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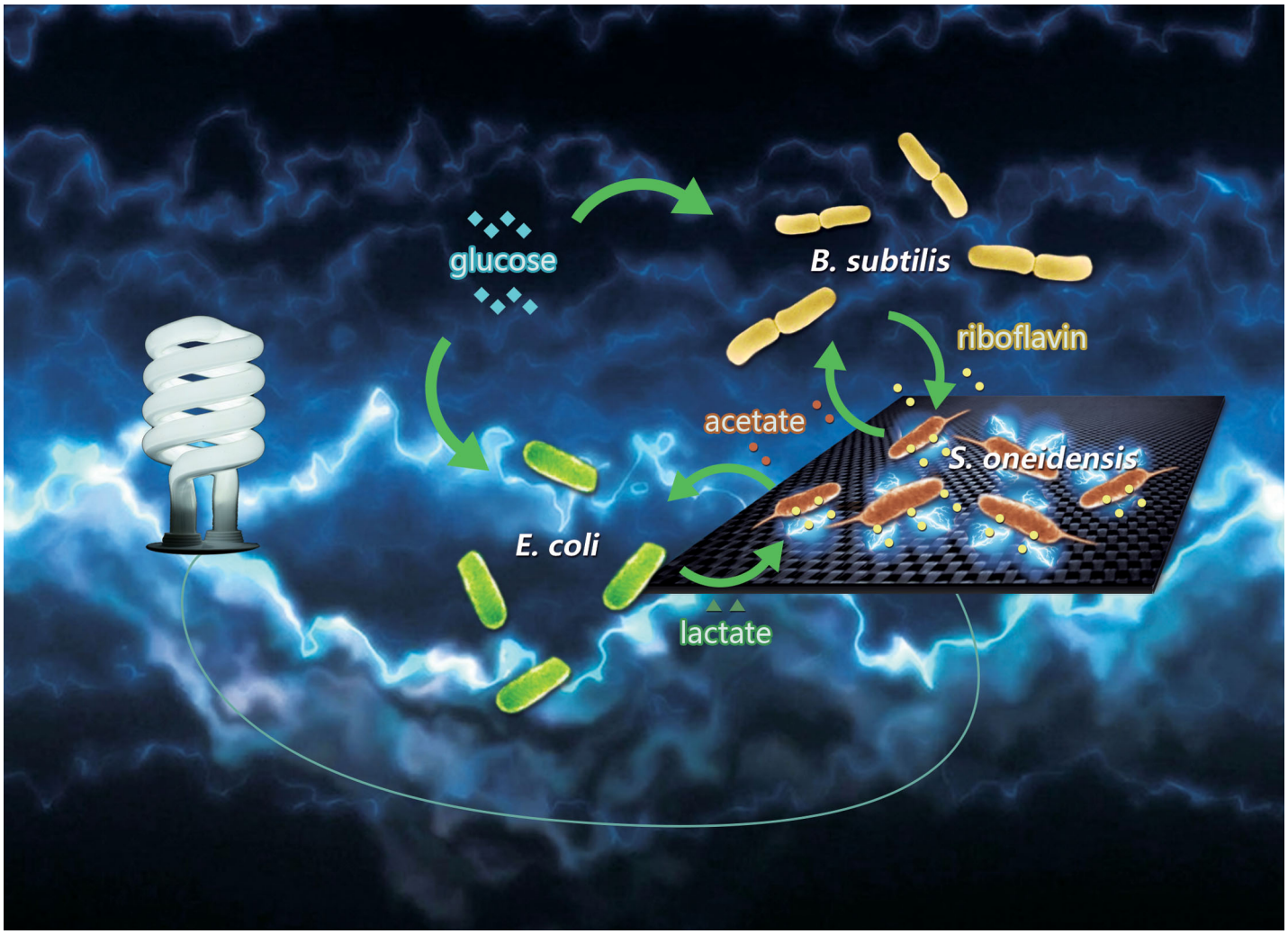

Showcasing research from Prof. Ying-Jin Yuan and Prof. Hao Song's group at Tianjin University, China.

A three-species microbial consortium for power generation

Synthetic microbial consortia with defined composition and interaction modes hold great promise for diverse bioenergy and environmental applications. By exploring the principle of "division-of-labor", we rationally designed and constructed a synthetic three-species microbial consortium that enabled highly efficient and stable electricity generation from sugar.

