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Current Opinion in Biotechnology

Partners for life: building microbial consortia for the future

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New technologies have allowed researchers to better design, build, and analyze complex consortia. These developments are fueling a wider implementation of consortium-based bioprocessing by leveraging synthetic biology, delivering on the field's multitudinous promises of higher efficiencies, superior resiliency, augmented capabilities, and modular bioprocessing. Here we chronicle current progress by presenting a range of screening, computational, and biomolecular tools enabling robust population control, efficient division of labor, and programmatic spatial organization; furthermore, we detail corresponding advancements in areas including machine learning, biocontainment, and standardization. Additionally, we show applications in myriad sectors, including medicine, energy and waste sustainability, chemical production, agriculture, and biosensors. Concluding remarks outline areas of growth that will promote the utilization of complex community structures across the biotechnology spectrum.

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Current Opinion in Biotechnology 2020, **66**:292–300

This review comes from a themed issue on **Tissue, cell and pathway engineering**

Edited by **Li Tang**, **Peng Xu** and **Haoran Zhang**

For a complete overview see the Issue and the Editorial

Available online 14th November 2020

https://doi.org/10.1016/j.copbio.2020.10.001

Introduction

Symbiotic intercellular relationships are ubiquitous. Such cooperative ecosystems exist in diverse arenas, including plant soil, mammalian guts, and hydrothermal vents [1–3]. Inspired by nature, synthetic biologists have been leveraging the capabilities of cellular communities for over a decade [4–9]. Already, consortia have been exploited in common processes such as anaerobic digestion and food processing, but a deep understanding of their complex interplay and how to improve upon it for wider application is still in its infancy.

Rationally building synthetic multicellular communities represents a major hurdle in biotechnology. These systems are inherently more complex and multifaceted than their monoculture counterparts, yet this provides opportunities to extend our collective biotechnological reach. Communities offer the potential for increased efficiency [10,11], expanded capability [12•,13], greater resilience [14,15], and modular functionality [16,17]. As a result, the field has seen an explosion of interest, and it stands on the cusp of widespread fluency in system design. A number of tools and approaches have been developed to aid in the synthetic design and application of these consortia [18–25], but they need to be further developed to provide tractability, predictability, and reliability.

This review provides a general overview of recent developments in this burgeoning field by presenting novel technological advances in rational consortia design and analysis, followed by a summary of the many contemporary applications.

Engineering tools

Analytical methods

Relative abundance data are the *de facto* reporting measure in cellular communities, but new methodologies have been proposed that could improve system insight and understanding. One instance of this is through the use of reference frames: by establishing a standard comparison microbe in the culture, data analysis yields more consistent results with deeper insight into abundance dynamics [26•]. Moreover, a less expensive, faster, and easier diagnostic RNA toehold switch sensor can detect and quantify specified biomarkers in microbiota [27].

Novel application of ^{13}C isotope labeling has allowed for specific determination of metabolic flux and inter-species metabolite exchange in microbial consortia [28], which can provide key insights into understanding species interaction and suggest specific engineering targets to optimize flux into desired products or to reduce wasteful pathways. Furthermore, knowing population ratios of species in consortia is key to normalizing metabolite abundance and protein or enzyme levels. Levels of consortium members in populations of unicellular organisms can be quantified using techniques such as fluorescence-activated cell sorting (FACS) flow cytometry [29]. However, for filamentous organisms or other microbes too similar to separate via flow cytometry, individual populations are often determined by qPCR quantification of well-characterized genes unique to each microbial partner

[30] or by quantification of a consistently expressed unique protein or metabolite.

Computational analysis

Significant progress has also been made with respect to *in silico* consortium analysis. One emerging area has been in the application of machine learning: biological datasets are now becoming large enough for this to be a viable approach, as demonstrated by the analysis of anaerobic digestion microbiome flow cytometry data [31] and the prediction of unobserved metabolites from paired metabolome–metagenome data [32•]. Additionally, software such as QIIME 2 [33] has been developed and updated to facilitate microbiome analysis, with plugin support for taxonomy classification using machine learning [34].

