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Synthetic microbial consortia: from systematic analysis to construction and applications

Hao Song, Ming-Zhu Ding, Xiao-Qiang Jia, Qian Ma and Ying-Jin Yuan*

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Synthetic biology is an emerging research field that focuses on using rational engineering strategies to program biological systems, conferring on them new functions and behaviours. By developing genetic parts and devices based on transcriptional, translational, post-translational modules, many genetic circuits and metabolic pathways had been programmed in single cells. Extending engineering capabilities from single-cell behaviours to multicellular microbial consortia represents a new frontier of synthetic biology. Herein, we first reviewed binary interaction modes of microorganisms in microbial consortia and their underlying molecular mechanisms, which lay the foundation of programming cell–cell interactions in synthetic microbial consortia. Systems biology studies on cellular systems enable systematic understanding of diverse physiological processes of cells and their interactions, which in turn offer insights into the optimal design of synthetic consortia. Based on such fundamental understanding, a comprehensive array of synthetic microbial consortia constructed in the last decade were reviewed, including isogenic microbial communities programmed by quorum sensing-based cell–cell communications, sender–receiver microbial communities with one-way communications, and microbial ecosystems wired by two-way (bi-directional) communications. Furthermore, many applications including using synthetic microbial consortia for distributed bio-computations, chemicals and bioenergy production, medicine and human health, and environments were reviewed. Synergistic development of systems and synthetic biology will provide both a thorough understanding of naturally occurring microbial consortia and rational engineering of these complicated consortia for novel applications.

Key Laboratory of Systems Bioengineering, Ministry of Education, School of Chemical Engineering and Technology, and Collaborative Innovation Centre of Chemical Science and Engineering (Tianjin), Tianjin University, Tianjin 300072, P. R. China. E-mail: yjyuan@tju.edu.cn; Fax: +86-22-27403888

1. Introduction

Synthetic biology is a nascent research field that employs engineering principles to program novel biological systems and their behaviours,[1–3] enabling a wide variety of applications

Hao Song

Hao Song received his PhD in Chemical Engineering from the University of Houston. After working as a postdoctoral research fellow at the University of Texas Health Science Center and a research associate at Duke University, he joined Nanyang Technological University at Singapore as an assistant professor. He is currently a professor in biochemical engineering in Tianjin University. His research interest includes synthetic biology, microbial electrosynthesis, and bionanotechnology. He has published over 50 peer-reviewed papers and reviews.

Ming-Zhu Ding

Ming-Zhu Ding received her PhD from Tianjin University under the direction of Professor Ying-Jin Yuan in 2011. Her research has focused on systems biology and synthetic biology. Dr Ding has been a fellow of Tianjin University since 2011. She is involved in Key Laboratory of Systems Bioengineering, Ministry of Education. She has published 15 papers in peer-reviewed international journals and 1 invited book chapter.

in chemical industries, energy, biomedicine, and environments.[4–10] A fundamental difference of synthetic biology from traditional molecular biology and genetic engineering is that synthetic biology focuses more on the design and construction of essential biological entities (such as genetic parts, devices, circuits, and biological pathways and networks) in a rational manner under the guidance of engineering principles, such that these biological entities and their assemblies can be mathematically modelled, quantitatively understood, standardized, controlled, optimized, and thus tuned to meet specific design purposes and performance criteria.[2] The initial development of synthetic biology witnessed the construction of a diverse spectrum of standard genetic parts and devices,[11,12] synthetic gene circuits,[1] and cell signalling and signal transduction networks.[13] A large number of genetic circuits (*e.g.*, Boolean logic gates, bistable switches, and oscillators, *etc.*) were developed in single cells in the last decade based on transcriptional control elements (*e.g.*, promoters), translational control elements (*e.g.*, RNAi, riboregulators, ribosomal binding sites (RBS), *etc.*), and post-translational control (*e.g.*, phosphorylation cascades, protein degradation, *etc.*).[2,4,14–17] Meanwhile, the adoption of synthetic biology strategies into rational engineering and optimization of metabolic pathways enabled enhanced production of many chemicals and drugs, which significantly advanced chemical and pharmaceutical industries.[5,7,18,19]

Extending from programming single cells to the cell–cell interactions of multicellular systems, the synthesis of microbial consortia opens a new horizon in synthetic biology in terms of system complexity and functionality.[20–26] Microorganisms rarely live in isolated niches, but usually live together with other microbial species to form microbial communities in natural environments and biospheres. More than 99% of microorganisms in environments cannot be successfully cultured by traditional cultivation technologies, one reason is that the maintenance of viability of these microbes may need supplementary metabolites or other signalling chemicals provided by other microbes in the ecosystems and communities.[27–29]

Engineering cell–cell interactions and communications is at the central point of engineering synthetic communities. We first review the interaction modes between microorganisms, and the molecular mechanisms underlying these interaction patterns, which provide the basis for the design of synthetic microbial consortia. Based on the interaction nature between engineered microorganisms (such as communications within isogenetic populations, one-way and two-way interactions between populations), we review microbial consortia synthesized in the last decade, which include many interaction modes such as commensalism, mutualism, competition, and predation. These synthetic consortia have well-defined genetic traits and allow direct manipulation of intrinsic parameters of the systems, thus making them ideal model systems to address fundamental ecological and evolutionary questions. Subsequently, diverse applications of synthetic microbial consortia are reviewed in

Xiao-Qiang Jia

Xiao-Qiang Jia is an associate professor from the Department of Biological Engineering, School of Chemical Engineering and Technology, Tianjin University. He received his PhD from the Tianjin University in 2008. His research expertise is in the multiphase bioreactor process model, environmental biotechnology and synthetic biology. He has published more than 50 peer reviewed international papers.

Qian Ma

Qian Ma is currently a PhD candidate in the Department of pharmaceutical engineering, Tianjin University. She has worked on the systems biology study of the microbial consortium of Ketogulonicigenium vulgare and Bacillus megaterium for vitamin C production in Prof. Ying-Jin Yuan's lab for 4 years. Her research interests include metabolomics, proteomics and fermentation. She has published 7 international peer-reviewed papers.

Ying-Jin Yuan

Ying-Jin Yuan is currently a professor of Biochemical Engineering in Tianjin University. He received his PhD from Tianjin University, China, in 1991. He is now the Director of Key Laboratory of Systems Bioengineering, Ministry of Education, China. His research interests include synthetic biology, systems biotechnology, bioenergy, biopharmaceutical engineering and plant cell culture. He has focused on the systematical characterization of a microbial consortium composed of Ketogulonicigenium vulgare and Bacillus megaterium by systematic analysis, and designing, simulating and constructing a synthetic ecosystem by using two quorum-sensing signal transduction circuits. He is the Fellow of IChemE. He has published more than 160 international peer-reviewed papers.

detail, including distributed bio-computations, production of chemicals, nutraceuticals and bioenergy, medicine and human health, and environments.

2. Analysis and design of microbial consortia

Based on the interaction modes between microorganisms, we discuss various underlying molecular mechanisms in forming microbial consortia, including cell–cell communications, and exchange of metabolites and energy, *etc.* We further discuss how systems biology can offer insights into the design and synthesis of microbial consortia.

2.1. Binary interaction modes between microorganisms

The interactions between microorganisms are ubiquitous and play a central role in determining the fate and evolutionary dynamics of individual organisms in microbial consortia, as well as system properties such as stability and dynamics of the entire communities.[30–33] Also, delineation of interaction modes between microorganisms in natural niches could facilitate engineering novel microbial consortia and their traits.

The interactions within the microbial ecological communities may have a null (0, or neutral), positive (+, beneficial or win), or negative (−, or detrimental or loss) impact on the partner microorganisms involved. Thus, the binary interaction outcomes could be classified into six different categories of pairwise interaction modes[32] (Table 1), *i.e.*, neutralism (0/0), commensalism (+/0), amensalism (−/0), mutualism (+/+), competition (−/−), and parasitism or predation (+/−). Dissecting such binary interactions among the community members is of great help in elucidating the properties and dynamics of the community as a whole system.

At one end of the spectrum of interaction modes is neutralism (0/0) with null interactions in the communities, in which the presence of one microorganism has basically no effect on the viability of another. For example, when two microorganisms that have different requirements of carbon sources or nutrients are mixed together and the viability of one species is not basically influenced by the other, they form a neutralistic ecosystem. Rigorous neutralism rarely occurs because it is hard to prove there is absolutely no interaction between microbial species, at least they compete for spaces, if not nutrients, in many scenarios. Thus, neutralism is often used to describe situations where interactions are insignificant or largely negligible.

Table 1 Binary interaction modes in microbial ecosystems

Binary interaction modes	Nature of interaction
Mutualism	+/+
Commensalism	+/0
Parasitism or predation	+/−
Competition	−/−
Amensalism	0/−
Neutralism	0/0

0 (null: neutral); + (positive: beneficial or win); − (negative: detrimental or loss).

Commensalism (+/0) is a relationship in which one partner derives benefits from the other, while the other partner is not affected (neither harmful nor beneficial) by the association. In contrast to commensalism, amensalistic interaction (−/0) refers to the association in which one partner is harmed by the other, but having no effect on the first. For example, *lactobacilli* could release H^+ ions as a metabolic by-product to lower the pH of its surrounding environment, thus leading to a detriment to other microorganisms.

The loss–loss relationship (−/−) between the community members is competition, which is one type of antagonistic interaction. Nutrients are usually the competition targets between microorganisms. In the resident microflora of human guts, probiotic bacteria (including *Bifidobacterium* and *Lactobacillus* spp.) are able to benefit host health in part through competition with pathogenic bacteria for nutrients and space, thus presenting a barrier to the infection by these pathogens.[34] Also, microorganisms may produce compounds as a means of chemical warfare to antagonize other microorganisms (*e.g.*, by inhibiting the growth or being detrimental to potential competitors). Other types of antagonistic interactions are the predator–prey (*i.e.*, predation, +/−) and the host–parasite (parasitism, +/−) relationships. Predation is crucial in forming the population structure and composition of many food webs.

Finally, the win–win relationship of symbiotic association is mutualism (+/+), in which both partner microorganisms derive benefits from one another.

2.2. Molecular mechanisms of interactions in microbial consortia

The partner members in microbial communities employ a diverse set of molecular interaction mechanisms to synchronize their behaviours, achieving collective behaviours of the entire communities. A detailed understanding of such molecular mechanisms not only provides insights into how the spread of interaction signals and information coordinate community members to achieve adaptive multicellular behaviours, but also facilitates engineering and constructing new traits of artificial consortia.

Microorganisms usually interact with each other (either within their own species, or across interspecies) *via* two main mechanisms, *i.e.*, the contact-based interaction by which the interchanges of biomolecules (*e.g.*, proteins, nucleic acids, *etc.*) and electrons between microorganisms occur *via* physical cell–cell contact, and the contact-independent interaction by which the exchanges of metabolites and information signals between microorganisms are carried out by diffusible chemicals and physical contact is not required.[35]

2.2.1. Contact-independent interactions between microorganisms. One of the most widely recognized contact-independent interactions among microorganisms is mediated by QS signalling molecules, by which microbial cells respond to a chemical input or stimulus only until a critical threshold of population density is reached. Thus, microorganisms use QS mechanisms to sense the existence of neighbouring species, gauge their cell densities, modulate their gene expressions, and

coordinate their group behaviours to elicit social traits of a microbial population accordingly.[36,37] The QS mechanism influences and controls many physiological behaviours of microorganisms, including virulence, competence, symbiotic interactions, motility, and biofilm formation.[38–41] The structure of diffusible QS signalling molecules is diverse, ranging from *N*-acyl homoserine lactones (*e.g.*, AHLs in *Vibrio fischeri* and *Pseudomonas aerugenosa*)[42–44] and quinolones (*e.g.*, PQS systems in *P. aerugenosa*),[42,45] furanosyl borate diester (autoinducer-2 (AI-2) systems, *e.g.*, in *Streptococcus oralis*),[44] to oligopeptides (*e.g.*, sporulation and competence regulation in some Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*).[46,47] Quenching QS signaling by inhibition or degradation of these QS molecules is a promising strategy in combating pathogenic microorganisms and interfering gene expressions involved in the microbial interactions.[48,49] The QS systems, in particular the AHL-based cell–cell communication systems, have been widely adopted in the construction and engineering of many synthetic gene circuits and microbial ecosystems, including population density control circuits,[50,51] synchronization of population-level oscillations,[52] synthetic consensus in microbial consortia,[53] and predator–prey ecosystems,[54] *etc.*

In addition to the QS signaling molecules, microorganisms also make use of a large repertoire of structurally different metabolites to establish cell–cell interactions and microbial ecosystems, including small molecules (such as hydrogen, methane, formate, lactate, sulfide, carbon dioxide, and indole, *etc.*) and large molecules (peptides and proteins such as hydrolases, protease, *etc.*).[26,35,55] For example, in methanogenesis (*i.e.*, anaerobic digestion of organics to methane), the production of methane by the microbial catabolism of organic substrates is dependent upon the synthrophic association between bacteria (*e.g.*, sulfate-reducing bacteria such as *Desulfovibrio vulgaris*) and archaea (*e.g.*, methanogens such as *Methanococcus maripaludis*).[56–59] In this ecosystem, electron exchanges occur between bacteria acting as electron-donating species that oxidize the organic substrates and release electron shuttles (*i.e.*, electron carrier molecules, such as hydrogen, formate, flavins and phenazines, *etc.*), and methanogens acting as electron-accepting species that accept the electrons to reduce carbon dioxide to methane. In the pure culture, *D. vulgaris* anaerobically catabolizes organic substrates and uses sulfate as an electron sink when it is available, while *M. maripaludis* grows anaerobically to assimilate and reduce carbon dioxide by consuming hydrogen as an electron donor, and neither of them can grow in the absence of sulfate or hydrogen, respectively. In the co-culture of the two syntrophic microorganisms, *D. vulgaris* acts as the ''fermenter'' to break down organic carbon sources and release metabolites such as hydrogen and formate as electron carriers, which are subsequently scavenged by *M. maripaludis* as an electron donor. Thus, such interspecies metabolites' exchange enables cooperative exchange of electrons during syntrophic metabolism, maintaining the viability of the co-culture.[57]

2.2.2. Contact-dependent interactions between microorganisms. In addition to the above mentioned diffusible electron shuttle-mediated electron transfer between microorganisms, recent studies showed that direct contact-based interspecies electron transfer also played crucial roles in methanogenesis.[60–64] In a defined co-culture of *Geobacter metallireducens* and *Methanosaeta harundinacea* that can convert ethanol to methane, in addition to acetate exchange, the two microorganisms are able to exchange electrons *via* direct interspecies electron transfer.[65] In this co-culture aggregate, *G. metallireducens* oxidizes ethanol and directly transfers electrons to *M. harundinacea via* its conductive pili, *M. harundinacea* in turn accepts electrons and reduces carbon dioxide to methane. Furthermore, conductive materials like granular activated carbon and magnetite can also facilitate direct-contact interspecies electron transfer between bacteria and methanogens, in which these highly conductive materials can serve as electrical conduits to promote electron flow in the syntrophic interactions and anaerobic digestion.[64,66–68]

Direct contact-based interactions between microbial cells can also be used to deliver macromolecules from the donor to the recipient cells.[69,70] For example, the bacterial type IV secretion systems (T4SS) use the pili-based system to mediate the DNA and/or proteins transfer from donor to recipient cells by forming a conjugation between these cells upon cell–cell contact, although this secretion channel and structure assembly were not completely elucidated.[71] The bacterial type VI secretion systems (T6SS) utilize a bacteriophage-like apparatus and cell-puncturing organelle to target prey cells for antagonistic inhibition *via* translocation of effector proteins.[72–75] For example, *Pseudomonas aeruginosa* is able to outcompete *P. putida* in their mixed cultures by translocation of effector proteins *via* T6SS.[76] It was shown recently that bacteria may also use intercellular nanotubes (tubular extensions) that bridge neighbouring cells to mediate cell–cell communication.[77] The nanotubes can serve as a route for exchanging cytoplasmic molecules (including plasmids, proteins and small molecules), conferring the recipient cells non-heritable or heritable traits. Such exchange of intracellular molecules *via* nanotubes occurs within and between microorganisms, for example, *Bacillus subtilis* can form nanotubes with *B. subtilis*, *S. aureus*, or *Escherichia coli.*[77]

2.3. Systems biology offers insights into the rational design of synthetic microbial ecosystems

To design and program target microbial consortia, a detailed understanding of molecular mechanisms underlying cell–cell interactions (including cell–cell communications, and exchange of metabolites and electrons between cells) and multicellular physiologies are prerequisites.[24] In a sense, systems biology approaches enable the systematic characterization of genetic and metabolic pathways in microbial consortia, which provide useful insights into understanding the comprehensive molecular mechanisms of interactions in microbial consortia. Such systematic understanding can provide clues on how to construct functional genetic parts and devices, as well as the consortia with defined traits and multicellular behaviors.[78] Thus, systems biology is not only helpful for the understanding, but also for the design and optimization of synthetic consortia (Fig. 1).