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A three-species microbial consortium for power generation†

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Design and construction of synthetic microbial consortia is a promising strategy to enhance the performance of bioelectrochemical systems (BESs) and to facilitate practical applications in bioenergy production. According to the design principle of "division-of-labor", we synthesized a three-species microbial consortium for power generation, consisting of engineered *Escherichia coli*, *Bacillus subtilis* and *Shewanella oneidensis*. In this consortium, *E. coli* digested glucose to produce lactate as a carbon source and an electron donor; *B. subtilis* produced riboflavin as an electron shuttle; and *S. oneidensis* served as the exoelectrogen to generate electricity. In return, *S. oneidensis* oxidized lactate to acetate, which fed *E. coli* and *B. subtilis* as the carbon source. Thus, the three species formed a cross-feeding microbial consortium, which performed "better together" for power generation. As a result, glucose (11 mM, total 0.28 g) was converted to electricity for more than 15 days with high energy conversion efficiency (up to 55.7%). The microbial composition and electricity output were stable throughout the operation cycle. Furthermore, the consortium exhibited highly functional robustness to fluctuations in the initial inoculation ratio of the three strains. This system provided new insight into the rational design of more efficient, stable, and robust synthetic microbial consortia applicable in bioenergy and environmental bioremediation.

Broader context

Bioelectrochemical systems (BESs) may benefit from microbial consortia that efficiently convert carbon sources to electricity. A key challenge with this system is how to manipulate the distribution of carbon source metabolism and energy generation to maximize electricity generation. Herein, we rationally designed, constructed and tested a synthetic three-species microbial consortium to enhance the energy conversion efficiency. In our system, engineered *Escherichia coli* and *Bacillus subtilis* efficiently converted a total of 0.28 g glucose to lactate (carbon source and electron donor) and riboflavin (electron shuttle), which enabled *Shewanella oneidensis* to generate a stable current for over 15 days. In return, *S. oneidensis* could oxidize lactate to acetate, which could be used by *E. coli* and *B. subtilis* as the carbon source. Our study established a novel synthetic three-species consortium by the design strategy of "division-of-labor" and each individual performed "better together" to achieve highly efficient conversion of sugar to electricity. Our results lay the foundation for further optimization of more complex microbial consortia for energy and environmental applications.

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Introduction

Synthetic microbial consortia with a defined composition and controllable functions hold great promises for diverse bioenergy and environmental applications,[1,2] including bioelectrochemical systems (BESs) for sustainable bioelectricity production in sediment microbial fuel cells, power distributed biosensors in the sea, microbial electro-fermentation and electrosynthesis systems for the production of chemicals, wastewater treatment, *etc.*[3–6]

Microbial consortia with wild-type microbes were used in many BESs to degrade complex and organic pollutants for the production of electricity and chemicals.[7–9] However, owing to unknown genetic backgrounds of many wild-type species and uncharacterized microbial interaction mechanisms in the BESs, the energy conversion efficiency of these microbial consortia was difficult to be further optimized, which greatly restricted their practical applications.

Many synthetic biology techniques and tools were developed and could be used to program electroactive microorganisms and microbial consortia (*e.g.*, interactions between fermenters and exoelectrogens) to promote the energy transfer efficiency of BESs.[10–14] So far, synthetic microbial consortia with two-species have been successfully applied to BESs to convert organic wastes into electricity.[15,16] For example, there have been a few reports on synthetic two-species consortia including *Shewanella oneidensis*, a model exoelectrogen, for power generation.[17,18] However, synthetic microbial consortia with three or more engineered species for BESs were less studied.[19] Programming synthetic microbial consortia with three or more species requires a detailed knowledge of how to manipulate the composition, function and interaction of these microbes, which posed great challenges in the design and construction of robust consortia to perform complicated tasks in dynamic environments of BESs.[20–25]

To elucidate the design principles of synthesizing more complicated and robust microbial consortia, we herein used synthetic biology approaches to design and construct a synthetic three-species microbial consortium for electricity generation, which consisted of engineered *Escherichia coli, Bacillus subtilis* and *Shewanella oneidensis*. We explored the principle of "division of labor"[20,26] to distribute the metabolism and extracellular electron transfer functions among the fermenters (engineered *E. coli* and *B. subtilis*) and the exoelectrogen (*S. oneidensis*) in this synthetic consortium. The three species could efficiently exchange essential metabolites to form a cross-feeding microbial consortium,[27] which performed "better together"[28] for power generation. This system could also enhance our understanding of complex microbial consortia in natural environments, and serve as a model system for designing more complicated and efficient synthetic microbial consortia for energy and environmental applications.

