



Design and construction of synthetic microbial consortia in China

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ABSTRACT

The rapid development of synthetic biology enables the design, construction and optimization of synthetic microbial consortia to achieve specific functions. In China, the “973” project—“Design and Construction of Microbial Consortia” was funded by the National Basic Research Program of China in January 2014. It was proposed to address the fundamental challenges in engineering natural microbial consortia and reconstructing microbial consortia to meet industrial demands. In this review, we will introduce this “973” project, including the significance of microbial consortia, the fundamental scientific issues, the recent research progresses, and some case studies about synthetic microbial consortia in the past two and a half years.

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1. Introduction

In natural environments, 99% microorganisms exist in the form of microbial consortia. However, some defects of naturally occurring microbial consortia, such as difficulty in culturing, long operation cycle, low conversion efficiency, and poor stability and

controllability, limited their practical applications in biotechnology industries. Synthetic microbial consortia constructed via synthetic biology approaches would be an alternative for programming novel complex behaviors and optimal features for practical biotechnology applications. Arnold [1] and Weiss [2,3] pointed out that synthetic microbial consortia could perform even more complicated tasks and endure more changeable environments than that of monocultures, thus providing an important new frontier for synthetic biology. A better knowledge of the multicellular systems that drive cell-cell interactions in the consortia was highly needed [4,5]. Engineering novel cell-cell interaction capabilities became crucial in the nascent field of synthetic biology [2].

Recently, scientists have made great progresses about analysis,

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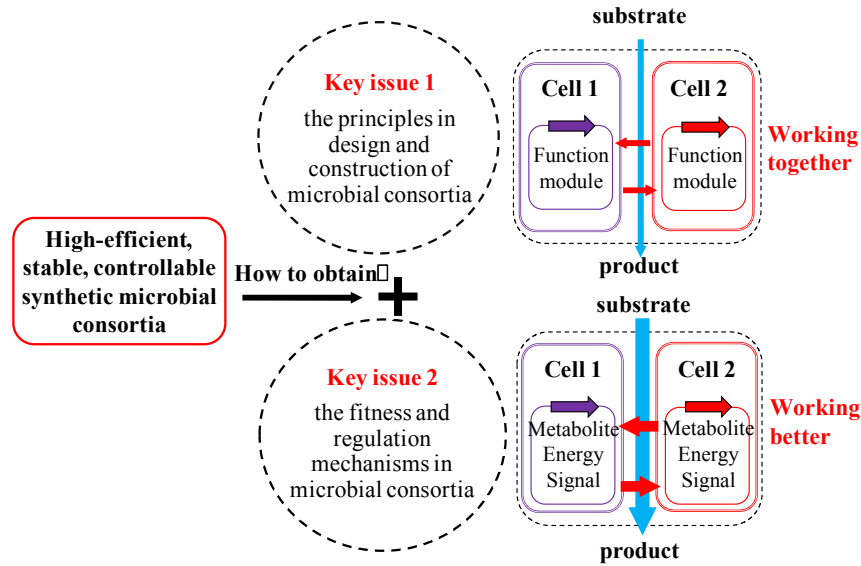


Fig. 2. Key issues for achieving high-efficient, stable, and controllable synthetic microbial consortia.

the transformation of metabolite, the restriction on transformation of energy and signal molecules. Thus, optimization of synthetic microbial consortia on metabolic balance, energy utilization and signal recognition would be beneficial for the synergistic effect between cells (Fig. 3). Interactions between microbial species are generally mediated by the exchange of small molecules, secreted by one species and consumed by another. In addition, the balance of the electron transfer system and the energy barrier, and signal molecules interaction are also important for the construction of microbial ecosystems. Focusing on the key signal molecules and the production, secretion, diffusion, absorption, consumption and response of metabolites, systematic analysis is essential for design, construction and improvement of the microbial consortia.

3. Research progress in “973” project about microbial consortia

3.1. Case study 1: one-step fermentation of vitamin C

To achieve rational design and construction of a target microbial consortium, a detailed understanding of the multicellular interaction is a prerequisite. Systematic understanding of the interaction can provide comprehensive and in-depth clues into the design and construction of the microbial consortia [28–32]. On the other hand, systems-level knowledge of synthetic microbial consortia will

provide in-depth understanding of the consortia and thus redesigning the biological functions of artificial consortia via synthetic biology [33,34].