The computational design of consortia has similarly seen recent improvements. Constraint-based community metabolic models have proven effective in elucidating mechanisms underlying phototrophic–heterotrophic co-cultures [35] and could be applied to better control the population dynamics of multicellular biological systems. Mixed-integer linear programming has also predicted trade-offs between up to three *Escherichia coli* strains in a consortium [36]. Furthermore, flux balance analysis has been done on genome-scale metabolic models simulating co-cultures of 773 human gut microbes to screen for the overproduction of a compound of interest [12•], demonstrating the potential for consortium analysis to extend beyond current feasible experimental capabilities. Dynamic flux balance analysis techniques can similarly help screen consortia, even with non-model organisms, by coupling it with minimal experimental data about the strain and iteratively simulating through time [37]. A new version of the COBRA Toolbox, which is commonly leveraged to solve this family of optimization problems, has been released and includes new functionalities, including the ability to integrate metabolomic, proteomic, transcriptomic, chemoinformatic, and thermochemical data [38].

Combinatorial testing of artificial consortia can quickly exceed our computational capabilities. This forces us to rethink this approach for larger systems containing more strains, perhaps by limiting higher-order analyses — in other words, analyses with more strains in symbiosis — to systems containing the best-performing consortia with fewer members (Figure 1). Moreover, by analyzing the number of artificial consortia that would need to be tested, we can approximate how current computational limits restrict exhaustively simulating a custom consortium of given size from a pool of microbial candidates. Adopting a similar approach as Perisin and Sund [12•], we estimate that there are about 10^5 two-member combinations of the 773 characterized human gut microbes [39], but about 10^8 three-member and 10^{22} ten-member combinations (Table 1). The COBRA Toolbox can determine a solution in the range of one second to two minutes [38]. Thus, at a minimum, one could expect combinatorially testing all two-member pairings to take approximately 3.5 days, but combinatorially testing all three-member groupings to take 888 days; a four-member system would be well outside the range of feasibility, taking approximately 468 years. Supercomputers could possibly reduce these times, but they would likely still struggle to test beyond 10-member system combinations, as the fastest supercomputer at the time of writing is capable of about

Figure 1

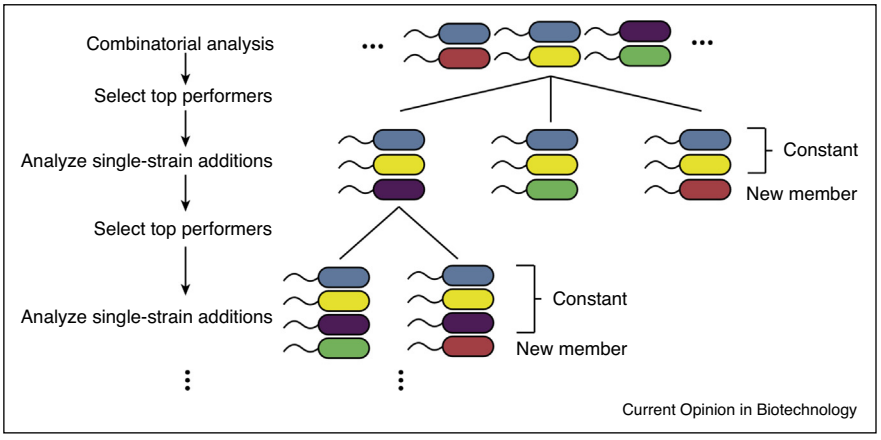


Diagram illustrating a reduced testing strategy. First, an initial combinatorial analysis would establish a set of co-cultures and assess a desired characteristic, such as growth rate or metabolite production. Either a percentage or fixed number of the top performers, as determined by the previous assessment, would then be further analyzed by testing all single-strain additions. This process would be continued until the desired number of strains are present.

Table 1

Estimates of the number of artificial consortia that would have to be tested for various computational reduction approaches. Previous work has observed that three-member syntrophic systems with the greatest growth tend to have highly cooperative two-member subsets [77], suggesting that one possibility for reducing computational burden could be by limiting analyses of consortia containing *n* members to the top-performing consortia of $n-1$ members, whereby top-performing consortia are determined by their growth rate, metabolite production, or another property of interest. This table shows the effects of selecting a relative or absolute number of top-performing candidates. Using 773 genome-scale models of human gut microbes as a basis [39], we assume the scenario in which all subsequent consortium constructions are unique. This assumption means that the true number of consortia to test will likely be less than the number presented here, as top-performing groupings likely share many common members.