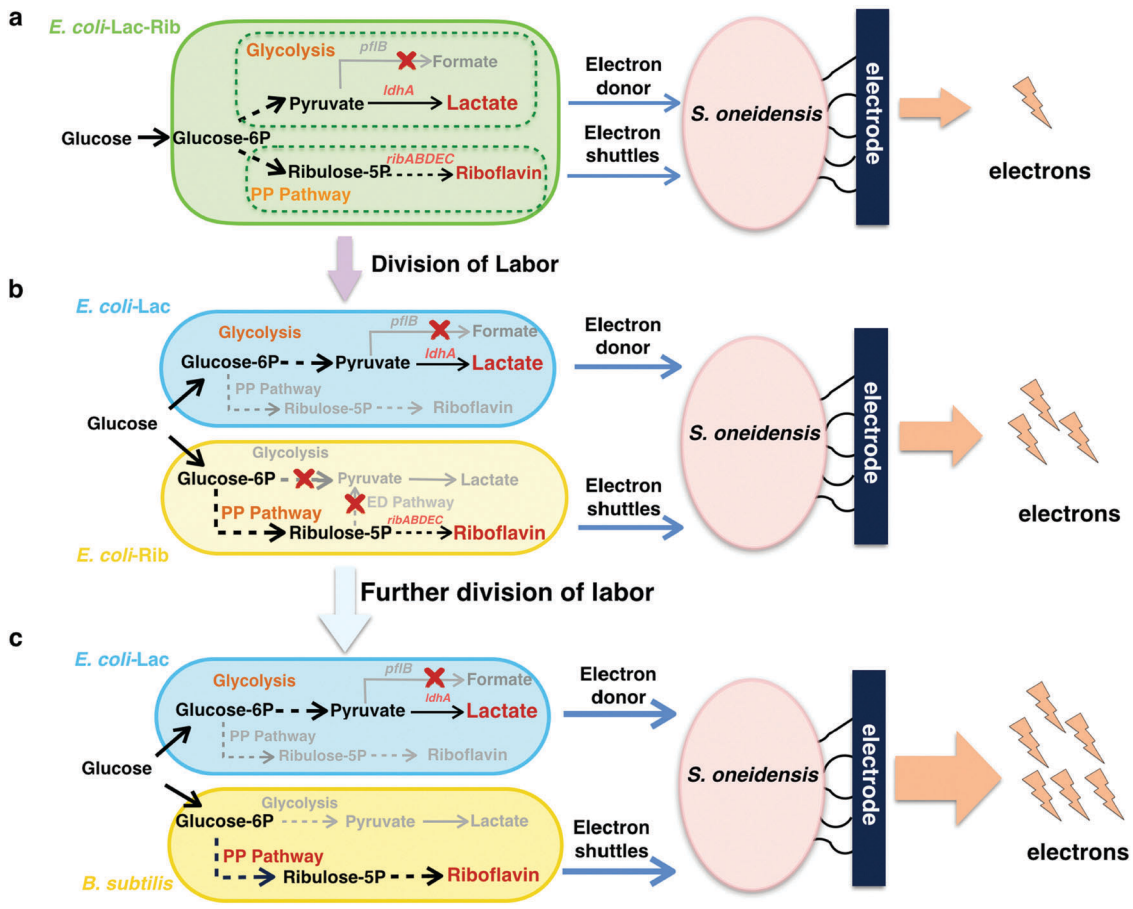

Fig. 1 Schematic of synthetic microbial consortia based on division of labor. (a) *E. coli*-Lac–Rib was engineered to produce lactate and riboflavin as a carbon source and an electron shuttle for *S. oneidensis*. (b) Based on division of labor, metabolic functions of lactate and riboflavin production were divided into two *E. coli*. *E. coli*-Lac was engineered to produce lactate as a solo carbon source by knockout of the *pflB* gene. *E. coli*-Rib was engineered to overproduce riboflavin by overexpressing the *ribABDEC* cluster and reducing glycolysis and ED pathways. (c) For further labor specialization, *B. subtilis* with higher riboflavin production capability was introduced into the ecosystem because *B. subtilis* could also produce a certain amount of lactate.