3.1.1. Genomics analysis of the bacteria in microbial consortium

The conventional two-step fermentation, consisting of *Glucobacter oxydans*, *Bacillus* spp. and *Ketogulonicigenium vulgare*, was widely adopted in the industrial production of 2-keto-L-gulonic acid (2-KGA), the precursor of vitamin C. During the second step, *K. vulgare* is responsible for the biosynthesis of 2-KGA from L-sorbose, and *Bacillus* spp., as a companion, promotes the growth and production efficiency of *K. vulgare*. The in-depth analysis of the interaction between *Bacillus* spp. and *K. vulgare* was conducted. Firstly, the genome sequence of *B. megaterium* [35] and *K. vulgare* [36,37] was analyzed and annotated, which provided a better-defined genetic background for studies in gene expression and regulation, especially the genome-scale metabolic network construction. The metabolomic and proteomic analyses were also carried out on the *B. megaterium*-*K. vulgare* consortium [29–32]. The systematic analyses on vitamin C-producing strains provided deeply insights into the relationship between the two microorganisms, which benefited our reconstruction of the microbial consortium.

The complete genome of *K. vulgare* Hbe602 was deciphered to provide insights into the symbiosis mechanism and the versatile metabolism [36]. Comparative genomic analyses of *K. vulgare*

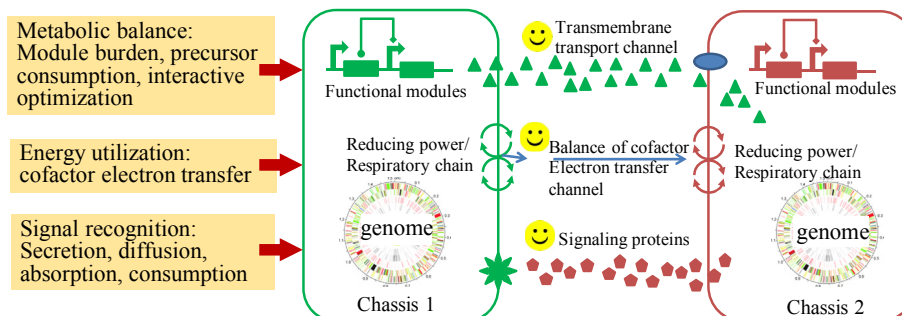


Fig. 3. Fitness and regulation of synthetic microbial consortia.

Hbe602, WSH-001 and Y25 were carried out, and the differences with WSH-001 and Y25 were labeled in the red and yellow dots, respectively (Fig. 4). Through the whole genome comparison, the different genes with the nucleotide identities lower than 90% were obtained, mostly focusing on proteolytic enzymes and transporters. The complete genome sequencing of *B. thuringiensis* Bc601³⁵ and *B. endophyticus* Hbe603³⁷ were reported, and the comparative genomics analysis was carried out, which enabled a deeper understanding of the cooperative mechanism with *K. vulgare*, and facilitated the optimization of bacterial consortium. In all, *B. endophyticus* provided essential functions that *K. vulgare* lacked to reach its maximum growth rate and acted as an alternative source of environmental nutrients in the consortium.

Furthermore, metabolic network of *B. thuringiensis* Bc601 and *B. endophyticus* Hbe603s was obtained, including the central carbon metabolism, amino acid metabolism and cofactor metabolism (Fig. 4). In the central carbon metabolism, the complete glycolysis, citrate cycle (TCA cycle) and pentose phosphate pathway in the two microbial species were identified. In the amino acid metabolism, *B. endophyticus* Hbe603 has more lysine degradation related genes than *B. thuringiensis* Bc601. In the cofactor and vitamin metabolism, both species have the complete biosynthesis pathway of folate, protoheme, pantothenate and CoA, while defect in the lipoic acid and biotin biosynthesis pathway.