2.3.1. Elucidating molecular mechanisms of interactions in microbial consortia by systems biology. Cell–cell interactions

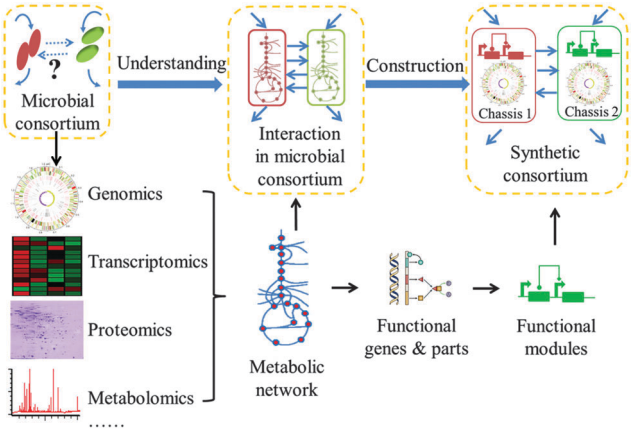

Fig. 1 Strategies for systematic analysis and rational design of synthetic microbial consortia. The molecular mechanism of interactions between microorganisms in microbial consortia could be elucidated using systems biology approaches (*e.g.*, genomics, transcriptomics, proteomics, metabolomics, *etc.*), which could provide insights into the design and construction of functional genes, parts, modules and the entire synthetic consortia.

play central roles in determining population behaviours of microbial consortia. Many interaction modes in microbial consortia (*e.g.*, commensalism, mutualism, competition, neutralism, amensalism, parasitism or predation) are mediated by the exchange of metabolites or QS signalling molecules. Systems analyses[79,80] (*e.g.*, genomics,[81] transcriptomics,[82] proteomics[83–86] and metabolomics[87–90]) were used for analysing various biological systems, including natural and artificial microbial communities[91] (Fig. 1). In particular, meta-omics techniques (such as meta-genomics,[81,92–95] meta-proteomics,[83,96–100] meta-transcriptomics[101,102] and meta-metabolomics[103,104]) were intensively used for characterizing the composition structure, genetic and metabolic pathways involved in the interactions of microbial consortia found in natural niches, such as marine, soil, and gut, *etc.*

For example, Woyke *et al.* used the meta-genomic method to systematically characterize the gutless marine worm *Olavius algarvensis* and its four chemosynthetic symbionts in its body wall.[81] This meta-genomic analysis revealed that the symbionts contained four bacteria, *i.e.*, two sulfur oxidizers (γ-proteobacterial symbionts: γ1- and γ3-symbionts) and two sulfate reducers (δ-proteobacterial symbionts: δ1- and δ4-symbionts), which provided multiple sources of nutrition to the host. The interaction between the symbionts and the host was reconstructed and a model for energy metabolism in the symbiosis was proposed, which provided molecular evidence of the uptake and recycling of worm waste products by the symbionts.

The human gut microbiome has a profound impact on human health.[105] Meta-genomics was used to identify characteristic biomarkers of the gut microflora associated with obesity and inflammatory bowel diseases (IBD).[95] The meta-genomic data of gut microbiome from different host states were integrated with a system-level network analysis to view the interactions among the genes present in the microbiome. Topological analysis was used to examine the enzymes in community-level metabolic networks and to study its metagenome-scale metabolism. Both gene- and network-level topological differences associated with obesity and IBD were identified. This study suggested that lean and obese microbiomes had profound differences in their interaction patterns with the host metabolism.

2.3.2. Systems biology provides guidance in engineering synthetic consortia. Systematic understanding of molecular interaction mechanisms revealed by systems biology would provide novel insights into the design, construction, and optimization of synthetic consortia. Cell–cell interactions by exchanging metabolites and signalling molecules could unify and synergize population behaviours to form microbial consortia. Systems biology analyses of the cell–cell and cell–environment interactions provide detailed molecular mechanisms in organizing microbial consortia. Such information helps us identify the targets for the design of functional gene parts, devices, pathways and networks, which lay the foundation for optimizing microbial consortia (Fig. 1).

Taking the vitamin C fermentation process as an example, a systematic analysis of the molecular mechanism of the interactions between co-cultured *Bacillus megaterium* and *Ketogulonicigenium vulgare* guided the optimization of this consortium, which in turn increased the yield of vitamin C. Several systems biology approaches including genomics,[106,107] proteomics,[108] metabolomics[103,104,109] and trans-omics[110] were used to elucidate the metabolites' exchange between *K. vulgare* and *B. megaterium*. Du *et al.* used metabolomic profiling to elucidate the metabolite interaction dynamics in this consortium,[103] and found that *B. megaterium* enhanced the growth and proliferation of *K. vulgare* by promoting its TCA cycle and nucleotide and amino acid metabolisms, thus the biosynthesis rate of 2-keto-gulonic acid (2-KLG), the precursor of vitamin C. In addition, *K. vulgare* could either promote the growth of *B. megaterium* by providing amino acids, or inhibit the growth of *B. megaterium* by the biosynthesis of 2-KLG. Furthermore, Ma *et al.* used integrated proteomic and metabolomic analyses to study this consortium during co-fermentation,[110] and approximately 100 metabolites and 258 proteins were identified and quantified. They found that cell lysis of *B. megaterium* supplies purine substrates (adenine, guanine, xanthine and hypoxanthine) needed for the growth and 2-KLG production by *K. vulgare*, and the purine biosynthesis pathway in *K. vulgare* was also accelerated. In addition, *B. megaterium* was found to help *K. vulgare* to resist ROS, to enhance its energy production and to promote its growth and metabolism during sporulation.

These systems analysis results provided useful insights into the molecular interaction mechanisms underlying the vitamin C production in this microbial consortium, which guided further rational engineering the consortium to optimize the vitamin C production. Furthermore, the fully annotated genome sequences of *B. megaterium*[106,111] and *K. vulgare*[107,112] provided a clear genetic background to achieve targeted regulation of gene expression in these two microorganisms and their genome-scale metabolic network modelling.[106] Based on such systematic understanding, Du *et al.*[113] further reconstructed this consortium by a combinatorial expression of sorbose/sorbosone dehydrogenase genes (*sdh*/*sndh*) and the synthesis genes (*pqq*ABCDEN) of the cofactor pyrroloquinoline quinone

(PQQ) in *K. vulgare*, enabling a 20% increase in the production of 2-KLG.

3. Applications of synthetic microbial consortia

Following a description of interaction modes between microbial components and their underlying molecular mechanisms in microbial consortia, we describe in this section a wide variety of synthetic microbial consortia that have been constructed in the last decade, and the applications of synthetic microbial consortia in diverse fields, including addressing fundamental biological questions, bio-computations, production of chemicals and bioenergy, as well as applications in medicine and environments.

3.1. Synthetic microbial consortia

3.1.1. Engineering cell–cell communications in isogenic microbial communities. Microorganisms can communicate with their own species *via* quorum sensing (QS) mechanisms, which enables the population-level control of gene expression and coordinates the coherent behaviours of the entire isogenic population. These engineered isogenic microbial communities lay the foundation for engineering cell–cell interactions to program microbial consortia composed of multiple microorganisms. A few synthetic genetic circuits were programmed to engineer coherent population behaviours. A first microbial population density control circuit was constructed by You *et al.* by programming a gene circuit, in which the regulation of programmed killing was under the control of bacterial cell–cell communication (upper panel, Fig. 2A).[50] The LuxR/LuxI QS system originating from marine bacterium *Vibrio fischeri* was cloned in *Escherichia coli* to achieve cell–cell communication. The LuxI synthase enzymatically produces the *N*-(3-oxohexanoyl)-homoserine lactone (3OC6HSL) signalling molecule (an acyl-homoserine lactone, AHL), a small molecule that can diffuse across cell membranes. 3OC6HSL accumulates in the culture medium with the growth and increased cell density of *E. coli*. At a sufficiently high level of 3OC6HSL accumulated in the medium, it binds to the protein LuxR, and the LuxR–3OC6HSL complex activates the transcriptional expression of the killer protein LacZα-ccdB, which regulates the cell death and consequently controls the population density (middle panel, Fig. 2A). The circuit is under the control of a synthetic P_{lac} promoter, inducible by isopropyl-β-D-thiogalactopyranoside (IPTG). This population control circuit is robust and resistant to noise in gene expression and intercellular phenotypical variability owing to the adoption of the cell–cell communication mechanism. This circuit was further characterized in a microchemostat chip, exhibiting sustained oscillatory dynamics in cell density over hundreds of hours (lower panel, Fig. 2A).[51] Also, such population-level oscillations are more persistent and stable than those oscillators programmed in individual cells.[51,114,115]

As an extension of the population-control circuit, Smith *et al.* programmed an Allee effect circuit in *E. coli*.[116] The strong Allee effect refers to the scenario that a population has a negative fitness level below a critical threshold of species

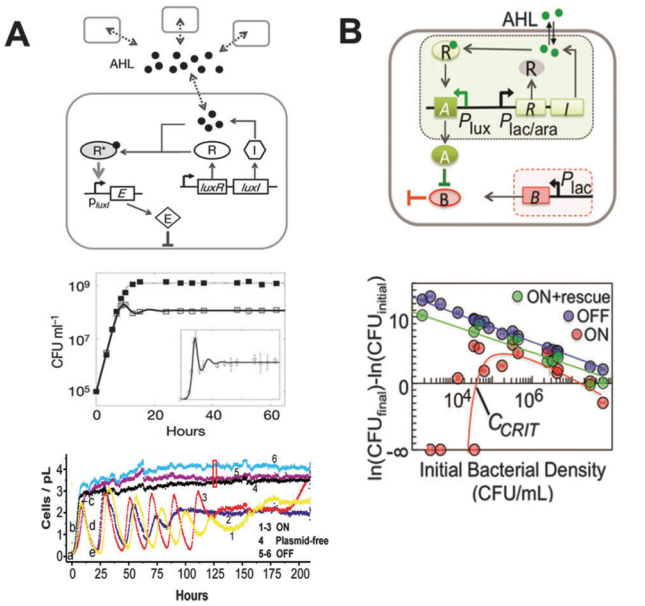

Fig. 2 Engineering cell–cell communication (*i.e.*, quorum sensing modules here) in isogenic microbial communities. (A) Schematic of a population control circuit programmed in *E. coli* (upper panel). The LuxR-LuxI QS module is used to control the expression of a killer gene *E*. The growth curves of the *E. coli* cells harboring the population control circuit (middle panel). Oscillatory dynamics of the engineered *E. coli* population characterized by microchemostat (lower panel). (B) The genetic circuit that gives rise to a strong Allee effect in engineered *E. coli* (upper panel). The LuxR-LuxI QS module induces the expression of an antidote CcdA (indicated by A) that neutralizes the toxicity of a killer protein CcdB (indicated by B). Experiments showed the strong Allee effect (lower panel). Reproduced with permission from ref. 50, 51 and 116. Copyright 2004 Nature Publishing Group, 2005 The American Association for the Advancement of Science, and 2014 The National Academy of Sciences of the USA, respectively.

density (C_{crit}).[117] To achieve the strong Allee effect, the LuxR/LuxI QS system and the CcdA/CcdB toxin–antitoxin module were programmed in *E. coli*, such that the proteins of LuxR/LuxI and CcdB were under the control of the $P_{lac/ara}$ promoter, and the protein CcdA (antidote of killer protein CcdB) was under the control of the P_{lux} promoter in response to the QS module (upper panel, Fig. 2B). When the circuit is ON (IPTG induction), the specific growth rate of the population is negative for an initial cell density C_0 below C_{crit}, and the specific growth rate is positive for $C_0 > C_{crit}$ (lower panel, Fig. 2B). Thus, this programmed *E. coli* population with the strong Allee effect follows a bistable growth: the population grows only when its initial density is above C_{crit}, while extinguishes when its initial density is below C_{crit}. Using this gene circuit with the strong Allee effect, Smith *et al.* further experimentally validated the biphasic dependence of population spread on dispersal (dispersal is necessary for the spread of a population into a new territory, but dispersal may inhibit spread), resolving a counter-intuitive ecological observation. This research also demonstrated the advantage of synthetic ecosystems over field studies in nature setting in addressing fundamental ecological questions, *i.e.*, synthetic ecological systems have well-defined genetic traits and interaction modes among microbes, allowing

direct mapping between mathematical modelling and experiments in a more definitive manner.

Instead of using a QS circuit to control programmed cell death, Anderson *et al.*[61] programmed an invasin gene (from *Yersinia pseudotuberculosis*) in *E. coli* by placing it under the control of the LuxR/LuxI QS circuit such that this *E. coli* strain harbouring the invasin is able to invade cancer-derived cells (*e.g.*, HeLa cells) in a cell-density-dependent manner, *i.e.*, invasion of cancerous cells occurs as *E. coli* reaches a sufficiently high cell density.

3.1.2. Engineering pattern formation *via* cell–cell communications in isogenic microbial communities. To control spatiotemporal behaviours of microbial populations, several cell–cell communication-based genetic circuits were synthesized to form spatiotemporal patterns. Liu *et al.*[118] constructed a gene circuit to suppress the motility of *E. coli* at high cell density, which is composed of two modules, a density-sensing module and a motility-control module (left panel, Fig. 3A). The LuxR/LuxI QS module is used to drive the expression of the lambda repressor (CI), which in turn represses the transcription of *cheZ* (*cheZ* controls the motility of *E. coli* in semisolid agar). Upon inoculation of the engineered *E. coli* harbouring this gene circuit at the centre of a Petri dish containing semisolid agar, a striking periodic pattern with alternating stripes of high and low cell densities formed sequentially and autonomously, arising from a recurrent aggregation at the front of the expanding cell population dictated by the programmed gene circuit (right panel, Fig. 3A). The number of stripes can be further modulated by the introduction of an anhydrotetracycline (aTc)-inducible CI expression module.

Payne *et al.* programmed a synthetic gene circuit in *E. coli*, which can generate robust, self-organized ring patterns in the absence of morphogen (*i.e.*, AHL molecule here) gradient.[119] The circuit is structured as coupled positive and negative feedback loops, in which a mutant T7 RNA polymerase (T7 RNAP) activates its own expression *via* a T7 promoter carrying a *lac* operator (forming the positive feedback), and the LuxR/LuxI QS module induces the expression of a T7 lysozyme that in turn inhibits the T7 RNAP (forming the negative feedback) (left panel, Fig. 3B). To report the dynamics of this circuit, cyan fluorescent protein (CFP) is co-expressed with T7 RNAP, and

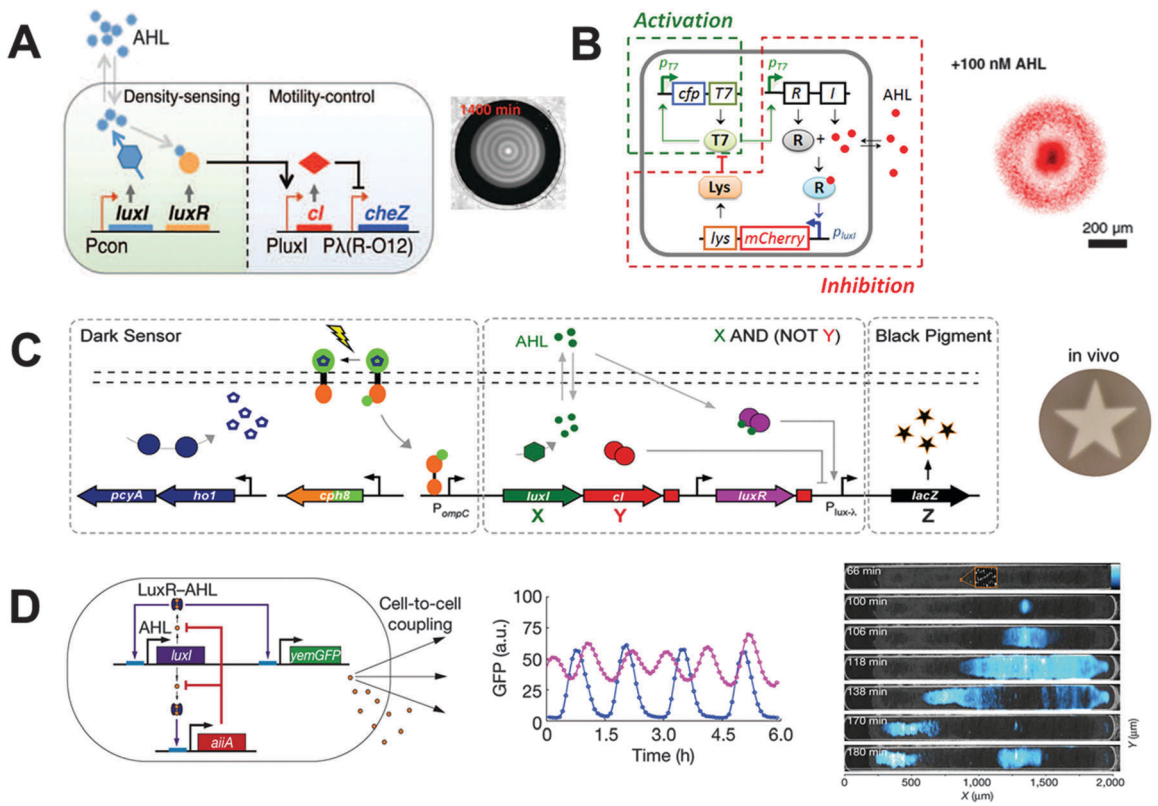

Fig. 3 Programmed pattern formation *via* cell–cell communications in isogenic microbial communities. (A) The gene circuit engineered in *E. coli*, where the LuxR-LuxI QS module induces the expression of CI that further inhibits *cheZ* expression (left panel), and the formed stripe pattern (right). (B) The gene circuit programmed in *E. coli* consists of an activator T7 RNAP that activates itself (activation), and a LuxR-LuxI QS module induced expression of T7 lysozyme (inhibition) (left panel). A self-organized mCherry bullseye pattern formed by the circuit (right panel). (C) Edge detection gene circuitry in *E. coli* (left panel). The black pigment was produced only in the engineered cells located at the boundary between light and dark regions (right panel). (D) Circuit diagram of the synchronized genetic clocks programmed in *E. coli*, where the LuxR-LuxI QS module induces the expression of the LuxI and aiiA (left panel). The period and amplitude of oscillations increase with the increased flow rate of a microfluidic device (magenta curve: 240 μm min^{-1}; blue curve: 280 μm min^{-1}; middle panel). Traveling waves of GFP fluorescence were observed in a microfluidic device (right panel). Reproduced with permission from ref. 52, 118–120. Copyright 2011 The American Association for the Advancement of Science, 2013 John Wiley and Sons, 2009 Elsevier, and 2010 Nature Publishing Group, respectively.

mCherry fluorescent protein is co-expressed with the T7 lysozyme. This circuit exhibited a self-organized pattern (a length scale of 500 μm) of gene expressions with a core of CFP protein and a ring of mCherry protein, whose formation is coupled with colony expansion in soft agar (right panel, Fig. 3B). Their agent-based modelling and experiments all showed that the AHL molecule (3OC6SHL) served as a timing cue to trigger the formation and maintain the ring patterns. These synthetic gene circuit-mediated spatiotemporal pattern formation processes provide novel insights into elucidating the fundamental mechanisms of natural developmental processes.