Results and discussion

Design and construction of synthetic three-species microbial consortium

The principle of ''division-of-labor'' has been commonly used to construct synthetic microbial consortia with two constituent individuals to accomplish complex tasks and to increase productivity.[29,30] Herein, we explored and applied this principle in a step-by-step manner to design a synthetic three-species consortium that was highly efficient for electricity generation. First, *E. coli* was engineered to convert glucose to lactate and riboflavin (named *E. coli*-Lac–Rib) for *S. oneidensis* to use as a carbon source and an electron shuttle to generate electricity, respectively (Fig. 1a).

To reduce individual metabolic burden and enhance productivity, the distinct functions of lactate and riboflavin production were then separated into two *E. coli*, using ''division-of-labor'' principles (Fig. 1b). *E. coli*-Lac was obtained by knockout of the *pflB* gene to produce lactate. Another *E. coli* was engineered by overexpressing *ribABDEC* genes and rerouting carbon flux to the pentose phosphate pathway (PP pathway) (named *E. coli*-Rib).[31] The production capacity of lactate (*i.e.*, the produced lactate concentration normalized by the total cell number) was increased by tenfold from 6.19×10^{-10} mM per cell to 6.24×10^{-9} mM per cell (Fig. 2a). *E. coli*-Rib converted 11 mM glucose to 19.26 μM riboflavin, which was much higher than that converted by *E. coli*-Lac–Rib (3.24 μM) (Fig. 2c).

For further division of labor, the most competitive riboflavin producer, engineered *B. subtilis* was introduced to replace *E. coli*-Rib (Fig. 1c).[32] The synthetic three-species microbial consortium consisting of *S. oneidensis*, *B. subtilis* and *E. coli* could convert 11 mM glucose to a total of 17.7 mM lactate, which was higher than that converted by the *S. oneidensis, E. coli*-Lac, and *E. coli*-Rib consortium (Fig. 2b). *B. subtilis* produced 28.3 μM of riboflavin, almost 1.5 times higher than that in the case of *E. coli*-Rib (Fig. 2c). In the above synthetic microbial consortia, the consortium with heterogeneous strains exhibited the best performance in terms of lactate and riboflavin production through ''division-of-labor''.

Mutualistic interactions by cross-feeding

Cross-feeding interactions in which bacterial cells exchange valuable and essential metabolites for the benefit of other species are ubiquitous in the microbial ecosystem.[33,34] Metabolic interactions, especially nutrient exchange, are key to building microbial consortia.[35–37] In our synthetic three-species microbial consortium, *E. coli* and *B. subtilis* were designed to convert glucose to lactate and riboflavin for *S. oneidensis*. In return, *S. oneidensis* oxidized lactate to acetate as a carbon source to feed *E. coli* and *B. subtilis* (Fig. 3a).[38,39] Each individual was separately cultivated under anaerobic conditions to test the metabolic interactions. Single strain *S. oneidensis* cultures could directly use lactate but not glucose for electricity generation and the efficiency of extracellular electron transfer (EET) was increased by adding riboflavin (Fig. 3b). *E. coli* converted glucose to 1.3 g l^{-1} lactate within 48 h (Fig. 3c). *B. subtilis* converted glucose to 28.3 μM