3.1.2. Reconstruction of one-step vitamin C fermentation

During the conventional two-step fermentation of vitamin C, the long incubation period and twice sterilization processes inhibited the further optimization significantly. Therefore, production of 2-KGA by mono-cultured *G. oxydans* [38] and a synthetic *G. oxydans*-*K. vulgare* consortium [22] were constructed and then enhanced by genetic modification. In the one-step fermentation of 2-KGA by *G. oxydans*-*K. vulgare*, the final titer of 2-KGA is 76.6 g/L,

and the yield of 2-KGA against D-sorbitol reached 89.7% within 36 h, which was comparable to that of the conventional two-step fermentation (about 90% within 48 h)²¹. Meanwhile, comparing with the traditional method, one-step fermentation shortens the fermentation time by about 25% and eliminates the need for a second sterilization process. In this way, the rate of equipment utilization could be significantly improved and the production cost could be notably saved. Moreover, the symbiotic interaction between the two microorganisms was optimized to perform better (Fig. 5). The relationship between the two microbes changed from commensalism & competition to mutualism. The metabolic interaction between the strains was further investigated by metabolomics, which verified the enhancement of the mutualism between the microbes and provided us potential strategies for further improving the synthetic consortium.

3.2. Synthesizing microbial consortia for power generation in microbial fuel cells

Microbial fuel cells (MFCs) were capable of converting chemical energy stored in chemical compounds to electrical energy by the metabolism of microorganisms. MFCs have functional and operational advantages over the technologies currently used for generating energy from a variety of organic substrates, including sugars, cellulose, organic acids and wastewater pollution. First, the direct conversion of varies of substrate energy to electricity enables high conversion efficiency much better than wind and solar energy. Second, MFCs can operate efficiently at ambient, and even at low, temperatures distinguishing them from all current bio-energy processes. Third, MFCs have potential for widespread application in locations lacking electrical infrastructures and also to expand the diversity of fuels we use to satisfy our energy requirements. *Shewanella oneidensis* MR-1 was one of the well-established model