Tabor *et al.* synthesized a genetically encoded edge detection algorithm in *E. coli*, which can sense light, communicate within the isogenic community of *E. coli* to identify the light-dark edges, and is able to present the edge visually.[120] This gene circuit consists of a dark sensor (NOT light logic gate), QS-type cell–cell communication, and an X and (NOT Y) logic gate (left panel, Fig. 3C). Red light inhibits the kinase activity of Cph8 protein, a chimeric sensor kinase that requires the covalently associated chromophore phycocyanobilin (PCB, produced from heme by two constitutively expressed genes *ho1* and *pcyA*), thus precluding the transcription of the *ompC* promoter (P_{ompC}). Therefore the dark sensor functions as a NOT light logic gate, which elicits the expression of LuxI (the synthase for the production of the QS signal 3-oxohexanoyl-homoserine lactone (3OC6HSL)) and CI (the transcriptional repressor from bacterial phage λ) in the absence of red light. 3OC6HSL binds to the constitutively expressed LuxR and activates a two-input tandem promoter $P_{lux\text{-}\lambda}$, while CI represses $P_{lux\text{-}\lambda}$. Thus, the promoter $P_{lux\text{-}\lambda}$ functions as an X and (NOT Y) logic gate. Finally, the promoter $P_{lux\text{-}\lambda}$ drives the expression of LacZ, a β-galactosidase that cleaves a substrate in the media to produce a black pigment. Therefore, a population of cells programmed with this edge detection circuit produces black pigment only at the boundary between light and dark regions (right panel, Fig. 3C).

Synchronization of oscillators *via* intercellular coupling governs many physiological processes, and is fundamentally important in the coordination of rhythmic behaviours among individuals in a community. Danino *et al.* programmed a global intercellular coupling gene network in *E. coli* using quorum sensing and quenching, which coordinates the gene oscillations of individual cells, enabling synchronized oscillations in the population level and traveling wave dynamics in the spatiotemporal domain.[52] The gene circuit has a negative feedback loop architecture, in which *LuxI* (from *V. fischeri*) and *aiiA* (from *Bacillus thurigensis*) and *yemGFP* genes were placed under the control of three identical copies of the *luxI* promoter (left panel, Fig. 3D) such that the LuxR/LuxI QS module activates the transcriptional expression of LuxI (and thus the synthesis of the AHL signalling molecule) and AiiA. AiiA is a quorum-quenching lactonase that catalyses the degradation of AHL, thus repressing the *LuxI* promoter, and closing the negative feedback loop. This negative feedback loop architecture and the coupling between cells *via* extracellular AHL signalling molecules underlie the occurrence and synchronization of oscillations in gene expression. Microfluidic devices were used to monitor the bulk synchronization of oscillations of *yemGFP* across the multicellular population, and the period and amplitude of the oscillations were found to increase with the increase of the flow rate in chambers (middle panel, Fig. 3D). Furthermore, traveling waves were observed to emerge spontaneously in the middle of the bacterial colony and propagate outward with a speed of $\sim$8–35 μm min^{-1} (right panel, Fig. 3D).

3.1.3. Engineering one-way communications in binary microbial consortia. To achieve the coordinated behaviors in synthetic microbial communities, a number of microbial consortia involving one-way and two-way communications among their component microorganisms were synthesized. The one-way communication systems usually refer to the sender–receiver (or sender–responder) multicellular bacterial populations. Basu *et al.* programmed a pulse-generating network in the receiver *E. coli* cells in response to a signaling molecule sent from the sender *E. coli* cells (upper panels, Fig. 4A).[121] The sender cells encode the LuxI protein (an AHL synthase) under the control of the $P_{LtetO\text{-}1}$ promoter, which is activated by anhydrotetracycline (aTc). The receiver cells comprise a LuxR protein under the control of the $luxP_L$ promoter, CI (LVA) (a destabilized CI repressor originated from bacteriophage λ) under the control of the $luxP_R$ promoter, and GFP (LVA) under the control of the $luxP_RcI\text{-}O_R\text{-}1$ promoter. At a low level of AHL synthesized in the sender cells, both CI and GFP are expressed. Once CI accumulates at sufficiently high levels, CI binds the hybrid promoter luxPR, which in turn inhibits further expression of GFP. Thus, this feed-forward regulatory motif generates a transient pulse dynamics of the expression of GFP in the receiver cells in response to the growth of the sender cells (lower panel, Fig. 4A).

Basu *et al.* further synthesized another one-way communication circuit between sender and receiver cells to program pattern formation in a synthetic multicellular system (upper panel, Fig. 4B).[122] The sender cells encode LuxI under the control of aTc-inducible $P_{LTet\text{-}O1}$ promoter, then the synthesized AHL signalling molecules diffuse through cell membranes and form an AHL gradient around the sender cells. The receiver cells carry a band-detect feed-forward gene network containing two plasmids (low-detect plasmid (pLD) and high-detect plasmid (pHD)) to respond to different concentration ranges of AHL from the sender cells. The pLD harbours a destabilized λ repressor (CI) under the control of $P_{lux(R)}$, and a lac repressor (LacI) under the control of the λP(R-O12) promoter; while the pHD encodes LuxR controlled by the $P_{lux(L)}$ promoter, $LacI_{M1}$ by the $P_{lux(R)}$ promoter, and GFP by the P_{lac} promoter. Thus, at close proximity to the sender cells, the receiver cells receive high concentrations of AHL, resulting in a high expression level of CI and $LacI_{M1}$ and repressed expression of GFP. At locations far from the sender cells, the receiver cells receive a low level of AHL, resulting in inhibited expression of CI and $LacI_{M1}$ and high-level expression of wild-type LacI, thus inhibiting the expression of GFP. Only at intermediate distances from the sender cells, an intermediate-level of AHL induces moderate-level expression of CI and $LacI_{M1}$, enabling a high-level expression of GFP. By expression of different fluorescent proteins as the output of the gene network, the initially uniformly spread receiver cells on the solid media will eventually form bullseye patterns around the inoculated sender cells at the centre of a Petri dish (lower panel, Fig. 4B). In addition, other patterns

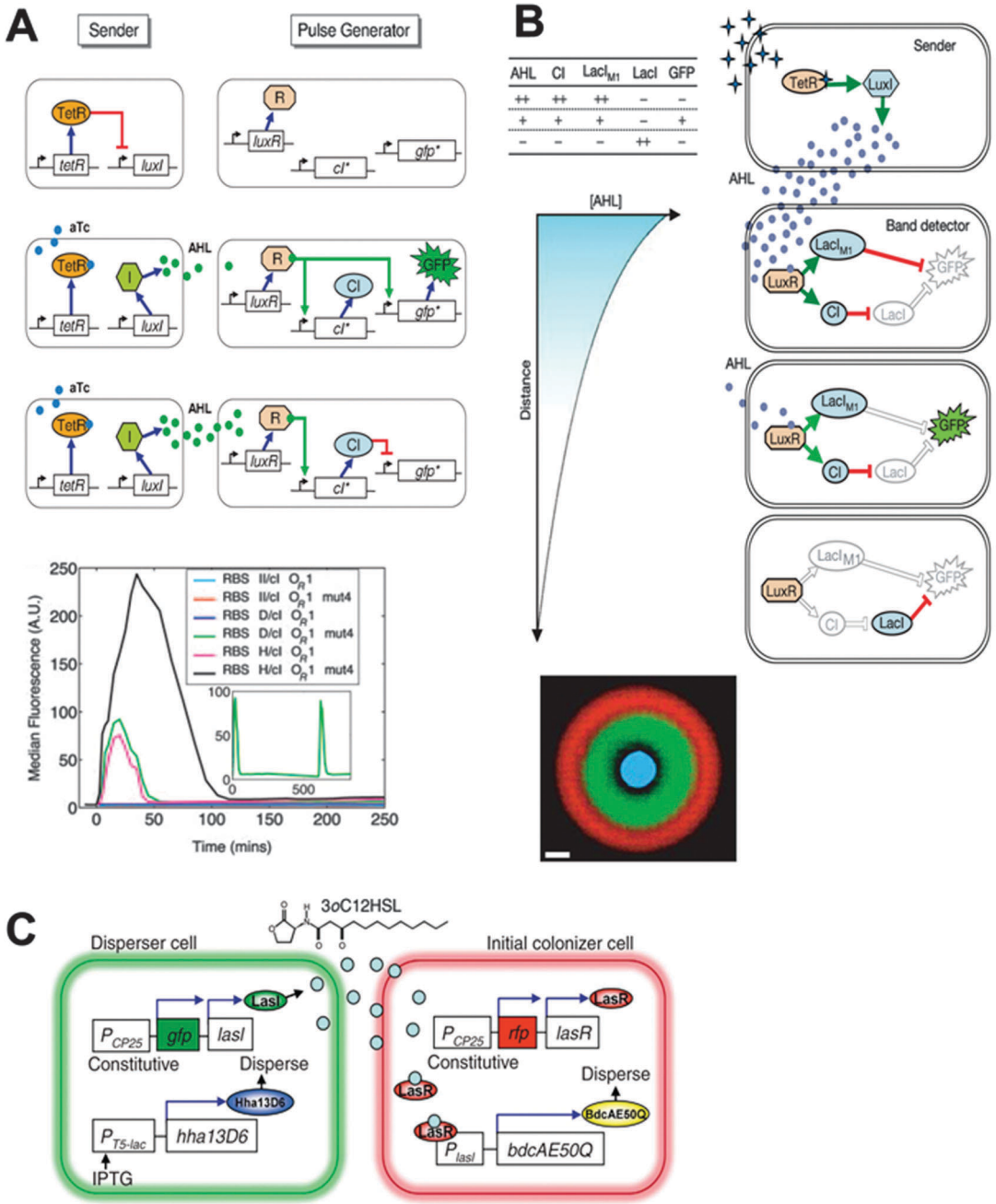

Fig. 4 Microbial consortia with one-way interaction between the sender and receiver cells. (A) Interaction logic of the programmed gene circuit in *E. coli*, where sender cells produce and excrete QS signalling molecule 3OC6HSL to induce a transient response in receiver cells (upper panels). The pulse-generating receiver cells respond to 3OC6HSL from sender cells and generate a transient expression of GFP fluorescence (lower panel). (B) Interaction logic of the band-detection multicellular consortium programmed in *E. coli*, where only the receiver cells located at intermediate distances from the receiver cells produce GFP fluorescence (upper panel). The bullseye pattern of an initially undifferentiated lawn of receiver cells formed around a sender colony (lower panel). (C) Diagram of the programmed interaction between the initial colonizer and disperser cells, by which introduction of disperser cells displaces the biofilm formed by the initial colonizer cells. Reproduced with permission from ref. 121, 122 and 125. Copyright 2004 The National Academy of Sciences of the USA, 2005 and 2012 Nature Publishing Group, respectively.

such as ellipses and clovers can be obtained, depending upon the seeding configurations of the sender and receiver cells.

Biofilm is an aggregation of cells on a surface embedded within a self-synthesized matrix of extracellular polymeric substances, including polysaccharides, proteins, DNA and lipids, *etc.*[123] The formation and dispersion of biofilms are controlled by QS signaling mechanisms.[124] Hong *et al.* used a similar concept to the sender–receiver systems to design a biofilm engineering signaling circuit in *E. coli* to achieve the controlled biofilm formation, displacement and removal.[125] This synthetic microbial consortium is composed of initial colonizer cells and disperser cells, communicated *via* the LasR/LasI QS system,

originating from *P. aeruginosa* (Fig. 4C). The disperser cells encode synthesis of the Hha13D6 protein (a biofilm-dispersing protein by activating proteases) under the control of $P_{T5\text{-}lac}$ (IPTG-inducible), and LasI (the synthase for the synthesis of auto-inducer *N*-(3-oxo-dodecanoyl)-L-homoserine lactone, 3OC12HSL) under the control of a constitutive promoter (P_{CP25}). The initial colonizer cells encode the synthesis of the BcdAE50Q protein (another biofilm-dispersing protein by binding cyclic diguanylate), and LasR. Firstly, the initial colonizer biofilm was formed in a microfluidic device. Upon introducing the disperser cells into the initial colonizer cells, LasR in the initial colonizer cells binds with 3OC12HSL from the disperser cells, and the LuxR–3OC12HSL complex induces BdcAE50Q by activating the P_{lasI} promoter, thus displacing the initial colonizer cells in the biofilm. Subsequently, IPTG can induce the expression of Hha13D6 to remove the disperser biofilm. Thus, the control of biofilm formation of a microbial consortium was accomplished by the programmed gene circuit.

Many biotechnology processes may involve interspecies communications between different species in microbial consortia.[126] Marchand *et al.* recently constructed an orthogonal interspecies sender–receiver communication system between Gram-negative (*E. coli*) and Gram-positive (*B. megaterium*) bacteria based on the production and response to autoinducing peptides (AIPs) (Fig. 5A).[127] The sender cells contain an established expression system of AgrB and AgrD proteins originating from Gram-positive *S. aureus* into *E. coli* to produce mature type-1 AIP (AIP-1),[128] which can be secreted into culture media. The receiver cells are engineered *B. megaterium*, harboring a two-component AgrCA system including two genes (*agrC* and *agrA*) and a P3 promoter (from *S. aureus*) in controlling the GFP expression (as the system output) by the AgrCA-dependent AIP recognition. This work shows that peptide-based communication systems are able to coordinate gene expression, metabolic pathways and growth in microbial consortia with different microbial species.

Beyond prokaryotic cells, an artificial sender–receiver communication network was also developed in a eukaryotic cell (*i.e.*, yeast *Saccharomyces cerevisiae*) by Chen *et al.* (Fig. 5B).[129] The sender yeast cells express AtlPT4 from *Arabidopsis thaliana* under the control of the GAL1 promoter. AtlPT4 catalyzes the isopentenylation of ATP to synthesize cytokinin isopentenyl-adenine (IP), an adenine derivative, which diffuses through cell membranes and enters the nearby receiver cells. Upon binding of IP to the cytokinin receptor AtCRE1 on the receiver cells (a *sln1Δ* mutant *S. cerevisiae* strain), the endogenous histidine phosphor-transfer protein YPD1 is phosphorylated to form phosphor-YPD1, which further phosphorylates the endogenous nuclear aspartate response regulator SKN7. Subsequently, phosphor-SKN7 activates the expression of GFP controlled by the P_{SSRE} promoter. Meanwhile, the receiver cells also overexpress PTP2 to inhibit the activity of HOG1 caused by the unphosphorylated SSK1 in the absence of cytokinin, because HOG1 activity can be lethal to cells under normal growth conditions. Thus, this artificial phosphorylation signaling pathway programmed the response of the receiver cells to the cytokinin IP synthesized from the sender cells, enabling the establishment of a sender–receiver multicellular communication in yeast.