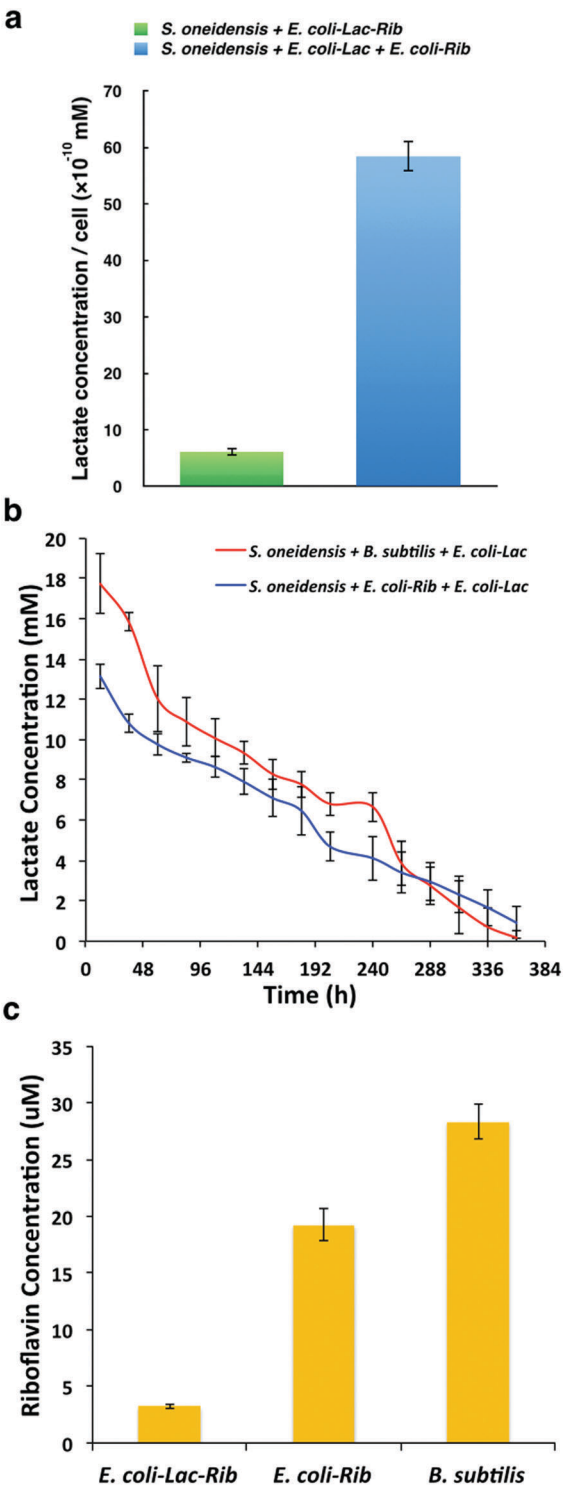

Fig. 2 Comparison of lactate and riboflavin production. (a) After division of labor, the lactate production capacity was increased tenfold from 6.19×10^{-10} mM per cell to 6.24×10^{-9} mM per cell. (b) Overall lactate concentration profiles of *S. oneidensis* + *B. subtilis* + *E. coli*-Lac, and *S. oneidensis* + *E. coli*-Rib + *E. coli*-Lac consortia. In both microbial consortia, lactate was consumed completely in 360 h. In the synthetic three-species microbial consortium, 18 mM lactate was completely converted from 11 mM glucose, 4 mM higher than that of the *S. oneidensis* + *E. coli*-Rib + *E. coli*-Lac consortium because *B. subtilis* also produced lactate. (c) Riboflavin production by *E. coli*-Lac–Rib, *E. coli*-Rib and *B. subtilis* after culturing with 11 mM glucose as a carbon source. All experiments were performed at least in triplicate; error bars indicate s.d.