	Number of tests			
Members in artificial consortia (*n* of 773)	Combinations[a]	Selecting top 1%[b]	Selecting top 0.25%[c]	Selecting top 100[d]
2	10^5	10^5	10^5	10^5
3	10^8	10^6	10^6	10^6
10	10^{22}	10^{13}	10^9	10^6
100	10^{128}	10^{95}	10^{49}	10^7

[a] $C_n = \binom{773}{n} = \frac{773!}{n!\,(773-n)!}$.

[b] $C_{n\geq 3} = C_{n-1} + 0.01 * C_{n-1} * [773-(n-1)]$, $C_2 = \binom{773}{2}$.

[c] $C_{n\geq 3} = C_{n-1} + 0.0025 * C_{n-1} * [773-(n-1)]$, $C_2 = \binom{773}{2}$.

[d] $C_{n\geq 3} = C_{n-1} + 100 * [773-(n-1)]$, $C_2 = \binom{773}{2}$.

10^{17} flops [40]. In contrast, only analyzing consortia groupings with the most potential could dramatically reduce testing requirements (Figure 2).

Biomolecular design

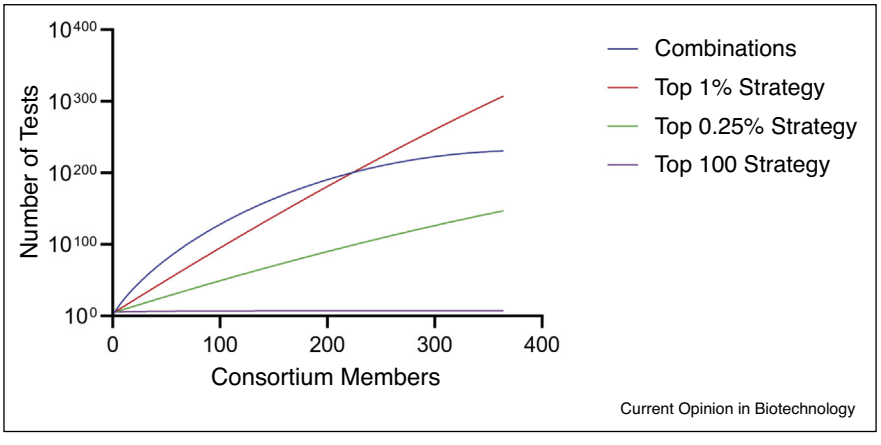
Three objectives often prevail when engineering consortia: controlling population ratios, splitting pathways across multiple organisms, and grouping cells in a programmed manner. Hence, several approaches have been developed in order to implement these functions in practice (Figure 3).

Population control

Cell-based

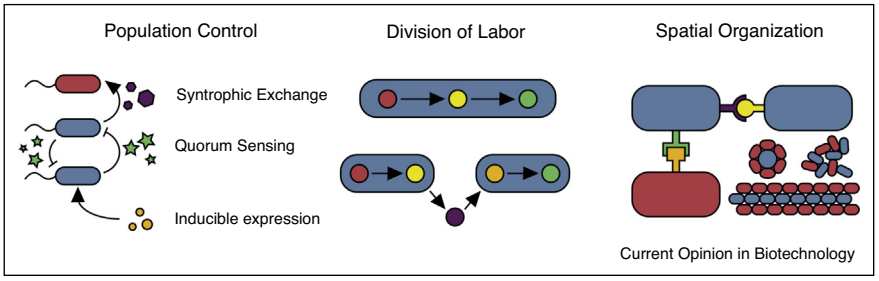
The maturation of consortia-building knowledge has led to a deluge of novel molecular tools to increase control of these communities. Quorum sensing is often the system of choice for engineering cell–cell communication, but it

Figure 2



Elaboration of the data in Table 1. The graph illustrates the number of artificial consortia that would need to be computationally analyzed using different reduction approaches. By assuming a fixed number of top candidates, the growth rate of the number of tests required is low. Additionally, the limits of our simplified independence assumption can be seen with the strategy selecting the top 1% of consortia: the number of groupings to test exceeds the total number of combinations. Unfortunately, because we do not know *a priori* how many strains will be commonly shared between top-performing consortia, it is impossible to calculate the actual number of tests required using these reductive approaches.