3.1.4. Engineering microbial consortia with two-way (bi-directional) communications

3.1.4.1. Synthetic microbial consortia via metabolite exchanges. Cell–cell interaction *via* metabolites is a common mechanism in natural microbial ecosystems. Such metabolite-mediated interactions were also programmed in a few synthetic ecosystems. Shou *et al.* designed a synthetic obligatory cooperation ecosystem in the genetically engineered yeast *S. cerevisiae*,[130] which includes a pair of nonmating yeast strains, each supplying an essential amino acid to the other strain. The strain $R^{\leftarrow A}_{\rightarrow L}$ overproduces lysine (by harbouring $Lys21^{op}$ enzyme) and requires adenine (lacking Ade8 enzyme) for its growth, while the strain $Y^{\leftarrow L}_{\rightarrow A}$

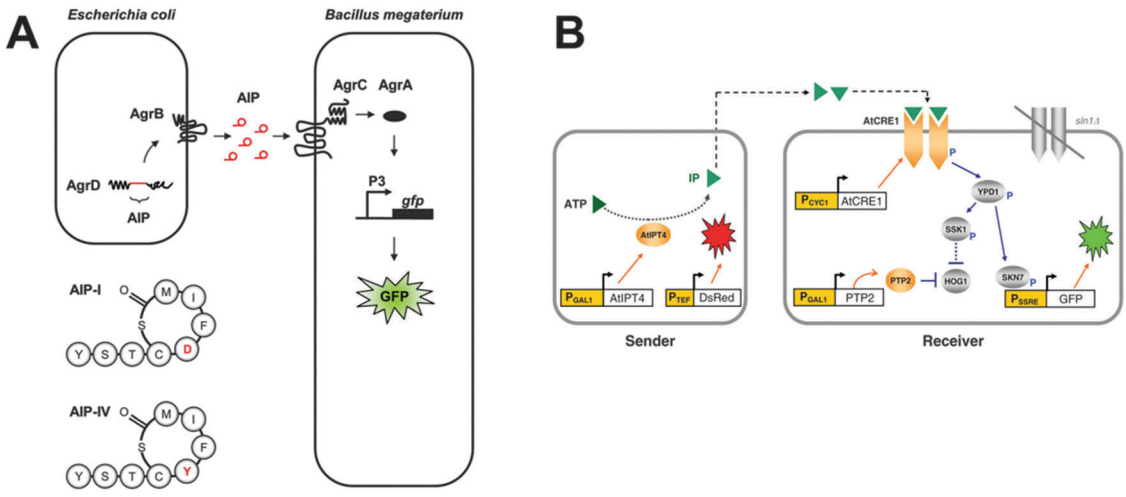

Fig. 5 Microbial consortia with one-way sender–receiver interaction between interspecies bacteria, or in a programmed eukaryotic cell. (A) A synthetic consortium consisting of engineered Gram-negative *E. coli* and Gram-positive *B. megaterium*, communicated by the autoinducing peptides (AIP). (B) Artificial sender–receiver consortium of engineered yeast *S. cerevisiae via* communication of plant hormone cytokinin from *Arabidopsis thaliana*. Reproduced with permission from ref. 127 and 129. Copyright 2013 John Wiley and Sons, and 2005 Nature Publishing Group, respectively.

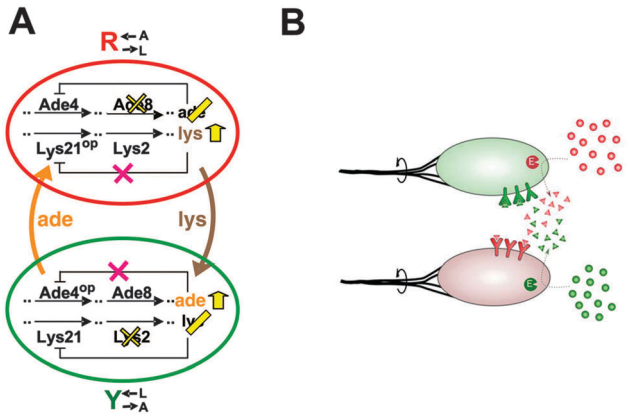

Fig. 6 Synthetic microbial consortia with two-way communication *via* metabolite-mediated interactions. (A) Wiring diagram of a synthetic mutualistic ecosystem programmed in *S. cerevisiae*, in which each strain supplies an essential amino acid to the other strain. The consortia can be viable only at the existence of the two cooperative cells, while each cell cannot grow independently in the absence of the other. (B) Schematic of a synthetic microbial consortium capable of cooperative chemotaxis, thus the two engineered *E. coli* strains can swim towards the gradients of two attractants, while each individual cannot. Reproduced with permission from ref. 130 and 133. Copyright 2007 The National Academy of Sciences of the USA, and 2009 John Wiley and Sons, respectively.

overproduces adenine (by harbouring ade4^{op} enzyme) and requires lysine (lacking Lys2 enzyme) to grow (Fig. 6A). Although each individual cannot grow, co-culture of the two strains establishes a mutualistic cooperation to maintain the survival and viability of the entire community. Using a similar strategy, Hosoda *et al.*[131] engineered a synthetic obligate mutualism using two nutrient autotrophs of *E. coli*, including an isoleucine auxotroph (I^-, lacking *ilvE* gene in synthesizing isoleucine) and a leucine auxotroph (L^-, lacking *leuB* gene in synthesizing leucine). Both auxotrophs cannot grow individually without addition of the corresponding amino acid, however, upon co-culturing, I^- can provide sufficient leucine for the growth of L^-, while L^- provided sufficient isoleucine for the growth of I^-, thus enabling simultaneous growth of the two strains.

Kerner *et al.* programmed a pair of auxotrophic *E. coli* strains that cross-feed amino acids with each other to form a synthetic symbiosis ecosystem, which enabled continuous tuning of the growth rate and population composition in the consortia.[132] This synthetic ecosystem includes a tryptophan (Trp) auxotroph *E. coli* strain W3 and a tyrosine (Tyr) auxotroph *E. coli* strain Y3, in which W3 harbors a plasmid to overexpress *yddG* gene (encoding an inner membrane protein YddG, under the control of the arabinose-inducible promoter P_{BAD}) to increase the export of tyrosine that feeds Y3; on the other hand, Y3 overexpresses a *trpEDfbr* gene (under the control of a propionate-inducible promoter P_{prpB}) to catalyze the first step in the Trp biosynthesis pathway to enhance the synthesis of Trp that feeds W3. By regulating the expression of these genes associated with the export or biosynthesis of these amino acids by different concentrations of these inducers, the metabolite exchange rates between the two strains can be tuned, enabling a wide range of growth rates and strain population ratios of the synthetic ecosystem.

Goldberg *et al.* engineered the chemotaxis system of *E. coli* to construct a microbial consortium (Fig. 6B), in which one *E. coli* strain (named tarPA+ ansB+) harbors the *ansB* gene to encode an asparaginase enzyme (a hydrolytic enzyme that converts asparagine to aspartate) and expresses a chemoreceptor TarPA that responds to the ligand phenylacetic acid (PAA), while the other *E. coli* strain (named tar+ pac+) harbors penicillin acylase (Pac) (a hydrolytic enzyme that converts phenylacetyl glycine (PAG) to PAA) and expresses a native aspartate receptor that responds to the ligand aspartate.[133] Thus, a mixture of the two strains is able to undergo chemotaxis along the combined gradient of the two attractants (PAG and asparagine), but it is not able to respond to either PAG or asparagine alone.

Metabolite-mediated cell–cell communication can also occur in inter-kingdom species. Weber *et al.* used airborne communications and signal transduction *via* volatile aldehydes, small vitamin-derived molecules or antibiotics to engineer a number of inter-kingdom communities across species.[134] Although these synthetic ecosystems are beyond microbial consortia due to the involvement of not only bacteria and yeast but also plant and mammalian cells, a diverse array of ecological interaction modes were constructed, including commensalism, amensalism, mutualism, parasitism, and predator–prey ecologies.[134]

3.1.4.2. Synthetic ecosystems via QS communications. QS systems were used for programming microbial ecosystems involving bi-directional communications, and several interaction modes were constructed, *e.g.*, mutualistic synergy,[53,135] commensalism,[135] and predation.[54]

Brenner *et al.* employed two QS systems, LasI/LasR and RhlI/RhlR systems from *P. aerugenosa*, to engineer *E. coli* to construct a consensus consortium (left panel, Fig. 7A).[53] In this consortium, one *E. coli* strain harbouring the gene circuit A expresses the LasI protein that catalyses the synthesis of 3-oxododecanoyl-HSL (3OC12HSL). Diffusion of 3OC12HSL to the other *E. coli* strain containing the gene circuit B led to the formation of the LasR–3OC12HSL complex and elicited the expression of the *rhlI* gene by activating the P_{las} promoter. RhlI catalysed the synthesis of butanoyl-HSL (C4HSL) in circuit B. C4HSL diffuses into circuit A and forms the RhlR–C4HSL complex to activate the P_{rhl} promoter, leading to the expression of the *lasI* gene in circuit A. Thus, the co-culture of the two *E. coli* strains enables them to exchange QS signals bi-directionally, such that the consortium generates "consensus" gene expression if and only if both populations are present at sufficiently high cell densities, while neither population can respond in the absence of the QS signal synthesized from the other population (right panel, Fig. 7A).

Motivated by the synthetic emulation of naturally occurring predator–prey interactions, Balagadde *et al.* constructed a synthetic predator–prey system *via* programming bi-directionally cell–cell communication.[54] Two QS signalling systems, LusR/LuxI from *V. fischeri* and LasI/LasR from *P. aerugenosa*, were used to program the interactions between the predator and prey populations (left panel, Fig. 7B). The predator population will die owing to

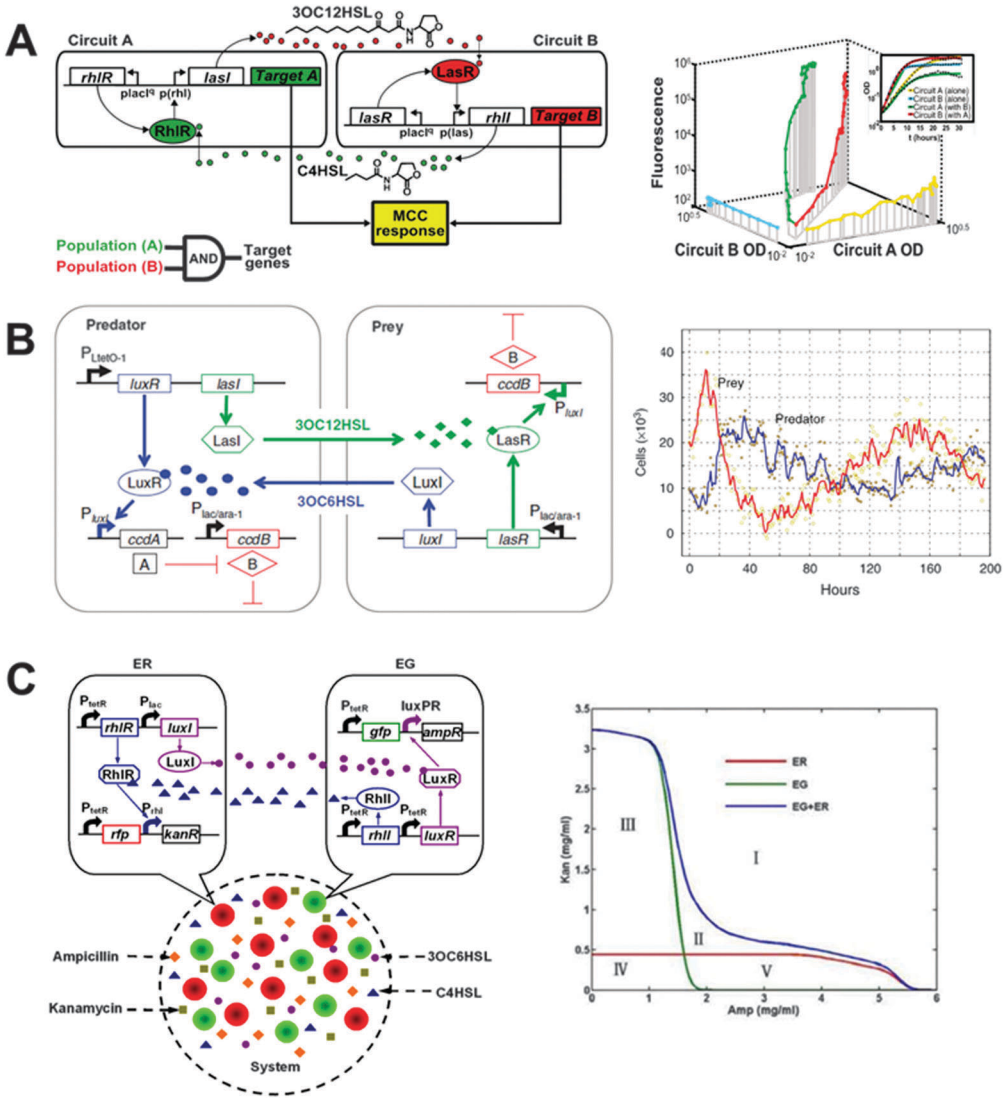

Fig. 7 Synthetic microbial ecosystems *via* bi-directional (two-way) quorum sensing communications. (A) Wiring diagram of a consensus microbial consortium *via* programmed QS communication by circuits A and B in engineered *E. coli*, in which the two populations generate consensus gene-expression response if and only if both are present at sufficiently high cell density, while neither population responds in the absence of the other (left panel). The fluorescence intensity of the co-cultured cells harbouring the gene circuit A and B that allow them to communicate is over 100-fold higher than when they grow in isolation (right panel). (B) The synthetic predator–prey ecosystem in *E. coli*, where the predator produces one QS signal 3OC12HSL to lead to the killing of prey, while the prey in turn produces another QS signal 3OC6HSL to rescue the predator (left panel). Oscillatory dynamics of the predator and prey populations in a microchemostat (right panel). (C) Programmed interactions *via* two sets of QS modules (LuxR-LuxI, and RhlR-RhlI) in an environment (*i.e.*, antibiotics)-sensitive synthetic *E. coli* ecosystem, consisting of ER and EG cells. ER has background resistance to ampicillin and can produce 3OC6HSL to induce the expression of EG to generate ampicillin resistance, while EG has background resistance to kanamycin and can produce C4HSL to induce ER to generate kanamycin resistance (left panel). Model simulation revealed that this ecosystem may exhibit five interaction modes at different antibiotic concentrations: (I) extinction, (II) obligatory mutualism, (III) commensalism for EG, (IV) facultative mutualism, and (V) commensalism to ER (right panel). Reproduced with permission from ref. 53, 54 and 135. Copyright 2007 The National Academy of Sciences of the USA, and 2008 John Wiley and Sons, respectively.

the IPTG-inducible expression of the ccdB killer protein when the cell density of prey is low. Prey cells harbour the *LuxI* gene, and its expression catalyses the synthesis of 3OC6SHL by the prey. When the prey cells reach a high density, the accumulated 3OC6HSL diffuses into the predator cells and, upon binding with the LuxR protein, elicits the expression of an antidote CcdA that interacts with CcdB to neutralize its toxicity, thus to rescue the predator population. The predator cells in turn express LasI to catalyse the synthesis of 3OC12HSL, which diffuses into the prey cells to induce the killer protein CcdB, leading to the killing of the prey. Long-term microchemostat experiments showed that extinction, coexistence and oscillatory dynamics (right panel, Fig. 7B) of the predator and prey populations are possibly dependent upon the operation conditions, such as the IPTG induction level and the dilution rate of the microchemostat.

Hu *et al.* used similar QS signalling modules (LuxR/LuxI and RhlR/RhlI) to program a synthetic microbial ecosystem that shows different ecological interaction modes, including extinction, obligatory and facultative mutualism, under conditions of different antibiotics levels and initial cell densities.[135] This ecosystem consists of two engineered *E. coli* strains, ER and EG, that show background resistance to ampicillin and kanamycin, respectively (left panel, Fig. 7C). ER harbours the *LuxI* gene under the control of the P_{lac} promoter (IPTG inducible), which catalyses the synthesis of 3OC6HSL. 3OC6HSL diffuses into EG and binds to LuxR, which subsequently elicits the expression of *ampR* gene, enabling EG resistance to ampicillin. On the other hand, EG produces C4HSL *via* the expression of RhlI. C4HSL diffuses to ER, and binds to RhlR, which elicits the expression of *KanR* gene and confers kanamycin resistance to ER. The interaction modes and dynamics of the ecosystem are dependent upon the concentrations of ampicillin and kanamycin (right panel, Fig. 7C). At sufficiently high concentrations of the two antibiotics, both strains extinct. With the appropriate antibiotic level (*e.g.*, 4 mg ml^{-1} ampicillin, 0.64 mg ml^{-1} kanamycin), ER and EG cannot grow individually, but can grow together as a co-culture, forming an obligatory mutualistic ecosystem. When the antibiotic level is sufficiently low (*e.g.*, 1 mg ml^{-1} ampicillin, 0.16 mg ml^{-1} kanamycin), facultative mutualism dominates the system dynamics. Thus, different ecological interactions can be achieved in one synthetic ecosystem by altering a few major environmental factors.

3.1.5. Using synthetic microbial ecosystems to address ecological questions

3.1.5.1. Cell dispersion and spatial effects on the stability of ecosystems. One critical aim of ecology is to elucidate the mechanisms that maintain the stability and biodiversity of ecosystems. Synthetic microbial ecosystems were used to investigate cell dispersion and spatial effects on the stability and biodiversity of ecosystems. Kerr *et al.* synthesized a rock-paper-scissors ecosystem consisting of three competing populations of *E. coli*:[136] one is a colicinogenic (*i.e.*, colicin-producing) strain of *E. coli* (C) that harbors the genes encoding the colicin, a toxin that leads to cell death, one is a colicin-sensitive *E. coli* strain (S) that is able to be killed by colicin, and the other is a colicin-resistant *E. coli* strain (R). In this ecosystem, S has a growth advantage over R, which outcompetes C in the growth rate, and C in turn kills S. Thus, this C–S–R community satisfies the competitive relationship of rock-paper-scissors. By mathematical modeling and experiments, Kerr *et al.* found that in a well-mixed environment (*e.g.*, in a shaking flask containing liquid media) in which dispersion and interaction between these three cells are not exclusively local, both S and C dropped below detection limits upon a certain time of co-culturing.[136] However, in the environments where dispersion and interaction are local (*e.g.*, in a static plate), C, S and R strains are all maintained at high densities. It was thus concluded that cell dispersion and interactions must be local for coexistence of this C–S–R microbial community.