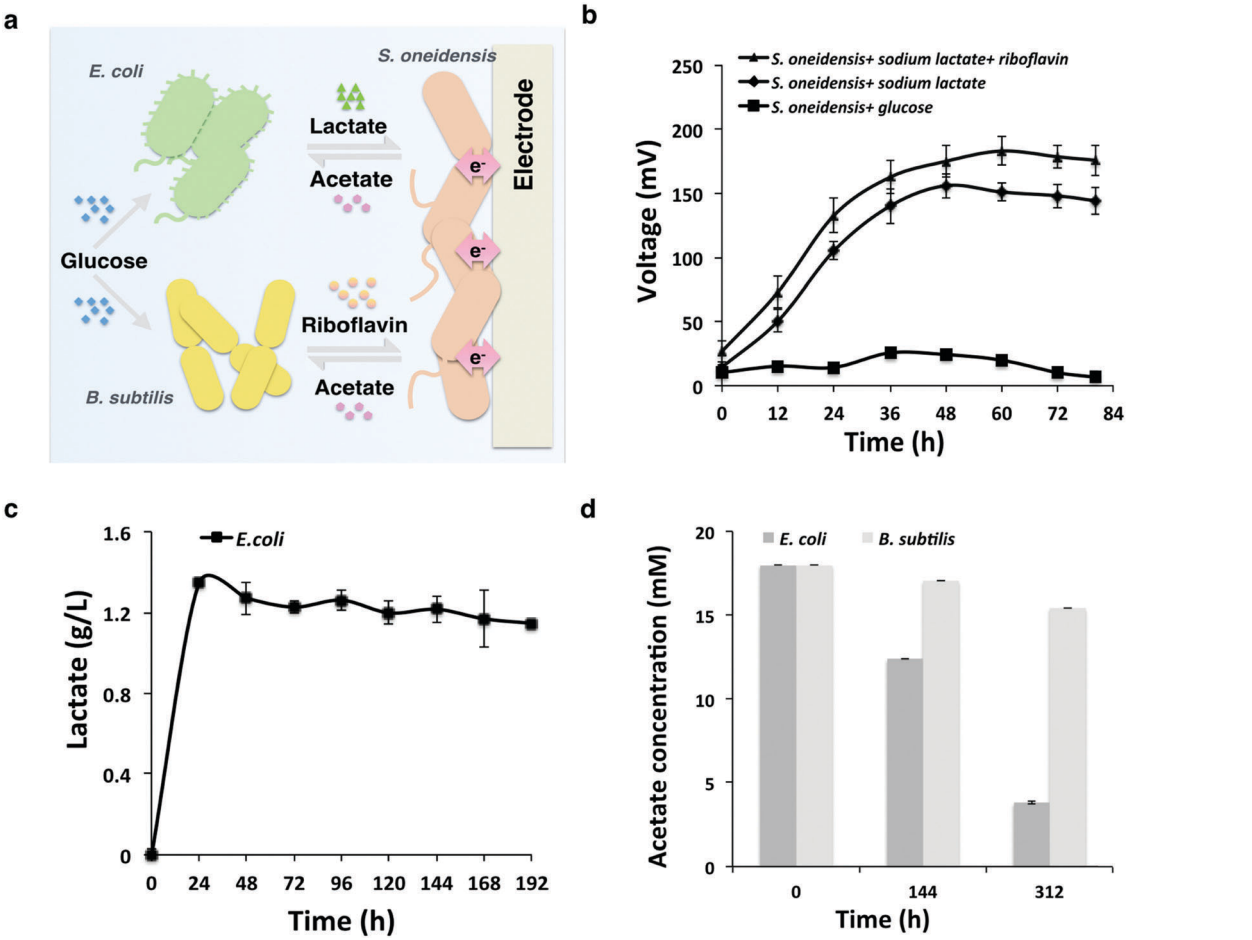

Fig. 3 Cross-feeding interactions in the synthetic three-species microbial consortium. (a) Schematic view of rationally designed cross-feeding interactions in the synthetic three-species microbial consortia. (b) *Shewanella oneidensis MR-1* could directly use lactate but not glucose for electricity generation and increase the efficiency of extracellular electron transfer (EET) by adding riboflavin. (c) Under anaerobic conditions, *E. coli* and *B. subtilis* both converted glucose to lactate. *E. coli* produced 1.3 g l^{-1} lactate in 48 h and *B. subtilis* produced 1.1 g l^{-1} lactate in 192 h, much slower than *E. coli*. (d) *E. coli* and *B. subtilis* used acetate as the carbon source for long-term survival. *E. coli* and *B. subtilis* were separately cultivated in M9 medium with 18 mM acetate as the carbon source. *E. coli* completely consumed 14.2 mM acetate in 312 h. *B. subtilis* used 3.6 mM acetate in 312 h, which was less than that by *E. coli*. All experiments were performed at least in triplicate; error bars indicate s.d.

riboflavin (Fig. 2c). *E. coli* and *B. subtilis* were separately cultivated with 18 mM acetate as the carbon source. *E. coli* consumed 14.2 mM acetate in 312 h, whereas *B. subtilis* used 3.6 mM acetate in 312 h (Fig. 3d). *Bacillus subtilis* produced riboflavin by using acetate (data not shown). Thus, in addition to constructing stable mutualistic interactions, cross-feeding between individual species also had the benefit of further using the limited source from incomplete oxidation of *S. oneidensis*.

Performance of the synthetic microbial consortia

Our synthetic three-species microbial consortium consisting of *E. coli, B. subtilis* and *S. oneidensis* executed the tasks of long-term glucose-to-electricity conversion, which performed much "better together" than as individuals in terms of productivity, stability, and robustness.

Considerable and stable voltage was detected during the discharge of this synthetic microbial consortium. The average voltage of the *S. oneidensis* and *E. coli* two-species consortium was about 280 mV. After division of labor for lactate and riboflavin production into two *E. coli*, the average voltage was increased to 350 mV (Fig. 4a). The synthetic three-species microbial consortium consisting of *S. oneidensis, B. subtilis* and *E. coli* produced the highest voltage (about 550 mV), which was up to 70% of the theoretical maximum (780 mV). The voltage output decreased until lactate was used up at about 360 h (Fig. 2b). The synthetic three-species microbial consortium converted 11 mM of 0.28 g total glucose to over 15 days of stable current without any supplement. The three-species consortium lasted much longer than a monoculture of *S. oneidensis* (300 h, 18 mM sodium lactate) and the voltage output curves were higher and more stable.[40] The synthetic three-species microbial consortium produced 331.2 coulombs of charge in total, which was 1.8 times higher than that of the *S. oneidensis* and *E. coli*-Lac–Rib two-species consortium (Table 1). In addition, the conversion efficiency was 55.7% of the theoretical maximum, which could be achieved by the conversion of glucose to electricity *via* cell

