

Chapter 4

Conclusions

Portions of this chapter are published as [1].

4.1 Challenges in engineering microbial consortia

There are several examples of synthetic microbial consortia in the literature [43, 44, 46], and in this thesis we have presented two engineered synthetic consortia that grow and function in biofilms [42]. However, there are significant challenges associated with engineering microbial consortia, and these will require attention as engineers consider their potential applications. Although many of the challenges are shared with those faced when engineering single microbial populations, some are particular to controlling the behavior of multiple, interacting populations. First, natural microbial communities can maintain homeostasis; members generally do not out-compete one another and do not exhaust the resources in their environments [151, 152]. However, it is difficult to design either long-term homeostasis or long-term extinction into a synthetic consortium, because long-term behavior, and even the long-term genetic composition of an engineered organism, is unpredictable. Thus, engineered consortia should be designed for contexts

in which members of the consortium can be re-introduced or eliminated as needed, and in which their behavior can be monitored over time. A second challenge is that, at least in nature, gene transfer between microbes is common [109]. As a result, engineered consortia should function despite horizontal gene transfer, or even exploit it. A third challenge will be to develop methods for incorporating stable changes into the genomes of microbes that are not currently commonly engineered. Horizontal gene transfer is limited when engineers make stable changes to the chromosome. In addition, organisms currently recalcitrant to genetic modification methods often perform very useful functions that are difficult to engineer into other organisms. For example, species of *Clostridia* (e.g., *Clostridium thermocellum*, for which there are no established genetic cloning protocols, and *Clostridium acetobutylicum*, the protocols for which are difficult and proprietary) live in consortia with other microbes and naturally secrete powerful cellulases [18]. A fourth major challenge inherent in engineering consortia is fine-tuning the performance of multiple populations. Techniques such as directed evolution that can optimize the behavior of a single population must be extended for application to multiple populations and varying environments. High-throughput screening methods and inexpensive gene-chip assay procedures will be extremely useful for the efficient construction and evaluation of synthetic consortia.

4.2 Synthetic consortia in healthcare

Microbial consortia can carry out more complex functions, and they might be more robust to changes in their environments than are individual populations. These two traits make microbial consortia attractive as platforms for a variety of technologies, if the

engineering challenges such as those listed above can be met. The field of medical technology stands to benefit greatly from the ability to engineer communities of microbes. Engineers have developed bacteria that serve as drug-delivery devices [153-155] and gene-delivery vehicles [154, 156, 157], but these technologies suffer a lack of precision in targeting and release. The greater complexity of function available, coupled with longevity and stability through environmental change, might make consortia a better starting-point for microbial drug-delivery and gene-delivery technologies. For example, a healthcare technology requiring the delivery of two therapeutic components in succession with a defined time-offset could potentially employ an oscillatory system (e.g., the predator-prey ecosystem of Balagadde et al. [43]) as a platform. Such an application would require much greater understanding of both the dynamics of mixed populations and how to control them in a robust fashion. Researchers have also introduced genetically engineered commensal bacteria into mammals as sentry cells. Such efforts have successfully prevented colonization by problematic organisms at epithelial barriers in the reproductive and digestive tracts of the mammals [158, 159]. A consortium of engineered commensal microbes might colonize and provide additional functionality, including detection warnings [160] or protection against multiple infectious agents, over longer periods of time. Similar strategies might also be considered to detect and prevent pathogenic colonization of wounds and the lungs.

4.3 Summary

Because members of microbial consortia communicate and differentiate, consortia can perform more complex tasks and can survive in more changeable environments than can

uniform populations. Simple engineered consortia might be described through mathematical models more easily than natural systems are, and they can be used to develop and validate models of more complex systems [161]. Furthermore, their behavior can be controlled by externally introduced signals (e.g., circuits can be induced by small molecules such as IPTG). To date, engineers have successfully constructed microbial consortia by implementing cell–cell communication and differentiation of function in traditional, laboratory microbes. To fully exploit the potential of engineered consortia, we must learn to stably engineer organisms that are currently recalcitrant to genetic manipulation. Furthermore, when engineering new technologies, we should prioritize safety by beginning with innocuous or commensal organisms. As a result of engineered communication and differentiation of function, engineered consortia do exhibit complex functions that can be difficult to engineer into single populations. If they are to be used in future technologies, engineered consortia will need to be tested and optimized for their ability to persist and withstand environmental fluctuations. In addition to “pushing the envelope” of synthetic biology, with promising health, environmental, and industrial applications, engineered microbial consortia are potentially powerful and versatile tools for studying microbial interactions and evolution.