To examine the role of micro-scale spatial structures in the stability of microbial ecosystems, Kim *et al.* constructed a synthetic community consisting of three wild-type soil microorganisms, *Azotobacter vinelandii* (Av), *Bacillus licheniformis* (Bl), and *Paenibacillus curdlanolyticus* (Pc) (upper panel, Fig. 8A).[137] Each of these microbes provides nutrients for the survival of the entire community, forming a reciprocal syntrophy of interactions. Av supplies nitrogen sources by fixing nitrogen into amino acids with a molybdenum-coupled nitrogenase, Bl degrades penicillin G with β-lactamases to remove antibiotic pressure to the community, and Pc supplies the carbon source (glucose) by cleaving carboxymethyl-cellulose. They used a microfluidic device to control spatial structure and chemical communications within this microbial community, and found that the community can stably coexist only at some intermediate separation distances in the order of a few hundred of micrometers, at which the combined consumption rate of a nutrient matches with its production rate (lower panel, Fig. 8A). This research showed that micro-scale spatial structure is a crucial factor in determining the stability and biodiversity of microbial communities.

To study the spatiotemporal modulation of biodiversity (or the relative abundance of species, a measure of the persistence of an ecosystem) in chemical-mediated ecosystems, Song *et al.* employed a synthetic *E. coli* predator–prey ecosystem *via* programmed QS communications as a model system to investigate how biodiversity of microbial communities can be modulated (upper panel, Fig. 8B).[138] Depending on the specific experimental conditions, the dominant interaction between the two engineered *E. coli* populations in the ecosystem could be competition for nutrients or predation *via* QS communications. They found that only at intermediate cell segregation distances, the response of the QS signal receiver cells is sensitive to the cell segregation distances (lower panel, Fig. 8B). Consequently, when the length scales of the population segregation and the chemical-mediated interaction are comparable, cell motility has a great impact on the biodiversity of this ecosystem. This research rationalized the biodiversity modulation in chemical-mediated ecosystems by habitat partitioning and cellular motility.

3.1.5.2. Cheating on the stability of ecosystems. A common-good producer in cooperative ecosystems may be challenged by the non-producer (*i.e.*, cheater), who benefits from the shared resources, but does not bear the production burden. It is intriguing to study how cooperative ecosystems maintain their stability when the common-good non-producer exists.[139,140] Chuang *et al.* constructed a synthetic microbial consortium consisting of two engineered *E. coli* strains of a common-good producer (P) and a non-producer (NP)[139] (Fig. 9A). P can constitutively express the autoinducer synthase RhlI that catalyzes the synthesis of the QS signaling molecule C4HSL (the common good in this system), however, NP cannot produce C4HSL. In both P and NP, C4HSL binds to the constitutively expressed RhlR to form the complex RhlR–C4HSL, which induces the artificial P_{rhl*} promoter to drive the expression of an unstable variant of the chloramphenicol (Cm) acetyltransferase gene, *catLVA.* Thus, the P and NP strains exhibit QS-dependent Cm resistance. Chuang *et al.* found that by varying the initial compositions of the P and NP, Simpson's paradoxical phenomenon could be observed, *i.e.*, NP grows faster within a mixture due to its growth

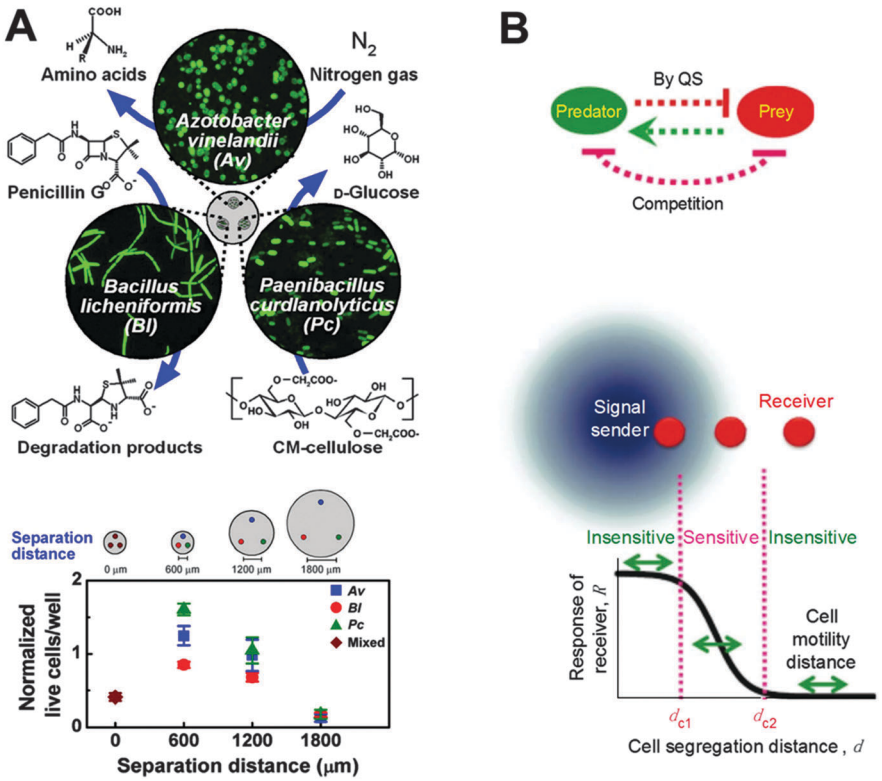

Fig. 8 Effect of spatial separation of components on the stability and biodiversity of microbial ecosystems. (A) A synthetic microbial community consisting of three soil microorganisms (Av, Bl, and Pc) with reciprocal syntrophy, where Av supplies nitrogen sources, Bl reduces antibiotic pressure, and Pc provides a carbon source (upper panel). This synthetic community coexists only at intermediate separation distances (lower panel). (B) A schematic of the chemical-mediated interaction modes in a synthesized *E. coli* predator–prey ecosystem (upper panel). Only at intermediate cell segregation distances, the response of the QS signal receiver cells is sensitive to the cell segregation distances (lower panel). Reproduced with permission from ref. 137 and 138. Copyright 2008 The National Academy of Sciences of the USA, and 2009 Nature Publishing Group, respectively.

advantage over P, however, the global proportion of P over a large initial proportion of P increased relative to its initial proportion.

In another study, Gore *et al.* constructed a cooperator–cheater consortium,[141] which includes the cooperator (budding yeast *S. cerevisiae*) being able to produce the enzyme invertase to hydrolyze sucrose to glucose for the growth of the cells, and the mutant cheater being not able to produce invertase. In the sucrose cultures, when the cooperator is initially a small fraction of the population, there will be little glucose available in the media, thus the cooperator has a growth advantage over the cheater because it can capture a small fraction of glucose it produces. This study elucidated a possible mechanism that cooperation is able to exist during evolution.

Cheaters could outcompete cooperators by not producing costly common goods, elucidation of the mechanisms underlying the stability of cooperative ecosystems under the challenge of cheaters is intriguing. Waite *et al.* recently constructed a synthetic cooperator–cheater ecosystem, enabling the elucidation of a mechanism that may purge cheaters stochastically to maintain the stability of cooperation.[142,143] This ecosystem consisted of three engineered *S. cerevisiae* strains, in which two are cooperators and the remaining one is a cheater. The cooperation occurs *via* the exchange of amino acids between two auxotrophic strains, *i.e.*, the $R^{\leftarrow L}_{\rightarrow A}$ strain (cooperator-1) requires lysine for growth and overproduces adenine, whereas the $Y^{\leftarrow A}_{\rightarrow L}$ strain (cooperator-2) requires adenine and overproduces lysine (Fig. 9B), thus the presence of both $R^{\leftarrow L}_{\rightarrow A}$ and $Y^{\leftarrow A}_{\rightarrow L}$ strains is a requirement for the survival of the two strains in the minimal media lacking adenine and lysine. The cheater is a $C^{\leftarrow L}$ strain that requires lysine (from cooperator-2) for its growth but do not overproduce any nutrients in exchange. The outcome of the competition between the three strains depends on whether the cooperator-1 or the cheater first acquires a mutant adaptive to the nutrient-limited cooperative environment, allowing the mutant to increase the rate of nutrient uptake. If the cheater first gains such a mutant, it eliminates the two cooperators and dominates the population. However, if the cooperator-1 first acquires such a mutant, the cooperator outcompetes the cheater and such cooperative populations grow faster than the cheater-dominated culture. This study elucidated a mechanism of the cheater control for the establishment and maintenance of cooperation.

3.2. Distributed bio-computations

Complex gene circuits that can perform information processing functions were programmed in single cells, enabling many predefined Boolean logic computations. However, the multiple wiring of biomolecules is usually difficult to implement reliably within one cell, and the resulting logic gates in single cells

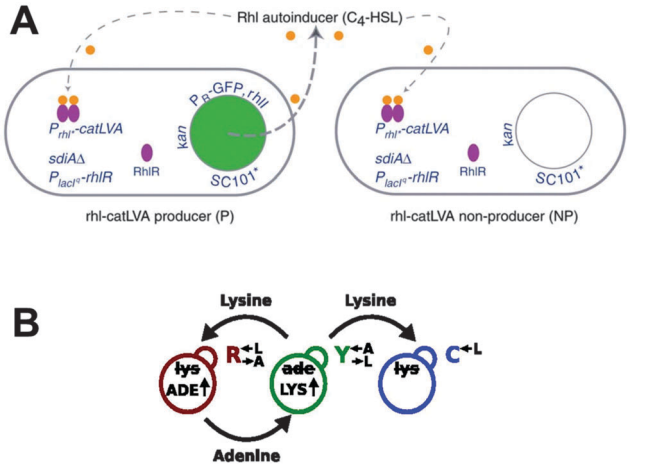

Fig. 9 Cheating on the stability of microbial ecosystems. (A) A synthetic cooperator–cheater consortium programmed in *E. coli*, in which the cheater does not produce the "public goods", *i.e.*, the QS signalling molecule C4HSL. (B) A synthetic yeast *S. cerevisiae* cooperator–cheater ecosystem. The cooperators supply essential amino acids to each other, while the cheater benefits from the lysine supplied by one cooperator, while does not provide adenine to that cooperator in return. Reproduced with permission from ref. 139 and 142. Copyright 2009 the American Association for the Advancement of Science, 2012 The National Academy of Sciences of the USA, respectively.

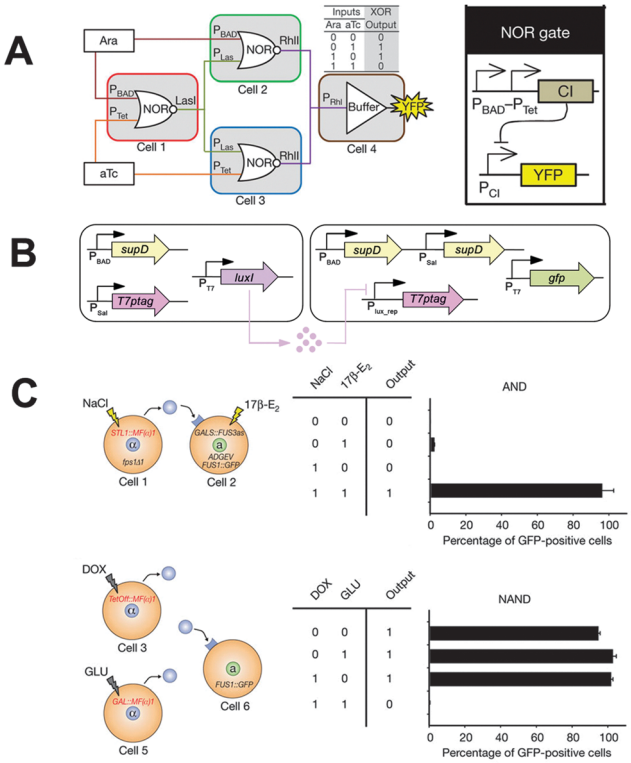

Fig. 10 Distributed bio-computations (Boolean logic gates) achieved by synthetic microbial consortia. (A) XOR logic gate achieved by programming QS-based communication between engineered *E. coli* colonies on a plate, in which the two inducers (arabinose and anhydrotetracyclin) act as the inputs, and YFP fluorescence is the output. Each of the colony strains contains a single logic gate, either NOR or Buffer (left panel). The gene circuit architecture of the NOR gate (right panel). (B) Schematic of a XOR logic gate programmed by a synthetic *E. coli* consortium. The sender cell produces a QS signal 3OC6HSL upon induction of the two inputs (arabinose and salicylate), which repressively transduces the expression of GFP fluorescence (output) *via* a repressive promoter P_{lux_rep}. (C) AND and NAND logic gates achieved by the pheromone (alpha factor)-based communication between two engineered yeast strains. 17β-E_2: oestradiol, DOX: doxycycline, GLU: glucose. Reproduced with permission from ref. 145–147. Copyright 2011 Nature Publishing Group.

cannot be easily used in other modules.[144] Instead, microbial consortia can be used to distribute the multiple wiring of biomolecules among different engineered cells, enabling distributed bio-computations.

Tamsir *et al.* achieved a complicated XOR gate by integrating multiple layers of simpler logic gates, *i.e.*, three NOR gates and a buffer gate programmed in four *E. coli* cells.[145] The NOR gate (right panel, Fig. 10A) is based on a tandem promoter with the same orientation to drive the expression of a transcriptional repressor, in which the CI-repressor gene is placed under the control of the P_{BAD}–P_{Tet} tandem promoter (being activated by either arabinose (Ara) or anhydrotetracycline (aTc)), and the output gene is placed under the control of the CI-repressible P_{CI} promoter. In the XOR gate design circuit (left panel, Fig. 10A), Cell 1 carries a NOR gate that uses Ara and aTc as inputs and the expression of LasI as the output, which catalyses the synthesis of 3OC12SHL to wire Cell 1 to the NOR gates in Cells 2 and 3. Similarly, Cells 2 and 3 respond to Ara and aTc as inputs, respectively, to express RhlI as their output, which produces C4HSL. Cell 4 responds to C4HSL as a buffer, *i.e.*, the output of Cell 4 is "ON" only if its input is "ON". Thus, the sequential programmed communications between the four colonies of engineered *E. coli* strains that were arranged on a plate accomplished the XOR logic gate.

On the other hand, Ji *et al.* constructed an *E. coli* consortium that is able to achieve the XOR logic gate[146] (Fig. 10B). In the consortium, one cell harbours an amber mutated T7 polymerase (*T7ptag*) under the control of the arabinose (Ara)-inducible promoter P_{BAD}, and *supD* tRNA under the control of the salicylate (Sal)-inducible promoter P_{Sal}, thus the T7 polymerase can be rescued by *supD* tRNA when both Ara and Sal are present, which in turn activate the P_{T7} promoter to induce the expression of LuxI, the synthase of 3OC6SHL. The other cell harbours an engineered repressive promoter P_{lux_rep}, the binding of which to the LuxR–3OC6SHL complex would inhibit the transcription of *T7ptag*. Upon optimization of the gene expressions involved in the circuit, this co-cultured microbial consortium can operate as a XOR logic gate.

Meanwhile, Regot *et al.* achieved a number of Boolean logic gates by programmed consortia including two, three, four or five engineered yeast cells.[147] For example, a two-cell consortium that can implement AND logic computation was designed (upper panel, Fig. 10C), in which Cell 1 produces pheromone under the stimulus of NaCl, and Cell 2 responds to the pheromone and another external input signal (oestradiol) to activate the production of Fus3 mitogen-activated protein kinase (MAPK) to express GFP and its fluorescence as the output. Thus, an AND gate is achieved in this microbial

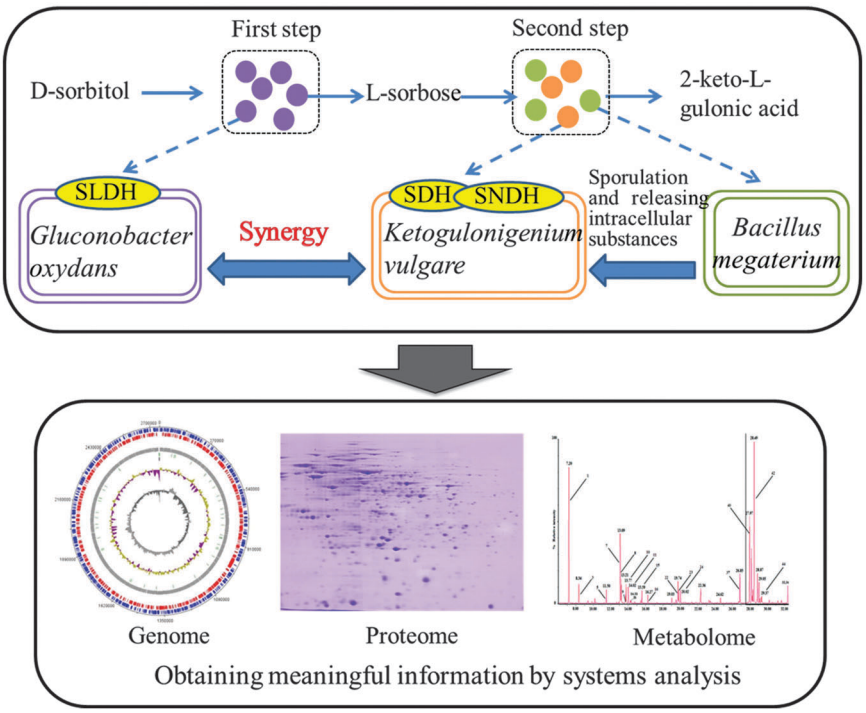

Fig. 11 Systems biology-guided design, construction and optimization of an artificial microbial consortium consisting of *Ketogulonicigenium vulgare* and *Bacillus megaterium* for vitamin C production. In the two-step fermentation production of vitamin C, *Gluconobacter oxydans* firstly converts sorbitol into sorbose, which is further metabolized into 2-keto-gulonic acid (the precursor of vitamin C) by co-cultured *B. megaterium* and *K. vulgare*. The molecular interaction mechanisms between microorganisms in the consortium can be elucidated by systems biology approaches, including genomics, proteomics, and metabolomics. SLDH, sorbitol dehydrogenase; SDH, sorbose dehydrogenase; SNDH, sorbosone dehydrogenase.

consortium consisting of two engineered yeast cells. With three engineered yeast cells, a NAND logic computation can be obtained (lower panel, Fig. 10C), in which Cells 3 and 5 display NOT logic and produce pheromone in the absence of doxycycline (DOX) and glucose (GLU) as an input, respectively; and Cell 6 responds to the pheromone from either Cell 3 or 5 by producing a GFP fluorescence (the output of the circuit). Therefore, only in the presence of both input signals (DOX and GLU), the GFP output cannot be produced, demonstrating the NAND logic computation. Furthermore, by implementing more complex consortia consisting of four or five communicated yeast cells, more complicated computational functions such as a multiplexer and a 1-bit adder with carry could be accomplished.