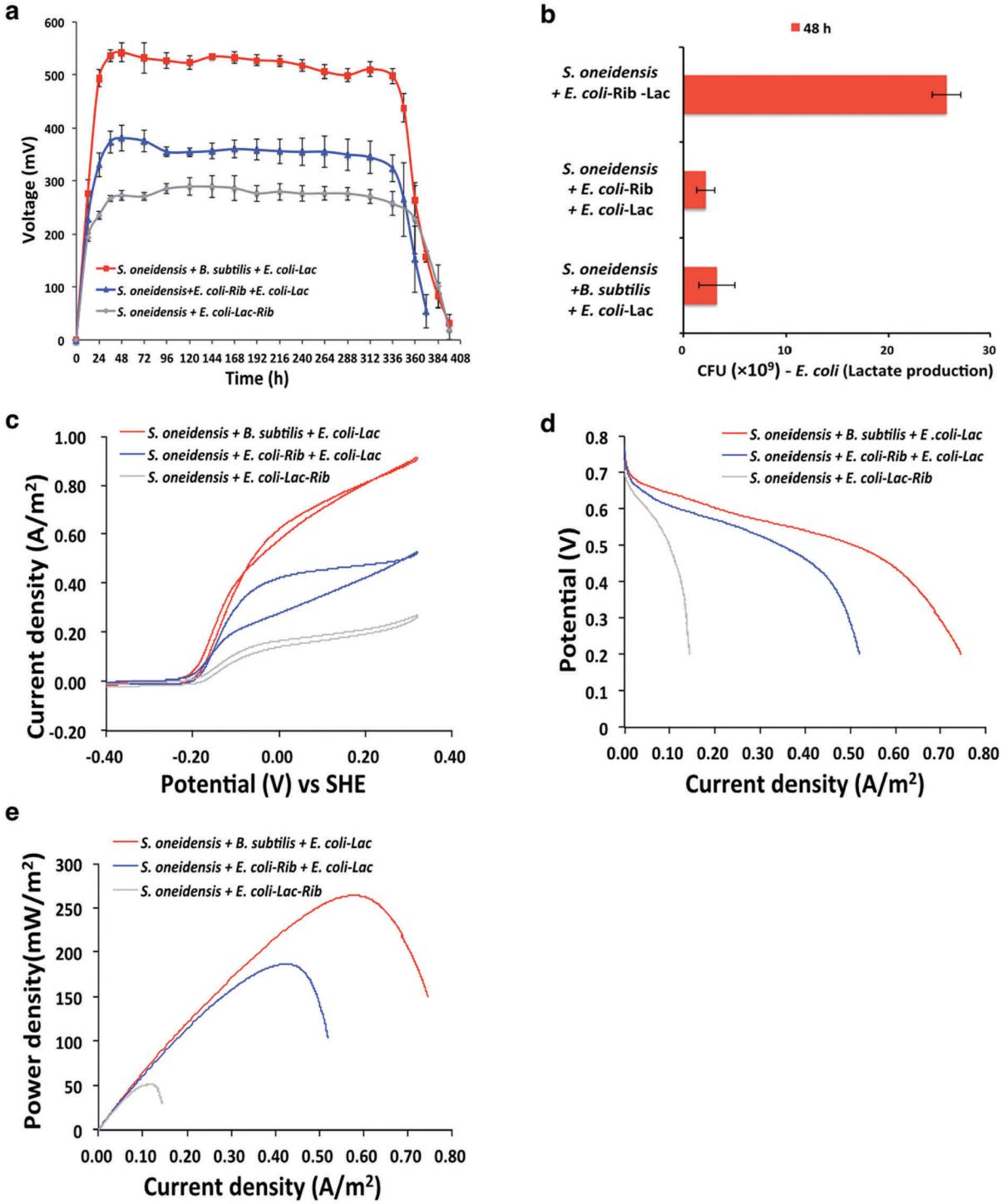

Fig. 4 Electrochemical performance and cell mass formation of synthetic microbial consortia. The synthetic three-species consortium consisting of *S. oneidensis, B. subtilis* and *E. coli* had the most efficient productivity due to the control of the energy loss during cell mass formation and low internal resistance. (a) Voltage output curves of three synthetic microbial consortia. The synthetic three-species consortium produced the highest voltage, which was about 550 mV, up to 70% of the theoretical maximum (780 mV). Voltage output decreased until lactate was used up at about 360 h. (b) Comparison of CFU counts of lactate producing *E. coli* in three microbial consortia at 48 h when glucose was consumed. After division of labor and labor specialization, the cell mass formation of *E. coli*-Lac was decreased to 91.4% and 87.3% respectively compared with *E. coli*-Lac–Rib because the metabolic flux was rearranged by division of labor. (c) Turnover cyclic voltammetry (CV) at a low scan rate of 1 mV s^{-1}. The catalytic current demonstrated saturating behavior with the increase of riboflavin concentration. The synthetic three-species microbial consortium including *B. subtilis* with the highest riboflavin production generated the highest catalytic current. (d) Polarization curves obtained by linear sweep voltammetry (LSV) with a low scan rate of 0.1 mV s^{-1}. The dropping slope of the polarization curve obtained from the three-species consortium was the smallest of all consortia, which means less internal resistance and energy loss. (e) Power density output curves. The synthetic three-species microbial consortium obtained a maximum power density of 241.5 ± 15.5 mW m^{-2}, higher than the power density of 183.7 ± 22.2 mW m^{-2} for the *S. oneidensis* + *E. coli*-Lac + *E. coli*-Rib consortium. All experiments were performed at least in triplicate; error bars indicate s.d.

Table 1 Systematic electricity output and conversion efficiency

	Coulombs of charge	Conversion efficiency (ε_m) (%)
S. oneidensis + *E. coli*-Lac–Rib	188.4	31.6
S. oneidensis + *E. coli*-Rib + *E. coli*-Lac	222.4	37.4
S. oneidensis + *B. subtilis* + *E. coli*-Lac	331.2	55.7

metabolism of wild-type *S. oneidensis* MR-1. Our three-species consortium achieved the highest glucose–electricity conversion efficiency based on previously reported results for the exoelectrogen *S. oneidensis*.

