions such as protein engineering of membrane-bound complexes. Similarly, membrane-associated extracellular mini-cellulosomes that spatially colocalize can improve reaction rate and efficiency to improve performance of synthetic consortia.[73] A four-member cellulosome-generating yeast consortia was recently demonstrated for ethanol production, reaching 87% of theoretical yield − a three-fold increase over a monoculture strain that expressed all four enzymes.[74] Thus, utilizing synthetic consortia for modular and programmable biosynthesis of useful compounds remains very promising.

FUTURE PROSPECTS FOR SYNTHETIC ECOSYSTEMS

Here we have discussed many of the interesting applications that have recently emerged from the area of synthetic microbial ecosystems. In most cases, these represent the low hanging fruits of this area − systems involving only a handful of strains with relatively simple interactions. However, these advances can be further extended using new tools in synthetic biology, mathematical modeling, and molecular biotechnology. Future developments in this area have the potential to transform fields of medicine, bioproduction, bioprocessing, and environmental engineering. Precise manipulation and multiplexed control of community composition, capabilities, and dynamics will generate a suite of reconfigurable cellular modules that can meet the myriad of health and environmental challenges that we face in the near future. Additional insights that reveal how these engineered systems respond to evolutionary pressures in natural environments will guide their proper use in socially responsible ways.

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