3.3. Applications in the production of nutraceuticals, drugs, and biofuels

3.3.1. Vitamin C production by synthetic microbial consortia. The synthetic consortium consisting of *K. vulgare* and *B. megaterium* has been successfully applied in a two-step fermentation process for the industrial production of vitamin C, a nutraceutical for the well-being of humans with tremendous global demand.[148,149] This 2-step process includes the conversion from D-sorbitol to L-sorbose by *Gluconobacter oxydans* as the first step, and the conversion from L-sorbose to 2-keto-L-gulonic acid (2-KLG, the precursor of vitamin C) by a microbial consortium of *K. vulgare* and *B. megaterium* as the second step (upper panel, Fig. 11). *B. megaterium* can sporulate and release its intracellular substances during the co-culture with *K. vulgare*. Systems biology analyses, including genomic, proteomic and metabolomic profiling (lower panel, Fig. 11), of the vitamin C production process would provide important information for the reconstruction and optimization of the consortium.

Yuan's group made a comprehensive study on the molecular mechanisms of the interactions between the two bacteria of *K. vulgare* and *B. megaterium*, which is summarized in Fig. 12. Zhou *et al.* explored the metabolite exchange between *K. vulgare* and *B. megaterium* by physically separating them on an agar plate,[104] and found that *K. vulgare* could degrade peptides in the environment to provide amino acids (including Ala, Val, Ile, Pro, Asp, and Glu *etc.*) for the growth of *B. megaterium*, which could subsequently induce the swarm of *B. megaterium* towards *K. vulgare*. In return, *B. megaterium* released several metabolites (including erythrose, erythritol, guanine, fructose, and inositol) for the growth of *K. vulgare*. Besides such cooperation between the two bacteria, competition for nutrients (including sugars, amino acids *etc.*) for their respective growth also occurs, which was demonstrated by a significant increase in 2,6-dipicolinic acid (a biomarker for the sporulation of *B. megaterium*) produced by *B. megaterium* after its migration.

Furthermore, by an integrated time-series proteomic and metabolomic analysis of the industrial production process of vitamin C, Ma *et al.* further found that the sporulation of *B. megaterium* could provide essential purines to promote the growth of *K. vulgare* and its production of 2-KLG. Also, the

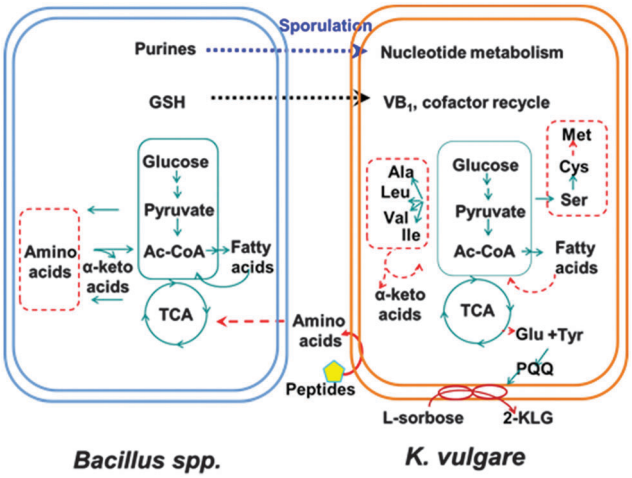

Fig. 12 Comprehensive molecular interaction mechanisms between *K. vulgare* and *B. megaterium* in the process of vitamin C production, as elucidated by the systems biology profiling analyses (*i.e.*, metabolomics and proteomics). The sporulation of *Bacillus* spp. could provide purines for *K. vulgare*. Glutathione (GSH) could promote the transport of vitamin B_1 (VB_1), and thus enhanced the cofactor recycling of thiamine pyrophosphate (TPP) in *K. vulgare*. *K. vulgare* could in turn degrade peptides in the environment into amino acids to facilitate the growth of *Bacillus* spp.

released proteins upon cell lysis of *B. megaterium* confer *K. vulgare* resistance to the stresses from reactive oxygen species (ROS).[110,150] These released proteins were mainly involved in the pentose phosphate pathway, sorbose metabolism, and the purine biosynthesis pathway *etc.*, and their expressions were significantly increased after the sporulation of *B. megaterium*. They also used proteomics to reveal the demand of transmembrane transport of substrates (in particular, thiamine and glutathione (GSH)) and antioxidant protection for the viability of *K. vulgare* in response to the GSH stimulation.[108] GSH was transported into the cell by the oligopeptide transport system (thiBPQ), which improved the thiamin/thiamin pyrophosphate transport. Thiamine pyrophosphate (TPP) is the cofactor of pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and transketolase, and thus enhances the tricarboxylic acid cycle and pentose phosphate pathway. It would generate more ATP and NADPH, which could then be used in combating against intracellular reactive oxygen stress (ROS).

Serial subculturing of the co-culture *B. cereus* and *K. vulgare* for 150 transfers was proven to greatly enhance the yield of 2-KLG,[151,152] where the evolved strains acquired a 16% increase in the yield of 2-KLG compared to the original strains. In order to elucidate the molecular mechanism of this subcultivation process, Ma *et al.* performed proteomic analyses of the 0th, 50th, 100th and 150th transfers of *K. vulgare*, *B. cereus*, and their co-culture, respectively.[152] By comparing the proteomes of the evolved strains and original strains, they found that evolved *K. vulgare* had enhanced the capability of sorbose conversion and amino acid biosynthesis, and *B. cereus* improved its capability to transport oligopeptides. It could be inferred that the two bacteria acquired enhanced cooperation in the utilization of amino acids, as it was reported that *K. vulgare* could supply amino acids to *B. megaterium*.[104] Inversely, sporulation of *B. cereus* was decreased upon subcultivation, suggesting an enhanced resistance of *B. cereus* to unfavorable environments.

Such information obtained from systems biology analyses was further used to guide the design, reconstruction and optimization of this microbial consortium for enhanced vitamin C production. Du *et al.* overexpressed two dehydrogenases in *K. vulgare* to convert L-sorbose to 2-KLG, *i.e.*, sorbose dehydrogenase (*sdh*) and sorbosone dehydrogenase (*sndh*) on a broad-host vector pBBR1MCS-2.[113] However, they found that the only expression of the two dehydrogenases cannot increase the production of 2-KLG by *K. vulgare*, because the function of these two dehydrogenases needs the cofactor PQQ, and the PQQ level in *K. vulgare* is limiting. PQQ is formed by the fusion of glutamate and tyrosine of the proteins encoded by the *pqq* gene cluster including *pqq*A, *pqq*B, *pqq*C, *pqq*D, *pqq*E and *pqq*N. Thus, Du *et al.* further constructed a combinatorial cloning of the *sdh*/*sndh* and the PQQ synthesis gene cluster (*pqq*ABCDEN) in *K. vulgare* (Fig. 13).[113] The dehydrogenase genes were constructed in three ways (*sdh*, *sndh* and *sdh*–*sndh*, respectively), and the PQQ cluster genes were also constructed in three ways (*pqq*A, *pqq*ABCDE and *pqq*ABCDEN, respectively). Thus, nine combinations were respectively cloned into *K. vulgare*. The fermentation results suggested that the combination of *sdh*–*sndh* with *pqq*ABCDEN accomplished the highest yield of 2-KLG, which enabled a 20% increase in the yield of 2-KLG over the wild-type *K. vulgare* upon co-cultivation with *B. cereus*.

3.3.2. Oligosaccharide production by synthetic microbial consortia. Synthetic microbial consortia were constructed for the production of sugar nucleotides and oligosaccharides, important biopharmaceutical agents for cancer therapeutics, anticoagulants, and vaccines with a global market of billions of dollars.[153–155] Koizumi *et al.*[156] developed a large-scale production system of UDP-galactose and globotriose from orotic acid, galactose and lactose using microbial consortia with the combination of two recombinant *E. coli* strains and *Corynebacterium ammoniagenes* (Fig. 14). In this bacteria coupling consortium, *E. coli* NM522/pNT25/pNT32 expressed *galT*, *galK*, *galU* and *ppa* to convert galactose into UDP-Gal, *E. coli* NM522/pGT5 expressed *IgtC* to convert lactose and UDP-Gal into globotriose, and *C. ammoniagenes* DN510 converted orotic acid to UTP, which was in turn metabolized by the *E. coli* strains of NM522 and pNT25/pNT32.

Cytidine 5′ monophospho-*N*-acetylneuraminic acid (CMP-NeuAc) and 3′-sialyllactose were also produced from orotic acid, *N*-acetylneuraminic acid, and lactose by the bacterial coupling of three *E. coli* strains (NM522/pTA23 expressing the CMP-NeuAc synthetase gene (*neuA*), NM522/pYP3 expressing the α-(2 → 3)-sialytransferase gene, and MM294/pMW6 expressing the CTP synthetase gene (*pyrG*)), and *C. ammoniagenes* DN510.[157]

Similarly, another large-scale system was established to produce α-Neu*p*5Ac-(2 → 6)-D-Gal*p*NAc, the carbohydrate portion of tumour-associated antigen from orotic acid, Neu*p*5Ac, and Gla*p*NAC, through the coupling of recombinant *E. coli* strains (NM522/pTA23, MM294/pMW6, and NM522/pYP13) and *C. ammoniagenes*.[158]

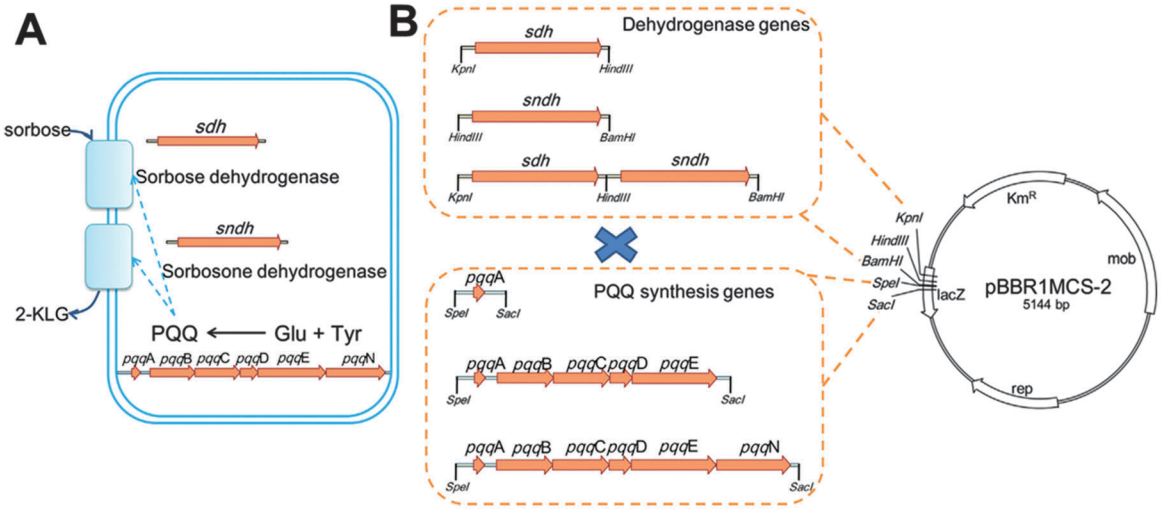

Fig. 13 Engineering *K. vulgare* by combinational expression of sorbose/sorbosone dehydrogenases (*sdh*/*sndh*) and cofactor pyrroloquinoline quinone (PQQ) to enhance the production of 2-keto-L-gulonic acid (2-KLG). (A) The L-sorbose conversion pathway was a redox reaction on sorbose to 2-KLG by the two membrane-bound enzymes: *sdh* and *sndh*. The cofactor PQQ is required in this redox reaction as a mediator of electron transfer. PQQ is formed by the fusion of glutamate (Glu) and tyrosine (Tyr) of the proteins encoded by the *pqq* gene cluster. (B) Schematic diagram of the engineered *K. vulgare* with different combination of genetic constructs in this study. The dehydrogenase genes were constructed in 3 ways: *sdh*, *sndh* and *sdh–sndh*. The PQQ synthesis genes were constructed in 3 ways: *pqqA*, *pqqABCDE* and *pqqABCDEN*. These two sets of genes were further combinatorially cloned into a broad-host vector pBBR1MCS-2, which led to 9 gene combinations in total. Reproduced with permission from ref. 113. Copyright 2013 Elsevier.

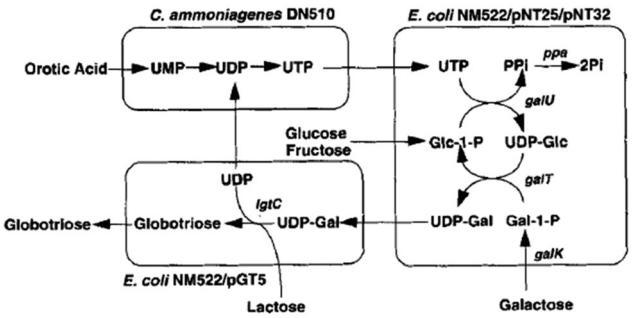

Fig. 14 A synthetic microbial consortium for the large-scale production of UDP-Gal and globotriose from orotic acid, galactose and lactose. *E. coli* NM522/pNT25/pNT32 expressed *galT*, *galK*, *galU* and *ppa* to convert galactose into UDP-Gal. *E. coli* NM522/pGT5 expressed *lgtC* to convert lactose into globotriose. Orotic acid was converted into UTP by *C. ammoniagenes* DN510. Reproduced with permission from ref. 156. Copyright 1998 Nature Publishing Group.

These studies further suggested that microbial consortia had great potential for oligosaccharide production on an industrial scale.

3.3.3. Biofuel production from lignocellulose by synthetic consortia. Significant progress has been made in the design and engineering of symbiotic consortia for the efficient production of biofuels from lignocellulosic biomass.[159] Park *et al.*[160] constructed a synthetic consortium consisting of a hyper cellulase producer *Acremonium cellulolyticus* C-1 and an ethanol producer *S. cerevisiae*, which can significantly improve the production rate of bioethanol from cellulose (Fig. 15A).[160] In this consortium, the cellulase was produced by *A. cellulolyticus* C-1 from Solka-Floc (SF), and the ethanol production was achieved by inoculating 10% (v/v) of *S. cerevisiae* and adding SF when the activity of cellulase was increased. The yield of ethanol by this one-pot co-culture method reached 0.18 (g ethanol per g SF) in a 3 L fermenter with 300 g SF per L.

Zuroff *et al.* developed a scalable, environmentally-mediated symbiotic co-culture ecosystem including *Clostridium phytofermentans* and yeast for the production of lignocellulosic ethanol (Fig. 15B).[161] The cellulose was hydrolyzed by *C. phytofermentans* to form cellodextrins, which could be further converted into ethanol by both *C. phytofermentans* and yeast. The growth of *C. phytofermentans* was inhibited by oxygen, and the consumption of oxygen by yeast including *S. cerevisiae* cdt-1 and *Candida molischiana* relieved such inhibition. This symbiotic consortium enabled an improved production of bioethanol (22 g L^{-1}) from α-cellulose in comparison to the mono-cultures.

Recently, Minty *et al.* developed a robust synthetic cooperator–cheater consortium (TrEc) consisting of fungus *Trichoderma reesei* RUTC30 (cooperator) and bacterium *E. coli NV3* pSA55/69 (cheater) for efficient biosynthesis of isobutanol from lignocellulosic feedstock (Fig. 15C).[162] In the TrEc consortium, *T. reesei* acts as a cooperator and secretes cellulases cellobiohydrolase I (CBHI), cellobiohydrolase II (CBHII), and endoglucanase I (EGI) to hydrolyze microcrystalline cellulose, thus pretreating corn stover to form soluble saccharides. Then, the oligosaccharides are further hydrolyzed to glucose by *T. reesei.* Soluble cellobiose and glucose serve as growth substrates for *T. reesei*, and glucose only for *E. coli*. Glucose is then metabolized by *E. coli* (as a cheater) to produce isobutanol, which inhibits microbial growth due to its toxicity. The highest isobutanol titer reached 1.88 g L^{-1} and the yield is up to 62% of theoretical maximum. This cooperator–cheater consortium could be stabilized and tuned under the guidance of quantitative dynamic modeling of this ecosystem. Such rationally designed ecosystems could be readily adapted for the production of other valuable chemicals.