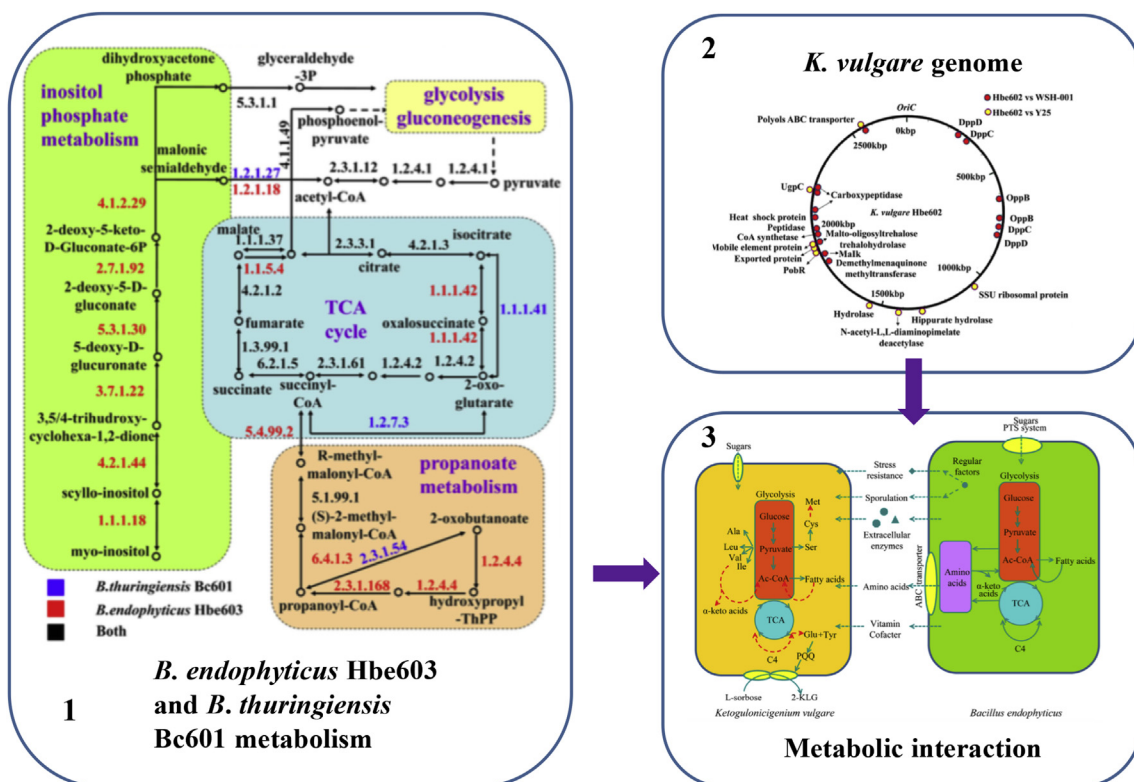


Fig. 4. Genomic and metabolomic analysis of bacteria and the cell-cell interaction in vitamin C fermentation. Part 1 is adapted by permission from Scientific Reports [35] ©. Part 2 is adapted by permission from Scientific Reports [36] ©. Part 3 is adapted by permission from PLoS One [37] ©.

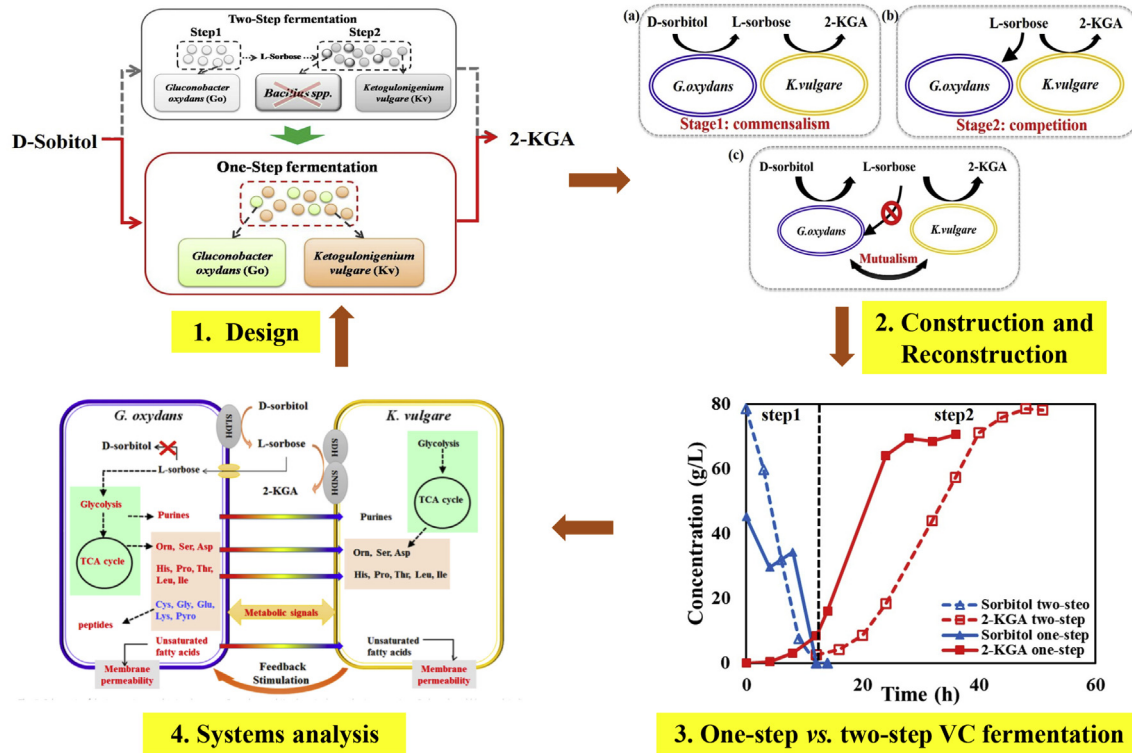


Fig. 5. Reconstruction of vitamin C one-step fermentation and optimization of the relationship between bacteria. Parts 1, 2, 3, 4 are adapted by permission from Microbial Cell Factories [22].