Figure 3



Overview of biomolecular techniques used to construct consortia. In order to control population levels, quorum sensing or syntrophic exchange systems have typically been used [11,41,42••,43]; recent work has enabled inducible quorum sensing systems [46••]. Division of labor is often an important part of a co-culture, with new research describing when this arrangement is advantageous and specific frameworks for how to divide a pathway [52•]. Modern work has further enabled controlled patterning and morphology, leveraging nanobodies to build predetermined structures [54••,55].

can suffer from crosstalk when using multiple sensing systems. Importantly, this approach has been shown to improve product yield, as demonstrated by a 60% increase in naringenin titer for an *E. coli* co-culture production system [11]. To increase the number of partners in a synthetic consortium, six acyl-homoserine lactone quorum-sensing communication channels have been analyzed for orthogonality and shown to effectively regulate gene expression in three strains [41].

Other work has shown that the six two-member modes of interaction (commensalism, amensalism, neutralism, cooperation, competition, and predation) can be built into consortia; the two-member models can subsequently be used to predict three-member and four-member population dynamics [42••]. Syntrophic exchange is another tool that has been adapted to build intercellular networks, often by making one microbe dependent on another for an essential compound. By cross-feeding amino acids, synthetic bacterial communities have exhibited enhanced evenness in environments as complex as the murine gut [43], offering another means of building stable communities.

Environment-based

While cell–cell signaling offers one approach for *de novo* consortium control, population ratios can also be responsive to environmental conditions. Fortunately, relatively simple control mechanisms using external mediators have been developed. A consortium of three *Saccharomyces cerevisiae* strains specializing in the fermentation of either glucose, xylose, or arabinose were shown to have more stable fermentation kinetics and were more able to respond to fluctuations in feedstock sugar concentration than a single generalist strain [15]. Population dynamics can also be achieved using external signal factors; for example, *Lactococcus lactis* strains were engineered to have a predator–prey relationship that would fluctuate in the presence of extrinsically mediated chloramphenicol concentrations [44]. Moreover, a pH-dependent promoter has been successfully used in *L. lactis* to modulate microbial community behavior [45], and an orthogonal and inducible quorum sensing system in *E. coli* has allowed for tighter peripheral control over community dynamics [46••].

Biocontainment

Community-scale design has allowed scientists to think about the creation of broader and longer-term solutions to challenges that would require the release of engineered organisms into the wild, as would be the case with human or plant microbiome engineering [47]. One common challenge when building engineered consortia is maintaining genotypic integrity, as new functionality often comes at the cost of fitness. Sequentially adding strains that both kill the previous strain and maintain the synthetic circuit of interest can help prevent a loss of this engineered functionality [48], resetting the culture's biological mutation clock. Sequestration is often another challenge, which has prompted discussion about gene drive development with regulated control on separate chromosomes that can limit spread across generations [49].

Consortia could uniquely address biosafety issues in a number of ways. By linking the survival of cell strains to one another by means of quorum sensing and syntrophic exchange, microbial communities can be necessarily confined to regions with its partners. These approaches can be coupled with single-strain strategies, such as environmentally responsive kill switches [50], to provide robust redundancies. Furthermore, novel toxin–intein antimicrobials can target individual strains in microbiota based on the presence of a unique transcriptional regulator [51••], allowing engineers to restore an augmented and unconfined consortium to its natural dynamic.