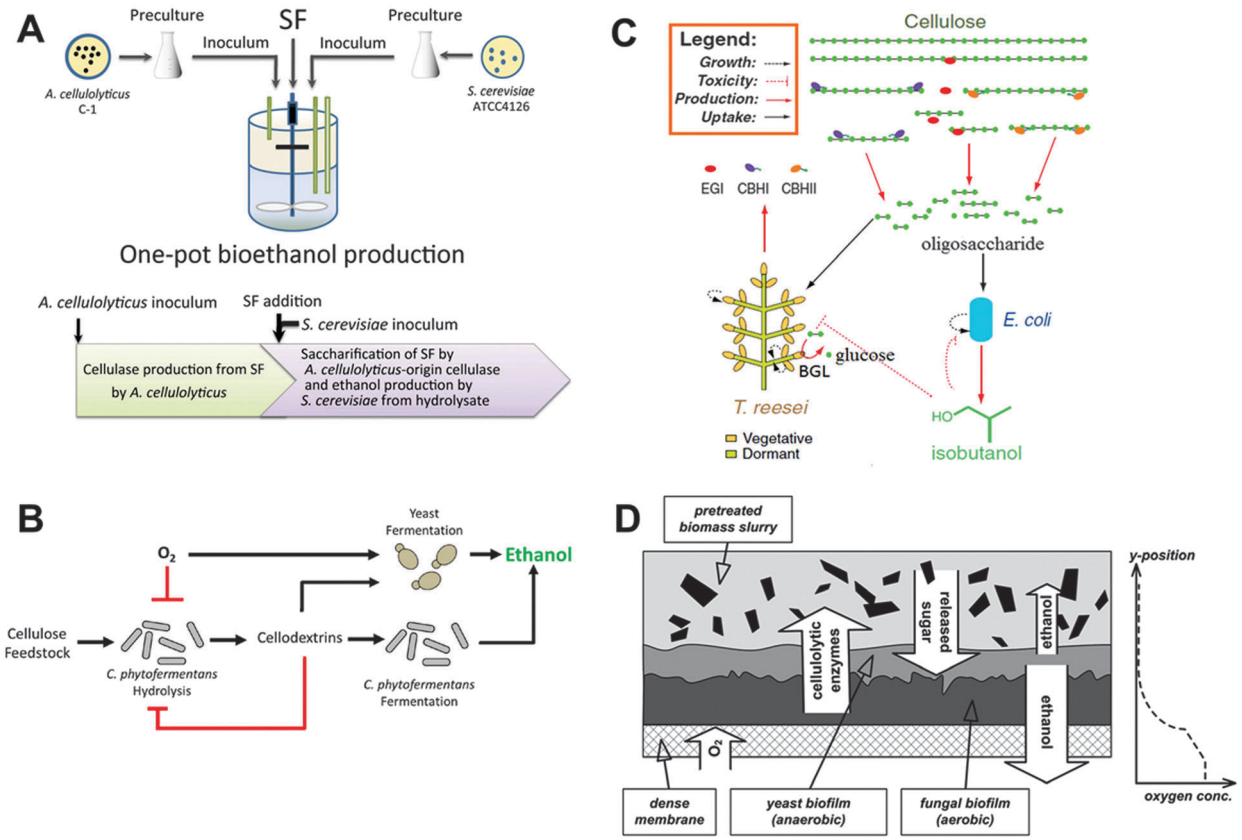

Fig. 15 Engineering symbiotic microbial consortia for bioethanol production from lignocellulosic biomass. (A) One-pot bioethanol production system by *S. cerevisiae* and *A. celluloytious.* The cellulase used for saccharification was produced by *A. cellulolyticus* C-1 from Solka-Floc (SF), and the ethanol production was achieved by *S. cerevisiae.* (B) A synthetic consortium for bioethanol production with a symbiotic *Clostridium phytofermentans*/yeast co-culture. The cellulose was hydrolyzed by *C. phytofermentans* into cellodextrin, which was converted into ethanol by both *C. phytofermentans* and yeast. Oxygen in this ecosystem that inhibits the growth of *C. phytofermentans* could be consumed by yeast during fermentation to relieve the inhibition, leading to a more efficient production of bioethanol. (C) *T. reesei* produces cellulases CBHI, CBHII and EGI to hydrolyze cellulose into soluble oligosaccharides which are metabolized into glucose *via* cell wall-localized β-glucosidase (BGL). Then, the glucose is converted by *E. coli* into isobutanol which inhibits the growth of *T. reesei*. (D) A multispecies biofilm membrane reactor for bioethanol production from the pretreated biomass slurry. Aerobic hydrolytic enzyme-producing fungus grows on the dense O_2 permeable membrane, and anaerobic yeast grows on top of it. Ethanol could be obtained from the liquid phase and the gas phase. Reproduced with permission from ref. 160–163, respectively. Copyright 2013 The National Academy of Sciences of the USA.

In a recent effort, Brethauer *et al.* developed a multi-species biofilm membrane reactor allowing the co-culture of aerobic fungus and anaerobic ethanol producing bacteria to achieve consolidated bioprocessing from lignocellulose to ethanol.[163] As shown in Fig. 15D, the hydrolytic enzyme producing fungus *T. reesei* grows directly on the O_2 permeable membrane that allows aerobic conditions; the ethanol producing *S. cerevisiae* grows on top of the fungal biofilm, which is in an oxygen deficient region. In this way, both aerobic and anaerobic conditions co-exist in one reactor. They tested this system using dilute acid pretreated wheat straw, and added a third microorganism *Scheffersomyces stipites*, a microaerophilic bacterium that could utilize pentose to produce ethanol with significant efficiency. The co-culture of the above three microorganisms achieved a 67% ethanol yield from the undetoxified dilute acid-pretreated wheat straw. These studies suggested that engineered symbiotic consortia are superior over monocultures in the efficiency and stability in the processes of converting lignocellulosic biomass to biofuels.

As an alternative approach, Chen's group[164,165] developed surface display technologies of a functional minicellulosome on the yeast cell membrane to construct a synthetic consortium that can produce bioethanol from cellulose. This yeast consortium C1 consists of four yeast strains by surface assembly, including a strain displaying mini-scaffoldin Scaf-ctf (SC), a strain secreting either an endoglucanase (AT), an exoglucanase (CB) or a β-glucosidase (BF), respectively. The functional minicellulosome displayed on the yeast surface was consistent with its reported synergy effect on cellulose hydrolysis, which was thus used for the synergistic saccharification and fermentation for efficiently converting cellulose to ethanol. The yield of ethanol from phosphoric acid swollen cellulose (PASC) by the consortium C1 was 3-fold higher than that of the yeast consortia C2 and C3 that secreted only the three cellulases.

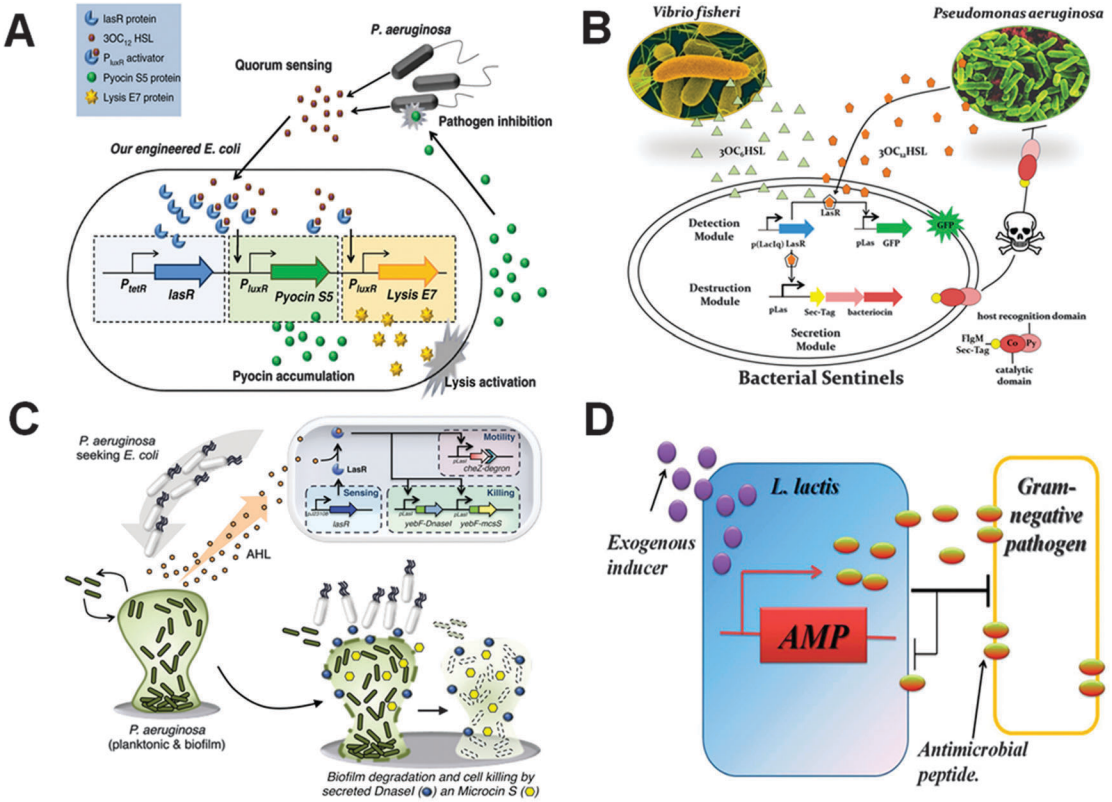

Fig. 16 Synthetic microbial ecosystems in pathogen sensing-killing: (A) engineered *E. coli* to sense and kill *P. aeruginosa* by the production and release of pyocin. The *luxR* promoter, after being activated by the LasR–3OC12HSL complex, led to the expression of E7 lysis protein and S5 pyocin in *E. coli*. (B) Engineered *E. coli* to sense and kill *P. aeruginosa* by synthesizing and secreting CoPy. The promoter *pLas* is initiated by the LasR–AHL complex, and then the production and secretion of CoPy is activated. (C) Engineered *E. coli* to sense, migrate and kill *P. aeruginosa*. The promoter *pLasI* is initiated by the LasR–AHL complex, then the gene *CheZ* (for controlling bacterial motility) and genes *DnaseI* and *mcsS* (for bacterial killing) are expressed to fulfil the programmed migration and killing. (D) Engineered *Lactococci lactis* to sense and kill Gram-negative pathogens by secreting antimicrobial peptides (AMPs). Reproduced with permission from ref. 170–173. Copyright 2011 John Wiley and Sons, and 2013 American Chemical Society, respectively.

These studies represent a significant step toward the cellulosic ethanol production by consortia-based bioprocessing systems.

3.4. Applications in medicine and human health

Synthetic biology has shown great potential in global health innovations,[166] in particular, the prevention, detection and treatment of many diseases.[167–169] Antimicrobial treatment using cells and phages is a broad research area with many great achievements, here we only limit our review on recent synthetic biology efforts in engineering targeted pathogen sensing with enhanced killing in microbes (bacteria and phages) upon the target bacteria by programmed genetic devices and circuits. It should also be noted that in such engineered microbial consortia, only the microbes (bacteria and phages) to kill target bacteria were genetically programmed, while the target bacteria to be killed were not engineered to interact with the synthetic microbes.

3.4.1. Synthetic pathogen sensing-killing. Saeidi *et al.* designed an engineered *E. coli* to sense and kill a pathogenic *P. aeruginosa* by expressing and releasing pyocin, which could induce the cell lysis of *P. aeruginosa* (Fig. 16A).[170] This synthetic microbial consortium consists of three genetic devices, *i.e.*, the sensing, killing and lysing devices. LasR produced under the control of the promoter P_{tetR} in *E. coli* could bind to the QS signaling molecule 3OC12HSL produced by *P. aeruginosa*, then the LasR–3OC12HSL complex binds the P_{luxR} promoter to activate the killing and lysing devices in *E. coli*, mediated by the pyocin S5 and lysis E7 protein expression modules. When 3OC12SHL reaches a threshold concentration in the co-culture medium, the lysis E7 protein induces the cell lysis of *E. coli*, and pyocin S5 is thus released to the medium to kill *P. aeruginosa*. This engineered *E. coli* could kill 99% planktonic *P. aeruginosa*, and inhibit biofilm formation of *P. aeruginosa* by 90%.

As a continuous effort, Gupta *et al.* further engineered *E. coli* to sense the AHL autoinducer 3OC12HSL produced by *P. aeruginosa*, and subsequently secrete a *P. aeruginosa*-specific chimeric bacteriocin CoPy to achieve the targeted killing of *P. aeruginosa* (Fig. 16B).[171] Similarly, this pathogen sensing-killing consortium consists of three genetic modules, *i.e.*, the detection, destruction and secretion modules. The autoinducer 3CO12SHL produced by *P. aeruginosa* was detected by the LuxR activated module in *E. coli*, in which the production of LasR was controlled under a *pLacIq* promoter. Upon binding of LasR to 3OC12HSL as the detection module, the engineered *E. coli* synthesizes CoPy, which is constructed by PCR SOEing the translocation and receptor domains from pyocin S3 to the nuclease and immunity domain of colicin E3. Pyocin S3 is a bacteriocin produced by *P. aeruginosa*

that kills other bacteria without hurting itself, and colicin E3 is such a specific bacteriocin of *E. coli*. Thus, CoPy could bind to the receptor on the cell surface of *P. aeruginosa* and kill *P. aeruginosa* due to lack of immunity, as the nuclease domain of colicin E3 is originally from *E. coli*. The CoPy toxicity is *P. aeruginosa*-specific without affecting sentinel *E. coli*. CoPy was secreted from *E. coli* by a flagellar system with FlgM fused to the N-terminus of CoPy controlled by the *pLas* promoter, which was transcribed by the LasR–3OC12HSL complex. By this design strategy, the growth of *P. aeruginosa* could be specifically inhibited by the engineered *E. coli* in the microbial consortia.

In a parallel study, Hwang *et al.* engineered *E. coli* to kill *P. aeruginosa* by synthesizing a sensing-killing consortium, in which *E. coli* was engineered to specifically recognize, migrate toward, and eradicate both dispersed and biofilm-encased *P. aeruginosa* (Fig. 16C).[172] This consortium consisted of three genetic devices, *i.e.*, the sensing, motility and killing modules. In the sensing device in *E. coli*, a promoter *pJ23108* was used to initiate the expression of LasR. Upon the binding of 3OC12HSL with LasR in *E. coli*, the *pLasI* promoter was activated to induce the expression of the motility and killing modules, such that the expression of CheZ, YebF-DnaseI and YebF-McsS was initiated. CheZ is an integral member in controlling the chemotaxis of *E. coli*, lack of which would make *E. coli* non-motile. The introduction of the *cheZ* module into the *cheZ*-deficient *E. coli* strain aimed to make the motility of *E. coli* responsive to AHL concentrations, thus moving towards *P. aeruginosa*. The expression of microcin S (an antimicrobial peptide) and nuclease DNaseI resulted in the cell killing and biofilm degradation of *P. aeruginosa*, respectively. The secretion of these two proteins in *E. coli* was modulated by a secreting tag YebF. Thus, the secreted YebF-DNaseI and YebF-MccS could lead to the inhibition against *P. aeruginosa* in the extracellular medium.

Lactococci lactis, as a probiotic bacterium, was also engineered to kill Gram-negative pathogens by secreting heterologous antimicrobial peptides (AMPs).[173,174] Volzing *et al.* engineered a microbial killing consortium, in which *L. lactis* expresses two AMPs (A3APO and Alyteserin) under the control of a nisin-inducible promoter *PnisA*, which have high antimicrobial activity against the pathogens *E. coli* and *Salmonella*, but have significantly low antimicrobial activities against *L. lactis* itself (Fig. 16D).[173] This work demonstrated the great potential of using engineered probiotic bacteria to deliver AMPs to treat specific pathogen as a complementary antibiotic therapy.