exoelectrogen, which was widely used in MFCs for wastewater treatment and power generation. Single-species MFCs faced many practical barriers in industrial applications, especially its low extracellular electron transfer (EET) rate and narrow range of substrates such as lactate, pyruvate and formate [39,40]. In addition, engineering single strain to perform multi-tasks would induce metabolic burden and low viability for power production in MFCs. To solve these issues, extending engineering capabilities from single cells to multicellular microbial consortia brought new inspiration and strategies for improving performance in MFCs. Yang

et al. [20] recently designed an *E. coli-S. oneidensis* co-culture system, in which the genetically engineered *E. coli* played as a fermenter to consume xylose for the synthesis of formate and flavins to feed the exoelectrogen (*S. oneidensis*) as the carbon source and electron donor, respectively. Thus, a high-performance xylose-fed MFC system for bioelectricity production in MFCs was developed. However, the fermenter *E. coli* would form biofilms to occupy active anode surfaces, which would reduce the power generation by exoelectrogen. Therefore, we used *S. cerevisiae* incapable of forming biofilm at anaerobic MFC conditions to replace *E. coli* to

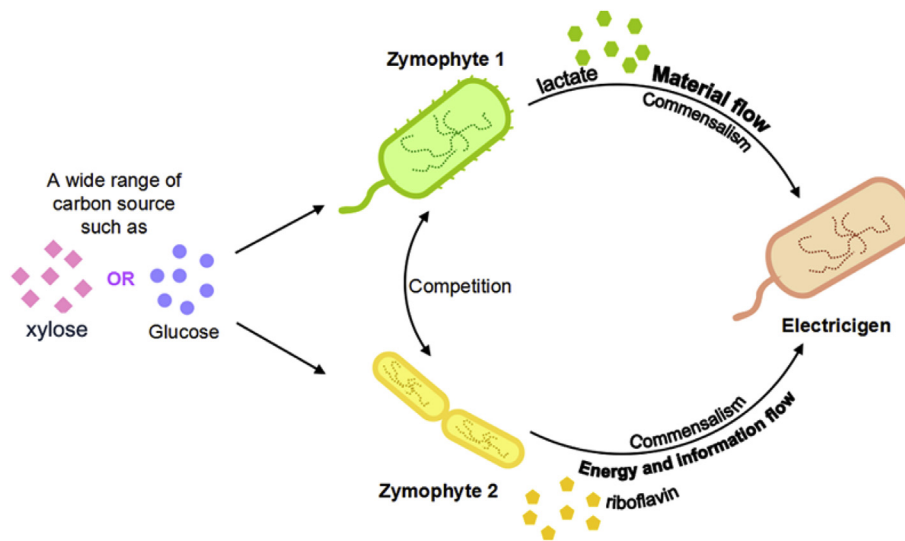


Fig. 6. A synthetic 3-species microbial consortium for high-performance microbial fuel cell (MFC) system. The three microbial species were *E. coli*, *B. subtilis*, and *S. oneidensis*.

develop a fungus (*S. cerevisiae*)-bacterium (*S. oneidensis*) co-culture, which avoided the fermenter cells to occupy anode surfaces. By engineering *S. cerevisiae* to metabolize glucose to produce lactate but not ethanol, the engineered *S. cerevisiae*-*S. oneidensis* consortium enabled a glucose-fed high performance MFCs.

To take advantage of the capability of *B. subtilis* for riboflavin (the electron shuttle of *S. oneidensis*) biosynthesis, the TJU iGEM Team designed and constructed a three-species co-culture system with *E. coli*, *B. subtilis* and *S. oneidensis* by the principle of “division of labor” (Fig. 6). To enable the three-species microbial consortium with high-performance MFC was established by optimizing the interaction of material, information and energy interactions between the three microbial species. This project won the best energy project in the 2015 iGEM competition (<http://2015.igem.org/Team:TJU/Overview>).

4. Perspective

Natural ecosystems have complex microbial community compositions and functions. Environmental variation may exert significant influence upon the metabolic exchange, energy flux and nutrient cycling within even the simplest microbial consortia. So far, most studies about synthetic microbial consortia mainly focus on “build a consortium to understand it”. For synthetic microbial consortia, more design principles and models are required to reveal the interactions and dynamic changes of high-performance synthetic systems. Based on the understanding of the interaction among microbes, the improvement of robustness, stability and reproducibility should be further explored. Further development on ecological principles, engineered strategies and modeling tools will help us to achieve significant progress in designing diverse synthetic ecology with the reproducibility, robustness, controllable and even self-regulating characterizations.

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