Division of labor

Engineers often hope to marry ease of use with the performance of complex tasks, but monocultures have an upper threshold on their ability to deliver when it comes to more challenging bioprocesses, frequently sacrificing simplicity for functionality. Consortia could address this discrepancy by either splitting a single pathway or a parallel process across multiple convenient organisms. Accordingly, more robust division-of-labor systems have been recently described that allow for improved synthetic consortia designs. For instance, helpful criteria have now been established based on 24 common metabolic pathway types — such as a two-step extracellular conversions and three-step intracellular conversions — describing when division of labor would benefit the system [52•]. Indeed, division of labor can outperform monocultures in processes with many steps, resource-intensive enzymes, toxic compounds, or extracellular pathway steps [52•]. Job specialization in consortia can reduce bioprocessing for complex molecules, as demonstrated by the single-culture production of pure translation machinery using up to 34 *E. coli* strains [53]. Multicellular circuits have also been demonstrated: in a proof-of-principle study, a cell-signaling control scheme was used to regulate sugar concentration, in which a specified cell type functioned as either the controller's sensor, modulator, or effector [13].

Spatial organization

Observing nature's intricate multicellular architecture has led to a desire to engineer custom cell-based structures and the subsequent development of programmed morphology. To this end, a facile nanobody-antigen system has been created in *E. coli*. This genetically encoded system can design myriad multicellular patterns, including phase separation, differential adhesion, and sequential layering [54••]. Similar work showing self-organization and programmed structure assembly was completed with murine fibroblasts [55]. Other work has demonstrated encoded control via AND-logic, in which *E. coli* cells expressed yellow or cyan fluorescent protein when one of two signaling lactones dominated, but red fluorescent protein when both were present [56]. Additional work has allowed for the physical separation of genetically distinct cell types based on motility by programming plasmid segregation into only one daughter cell during division [57].

Standardization

To accelerate the adoption and development of consortia technology, standardized systems will be required. As of now, the scientific community largely lacks model microbiomes for different use cases, hindering researchers' ability to gain a detailed and comprehensive understanding of a single consortium [58]. Additionally, metabolic network reconstructions have the potential to rapidly screen consortia, but the manual curation required to obtain high-quality models limits scalability. As a result, efforts such as AGORA [39] and CarveMe [59] have attempted to produce a multitude of accurate genome-scale models that can facilitate automated consortia testing. Biomolecular tools can often be freely utilized across different organisms, but low numbers of effective signaling mechanisms have hindered the development of designer consortia: to date, only three quorum sensing systems have been shown to be orthogonal [41]. In order to fully realize the capabilities of consortia, the research community must come together to address and overcome these standardization challenges to expand the generalizability of consortia across fields.

Applications

Medicine

The potential health impact of gut microbiota has prompted a flurry of research on potential interventions for various medical conditions. Oral administration of *L. lactis*, for example, was shown to reduce *Vibrio cholerae* and increase cholera survival rates of infant mice; *L. lactis* was further engineered to produce a reporter enzyme easily seen in fecal samples that could detect *V. cholerae* signals [60]. Similarly, *E. coli* was engineered to metabolize phenylalanine under anoxic conditions, and its use was illustrated in mouse and primate models as a potential therapeutic for phenylketonuria [61]. Although some work has explored engineered interspecies consortia in germ-free mice [43], microbiome interventions tend to be single-strain additions, whereas the addition of communities could more strongly regulate the desired response and enable more complex interventions. In contrast, co-cultures have been utilized for pharmaceutical and nutraceutical production, as illustrated by the production of sakuranetin [16], rosmarinic acid [17], and apigetrin [62].

Energy and waste sustainability

Modern concerns surrounding availability of non-renewable energy supplies and global climate conditions have triggered a greater demand for renewable energy sources. Algal bicultures, one example of a biological solution, can have a higher energy return on investment and lower greenhouse gas emissions compared to monocultures, based on life cycle assessment [63]. By extending the products of photosynthesis to heterotrophs, co-cultures between cyanobacteria and fungus have been used to generate greater biomass and altered lipid profiles compared to axenic cultures [64]. Microbial fuel cells are also an area of interest in which co-cultures generate electricity by using lactate produced from glucose and xylose as an electron donor [65].