Duan *et al.* engineered a bacterial communication consortium consisting of *Vibrio cholerae* and engineered *E. coli* to prevent the virulence of *V. cholerae*.[175] They transformed *E. coli* Nissle 1917 to express the autoinducer molecule cholera autoinducer 1 (CAI-1), which can prevent the expression of virulence gene HapR in *V. cholerae* when CAI-1 is present at a high concentration with another signaling molecule, autoinducer 2 from *V. cholerae*. In this way, pretreatment of mice for 8 h with this engineered *E. coli* significantly increased the survival of the mice (ingested with *V. cholera*) by 92%. Furthermore, they tested this engineered *E. coli* Nissle strain in Caco-2 epithelial cells incubated with *V. cholerae*, and the result suggested that co-culture with Nissle expressing CAI-1 activity reduced the binding of cholera toxin subunit B to Caco-2 cells by 63% compared with the co-culture with the wild-type Nissle.[176]

3.4.2. Synthetic phage-biofilm interactions. Bacterial biofilms are crucial in the pathogenesis of many clinical infections, and difficult to eradicate due to their great resistance to antimicrobial treatments and removal by immune systems. Bacteriophages are natural killers of bacteria by causing bacterial cell lysis, and thus have the potential for treating biofilm related diseases.[177] Lu *et al.* engineered bacteriophage-bacterial biofilm predation consortia to assist antibiotic therapy in biofilm treatments.[178,179] Biofilms are surface-associated communities encased in a hydrated extracellular polymeric substance (EPS) matrix comprising polysaccharides, proteins, nucleic acids, and lipids, *etc.* Lu *et al.* thus engineered a phage-bacteria predation ecosystem, in which the expression of dispersin B (DspB) in T7 bacteriophage can lead to the degradation of a cell-bound EPS polysaccharide adhesin β-1,6-*N*-acetyl-D-glucosamine in *Staphylococcus* and *E. coli*, and finally led to the killing of these pathogens' biofilms (Fig. 17A).[178] Engineered DspB-expressing T7 phage infected the *E. coli* biofilm, and replication of both phage and DspB would occur in the infected *E. coli*. Then both phage and DspB were released after cell lysis of *E. coli*, which led to the degradation of the crucial glucosamine component of biofilm EPS and the dispersal of *E. coli* biofilm by DspB and the subsequent infection of *E. coli* by the released phages. Thus, this engineered enzymatic bacteriophage could accomplish ~99.997% removal of biofilm, two orders of magnitude better than that of the non-enzymatic phage.

Lu *et al.* further developed phage-bacteria consortia by engineering bacteriophage as an effective antibiotic adjuvant that can overexpress proteins to target gene networks (*e.g.*, oxidative stress response systems, and biofilm formation regulation systems, *etc.*) that are not directly targeted by antibiotics.[179] As shown in Fig. 17B, Lu *et al.* engineered a bacteriophage to express genes of *CsrA* and *ompF* under the control of the P_L*tetO* promoter to target the *E. coli* biofilm formation process. CsrA is a global regulator that represses biofilm formation, thus making *E. coli* biofilm more susceptible to antibiotics (*e.g.*, quinolones) treatment, and OmpF is a porin for the penetration of quinolones into *E. coli* to enhance the antibiotic killing effect. Thus, the engineered phage with simultaneous expression of CsrA and OmpF significantly increases loxacin's bactericidal effect.

3.5. Applications in environments

3.5.1. Biodegradation of organic wastes by synthetic microbial consortia. One advantage of microbial consortia over single populations lies in their capability to perform more than one task, which is crucial in the biodegradation of pollutants since a complete mineralization of contaminants involves multiple enzymes that usually do not exist in one single strain, and neither strain in the consortia can carry out the overall tasks of pollutant biodegradation alone.[180] Naturally occurring microbial consortia have been applied in environment bioremediation for years. However, the biodegradation efficiency remains too low to fully meet industrial requirements, as the top priority for these consortia is survival, which might constrain their capability to

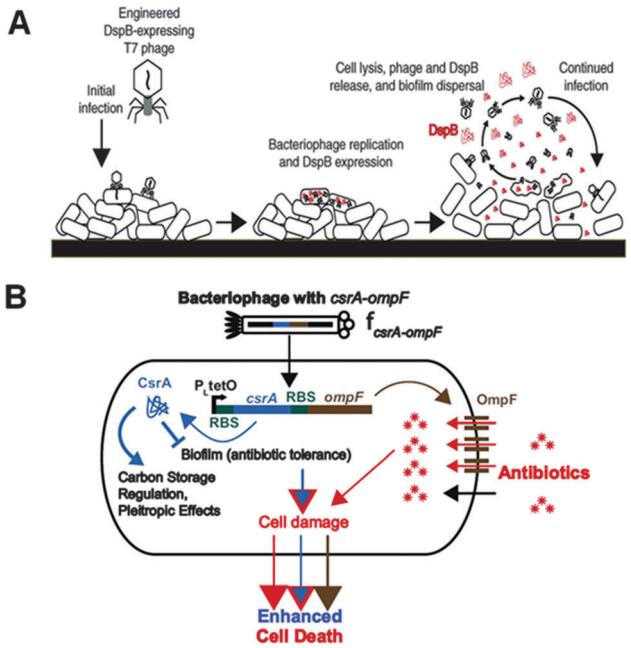

Fig. 17 Synthetic phage-bacterial biofilm predation consortia for efficient biofilm killing. (A) Engineered T7 bacteriophage to express dispersin B (DspB) to kill biofilms of *Staphylococcus* and *E. coli*. (B) Engineered bacteriophage to express CsrA that represses bacterial biofilm formation. Reproduced with permission from ref. 178 and 179. Copyright 2007 and 2009 The National Academy of Sciences of the USA.

biodegrade pollutants. Synthetic microbial consortia may help to accelerate the biodegradation efficiency in complex pollutant bioremediation by introducing new genetic devices and modules.

Commensal consortia were developed for the biodegradation of organic pollutants. Cowan *et al.*[181] constructed a commensal dual-species biofilm for the enhanced degradation of 2-chloroethanol and p-cresol. This consortium is composed of *Pseudomonas* sp. strain GJ1 (a 2-chloroethanol degrading microorganism) and *P. putida* DMP1 (a p-cresol degrading microorganism) (Fig. 18A). The consortium was in the tower- or mushroom-shaped biofilm clusters, in which GJ1 was surrounded by DMP1, which caused GJ1 to expose to a low concentration of p-cresol. Thus, DMP1 mitigated the inhibitory effects of p-cresol on GJ1, and these two strains maintained a commensal interaction. In another work, Fazzini *et al.*[182] constructed a commensal microbial consortium composed of *Pseudomonas reinekei* sp. strain MT1 and *Achromobacter xylosoxidans* strain MT3 for the enhanced biodegradation of 4-chlorosalicylate (4CS) (Fig. 18B). In this consortium, only *P. reinekei* MT1 is able to use 4CS as a carbon and energy source, and metabolic interactions between MT1 and *A. xylosoxidans* MT3 enabled the enhanced biodegradation of 4CS. 4CS generated a strong oxidative stress response in *P. reinekei* MT1 due to accumulation of toxic intermediates, 4-chlorocatechol and protoanemonin. Upon co-culturing of *A. xylosoxidans* MT3 and *P. reinekei* MT1, 4-chlorocatechol (a toxic intermediate) was partially taken up and further degraded by MT3, thus MT3 helped to prevent toxic intermediate accumulation that inhibited MT1. Meanwhile, in the mixed culture, *P. reinekei* MT1 expressed a major outer membrane porin OprF, which increased the substrate permeation and catabolism.

Chen *et al.*[183] synthesized a mutualistic microbial consortium to degrade petroleum pollutions by co-culturing two bacteria, *Acinetobacter* sp. XM-02, a hydrocarbon degradation bacterium, and *Pseudomonas* sp. XM-01 that is unable to grow on alkane hydrocarbons but can produce rhamnolipid with glycerol as the sole carbon source (Fig. 18C). *Acinetobacter* sp. XM-02 degraded hydrocarbons to produce intermediates which could be subsequently utilized by *Pseudomonas* sp. XM-01 to produce rhamnolipid, a biosurfactant that can enhance petroleum degradation by XM-02.

Dejonghe *et al.* developed a microbial consortium including five strains (*Variovorax* sp. strain WDL1, *Delftia acidovorans* WDL34, *Pseudomonas* sp. Strain WDL5, *Hyphomicrobium sulfonivorans* WDL6, and *Comamonas testosteroni* WDL7), which was able to subsequentially degrade linuron, a herbicide (Fig. 18D).[184] WDL1 could degrade linuron to 3,4-dichloroaniline (3,4-DCA) and *N*,*O*-dimethylhydroxylamine by using it as the carbon and energy source. WDL34 and WDL7 were responsible for the degradation of the intermediate 3,4-DCA, which thereby seemed to protect WDL1 from its toxicity. WDL6 was the only strain to degrade *N*,*O*-dimethylhydroxylamine. When these strains were mixed in a synthetic community, the rate of linuron degradation was significantly improved due to their synergistic interactions.

3.5.2. Bioelectricity harvest by synthetic microbial consortia in microbial fuel cells. Bioelectrochemical systems (BESs), including microbial fuel cells (MFCs), microbial electrolysis cells, and microbial electrosynthesis *etc.*, are sustainable and green technologies that enable simultaneous wastewater treatment and energy/chemical production.[185–189] Syntrophic microbial consortia were widely used in MFCs due to their capability to degrade a wide spectrum of complicated substrates by the fermentative microorganisms. The subsequent utilization of these fermentation end products by the exoelectrogens (producing electricity *via* their extracellular electron transfer pathways) can relieve feedback inhibition on the fermentative microbes, allowing rapid metabolism of substrates.[190]

Venkataraman *et al.* found that the current density produced by a symbiotic co-culture consisting of *Enterobacter aerogenes* (the fermentative microbe) and *P. aeruginosa* PA14 (the exoelectrogen) was at least 14-fold higher compared to either of the two bacteria alone.[191] Metabolic analysis revealed the metabolite-mediated mutualism between the two bacteria, in which 2,3-butanediol produced by glucose-fermenting *E. aerogenes* was subsequently consumed by *P. aeruginosa*. On the other hand, current production by *P. aeruginosa* using 2,3-butanediol as a carbon source was increased by 2-fold compared with glucose as a carbon source due to the enhanced production of phenazines, the electron shuttles using which *P. aerugenosa* performs its extracellular electron transfer and bioelectricity production.

Shewanella oneidensis MR-1 is one of the most well-studied exoelectrogens, which can only use lactate as an electron donor for current production.[192] To expand the carbon source (*e.g.*, glucose, unmetabolizable by *S. oneidensis*) as an electron donor, Rosenbaum *et al.* constructed a microbial consortium consisting

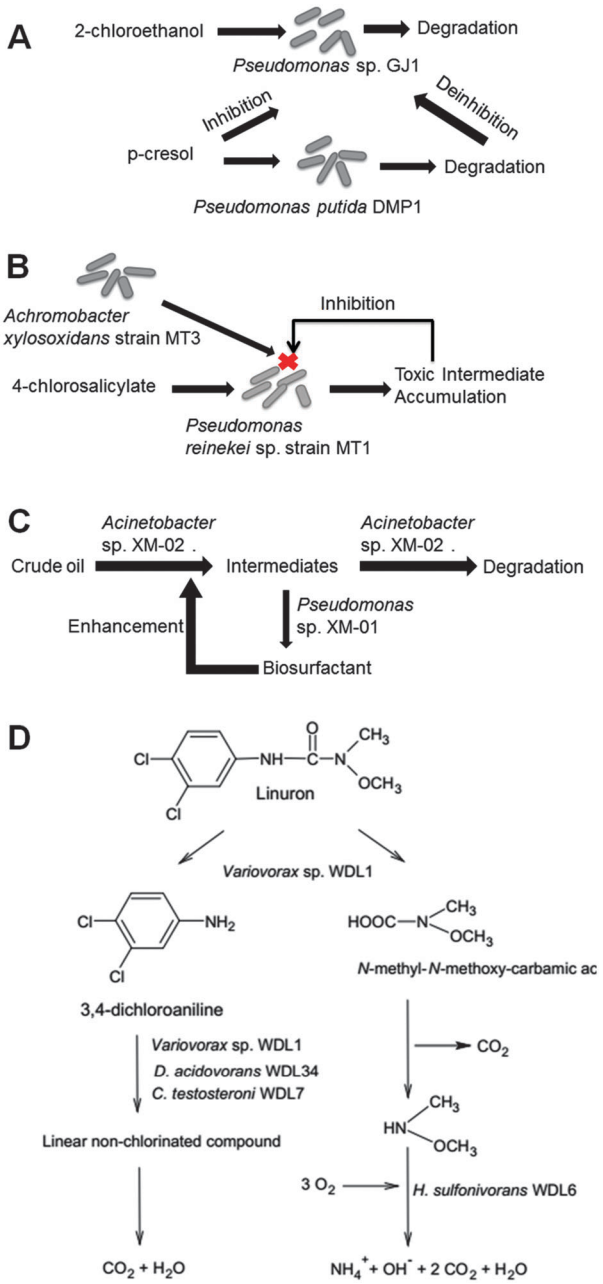

Fig. 18 Interspecific interactions in mixed microbial cultures in organic biodegradation. (A) *Pseudomonas sp.* strain GJ1, a 2-chloroethanol (2-CE) degrading organism, and *P. putida* DMP1, a p-cresol degrading organism, maintained a commensal relationship with DMP1 mitigating the inhibitory effects of p-cresol on GJ1 in a waste stream composed of 2-CE and p-cresol. (B) A commensal co-culture of the *P. reinekei* sp. strain MT1 with *A. xylosoxidans* strain MT3 could enhance the biodegradation of 4-chlorosalicylate. MT3 could degrade toxic intermediates produced by MT1, thus relieving their inhibitory effects on MT1. (C) *Acinetobacter sp.* XM-02 degraded hydrocarbons to intermediates, which were subsequently utilized by *Pseudomonas sp.* XM-01 producing rhamnolipid, a biosurfactant that can promote oil degradation by *Acinetobacter sp.* XM-02, forming a mutualistic consortium. (D) A microbial consortium for subsequential bioremediation of linuron. *Variovorax sp.* strain WDL1 first converted linuron to 3,4-dichloroaniline (3,4-DCA), *D. acidovorans* WDL34 and *Comamonas testosteroni* WDL7 were responsible for degradation of the intermediate 3,4-DCA, and WDL6 was the only strain to degrade *N,O*-dimethylhydroxylamine. Reproduced with permission from ref. 184. Copyright 2003 American Society for Microbiology.

of *S. oneidensis* and *Lactococcus lactis* to allow *S. oneidensis* to produce electricity with glucose as the primary fuel.[193] In this synergistic consortium, *L. lactis* breaks down glucose into lactate, which was subsequently used by *S. oneidensis* as a carbon source for current production.

Geobacter spp., the most efficient exoelectrogens, are strictly anaerobic bacteria.[194] However, in MFC processes, oxygen leaking from cathode chambers may enter anodic chambers, such that a substantial amount of substrates may not be able to generate current, thus columbic efficiency of MFCs can be significantly reduced. Qu *et al.* constructed a commensal consortium that consists of *Geobacter sulfurreducens* (exoelectrogen) and *E. coli* (nonexoelectrogen), in which *E. coli* can exhaust oxygen that may leak into the reactor, thus promoting the growth of *G. sulfurrducens* and its power generation.[195]

4. Concluding remarks

Many microbial consortia with diverse interaction modes such as mutualism, commensalism and predation were constructed by synthetic biology approaches in the last decade. Meanwhile, synthetic microbial consortia were applied in many fields, ranging from addressing fundamental ecological questions, distributed biocomputations, to chemical and bioenergy production, biomedicine, and environmental bioremediation. One great advantage of these synthetic microbial consortia is that they have well-defined genetic traits, and tractable and regulatable interactions between their components, which in turn enables optimization of their behaviors by many synthetic biology tools such as transcriptional, translational and post-translational devices.

However, these synthetic microbial consortia achieved so far are relatively simple, in which only two or three engineered microorganisms were recruited and coherently engineered. In many natural and engineered niches such as associated with environments (*e.g.*, marine, soil, sludge, and petroleum) and human health (*e.g.*, gut and skin), most naturally occurring microbial ecosystems are usually extremely complex in terms of their ecological structures, interaction patterns, and fluctuating environmental and evolutionary stresses, making rational engineering and optimization of these complicated ecosystems extremely challenging. It is envisioned, on the one hand, that rational design and synthesis of more complicated interspecies ecosystems capable of performing complex ecological or biological functions will be a trend. Such engineered ecosystems would act as novel model systems to emulate sophisticated spatiotemporal behaviours of their naturally occurring counterparts, to exploit intercellular communications and synergistic cooperation observed in nature, to provide novel insights into the influences of spatiotemporal microenvironments and modulation of metabolic interactions among species on the stable coexistence of multispecies microbial communities, thus to address many complex ecological questions. These rationally designed microbial ecosystems would also enable novel applications in the chemical industry, energy, environment, and healthcare.

On the other hand, exploiting synthetic cells or synthetic ecosystems to interfere and interact with existing ecological

systems in naturally occurring niches would be promising to modulate their behaviours and functions or create new functions that do not exist in nature, enabling novel applications. For example, introducing specially designed and synthesized cells or synthetic microbial consortia to interact with the microbial flora in patients gut would be a promising strategy in the treatment of many diseases associated with gut microbial flora. In bioremediation of recalcitrant pollutants and enhanced petroleum recovery, exogenous introduction of synthetic microbes or consortia into these natural ecosystems would be a novel strategy for achieving function augmentation and efficiency enhancement in these ecosystems. Meanwhile, a further mechanistic understanding of the interactions between these exogenously introduced microbial consortia and the intact microbial ecosystems would be crucial in the optimal and rational design of these synthetic microbes.

We envision that with the synergistic development and integration of systems biology tools (including meta'omics technologies for population profiling and analysis,[196–198] analytical tools for studying molecular interactions in microbial populations,[199,200] and genome-scale community modelling[57,198,201,202]) and synthetic biology tools (including genome-wide engineering and whole genome synthesis[203–206]), the interaction mechanisms of complex microbial communities will be thoroughly elucidated and understood, and rational engineering of these complicated microbial communities for many intriguing applications will be achieved in the foreseeable future.

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