Utilization of non-traditional feedstocks represents another topic of interest for co-cultures due to the impact on sustainability. Lignocellulosic biomass in particular could be a useful carbon source to upcycle, and *Bacillus megaterium* co-cultures secreting an endoglucanase and cellulase have been shown to degrade cellulose, providing a possible alternative to current high-cost pretreatment

and purification methods [66]. Analogously, work with fungus co-cultures of *Trichoderma reesei* and *Rhizopus delemar* has demonstrated how consolidated bioprocessing can convert cellulose and lignocellulosic biomass into fumaric and lactic acid [67]. Co-cultures have also been applied to bioremediation: co-culturing cyanobacteria *Synechococcus elongatus* with an engineered heterotrophic bacteria *Pseudomonas putida* resulted in degradation of 2,4-dinitrotoluene [68].

Chemicals

There is now an increased interest in bioprocessing because of its potential for green chemical manufacturing, a trend often characterized by ambient reaction conditions, the use of fewer harmful substances, and development of safer product alternatives [69]. Here too consortia can play a key role in producing platform chemicals. For example, inefficiencies in glutarate production from L-lysine were alleviated by splitting the pathway across two different *E. coli* strains [10]. Indeed, *E. coli* co-cultures have demonstrated versatility, able to produce compounds such as phenol [70•], pyranoanthocyanins [71], and naringenin [11]. In parallel, computational work on genome-scale metabolic models has also predicted microbial co-cultures that could provide a sound platform for converting food waste to commodity chemicals [12•].

Agriculture

Soil microbiota are important for a plant's health and have the capacity to be engineered to address agricultural concerns. Future food security has become a common concern, and plant microbiome engineering presents a promising approach to enhance current farming practices [72]. Current programs involve adding nitrogen fixation capabilities to bacteria naturally associated with a plant's soil microbiome [73]. Also, synthetic communication systems between plants and rhizosphere bacteria form the basis for laterally transferring foreign bacteria to a plant's soil microbiome while simultaneously providing a biocontainment mechanism [74].

Biosensors

Applying biologics to detect molecules has the potential to complement or even replace alternative analytical methods. Cell-based biosensing systems can offer portability, training, and flexibility advantages compared to traditional sensing systems [75]. Biosensors have yet to be broadly commercialized but continue to be slowly adopted, and consortia-based applications are gaining traction. For example, a co-culture system can detect organophosphorus pesticides with sensitivity on par with electrochemical sensors [76]. Additionally, by linking metabolite biosensors with growth-regulating genes, *E. coli* co-cultures have been shown to self-select for cells to increase phenol production [70•].

Conclusions

Consortial systems constitute a rapidly emerging field of biotechnology due to the advantages offered in terms of job specialization, adaptability to environmental changes, and expanded bioprocessing capabilities. Indeed, these natural and synthetic consortial systems are likely to become important players in the medical, agricultural, and green industries. Well-designed systems that take advantage of new tools to predict community behavior, program cell–cell interaction, and elucidate the rules governing microbial partnerships will be able to remove the bottlenecks currently limiting consortia. To be sure, these technologies still require significant improvements before they can transition into full-fledged industrial applications; proof-of-concept studies do not yet meet productivity or environmental benchmarks.

While there has been much progress, the field still has gaps that will dramatically accelerate technology implementation when resolved. The lack of robust orthogonal systems has stymied our ability to create predictable communities consisting of several strains. Additionally, a high-throughput means to effectively obtain detailed and accurate participant levels and bioinformatic data for consortia is lacking. As a result, computational tools to create and predict community dynamics have also lagged, only able to adopt contemporary techniques like machine learning in narrow instances. Increased standardization in various areas — such as the organization of microbiome data, architecture of genome-scale models, and use of more model consortia systems — would help unite a fractured informational landscape. Moreover, applications that involve engineering consortia outside of controlled environments will require the development of multiple redundant and robust biocontainment strategies — a formidable obstacle for the field.

Nonetheless, recent advances serve as harbingers of the field's future: the successful design of artificial communities that are robust, efficient, and flexible, just like the natural ones after which they are modeled. In such a similar manner, researchers in our field must continue to communicate and collaborate in order to ensure that engineered microbial communities become an integral part of the biotechnology and biomanufacturing landscape in what promises to be a consortium-based biotechnological revolution.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors of this article have been supported, in part, by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomic Science Program under Award Number DE-SC0019388 and by the U.S. National Science Foundation grant number 1804733.

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