The conversion efficiency was increased from 31.6% to 55.7% (Table 1) mainly by controlling the energy loss of cell mass production and internal resistance. With a certain input of resources, bacteria that produce low cell mass yields will have high conversion efficiencies.[41] Since lactate and cell mass production were coupled in *E. coli*, the CFU (colony forming unit) counts of *E. coli*-Lac–Rib were decreased by 91.4% compared with *E. coli*-Lac at 48 h. This decrease occurred because glucose was consumed due to rearrangement of the metabolic flux after division of labor (Fig. 4b). In the synthetic three-species microbial consortium, the counts of *E. coli*-Lac were decreased by about 87.3%.

Electrochemical analysis was conducted to study the electron transfer efficiency of the synthetic microbial consortia. Cyclic voltammetry (CV) at a low scan rate was applied to reveal redox reaction kinetics at cell–electrode interfaces. The catalytic current was demonstrated to have a saturating behavior with an increase in the concentration of riboflavin. Thus, the synthetic three-species microbial consortium including *B. subtilis* generated the highest catalytic current (Fig. 4c). Linear sweep voltammetry (LSV) at a low scan rate (0.1 mV s^{-1}) was used to obtain polarization curves. The slope of the linear part in the polarization curve represents the internal resistance of the corresponding system. Our experimental data showed that the dropping slope of the polarization curve obtained from the three-species consortium was the smallest in comparison to the other consortia, indicating less internal resistance (Fig. 4d). The improvement in riboflavin production decreased the internal resistance and thus reduced the energy loss of the system.[18,42] The power density output profiles (Fig. 4e) showed that the synthetic three-species microbial consortium achieved a maximum power density of 241.5 ± 15.5 mW m^{-2}, which was higher than the power density of the *S. oneidensis* with two *E. coli* consortium (183.7 ± 22.2 mW m^{-2}). The results of independent replications were consistent among tests as shown in Fig. S2–S4 (ESI†).

Microbial composition had strong and sensitive effects on the consortium function.[26] The stable composition of the three species in the consortium was crucial for electricity output to remain stable at 550 mV for the whole cycle of power generation. The CFU count of *E. coli* increased over the initial 48 h due to the carbon metabolic flux from glycolysis and remained stable (Fig. 5a). The CFU count of *B. subtilis* remained stable after glucose was exhausted at 48 h (Fig. 5b). *S. oneidensis* remained attached on the anode until lactate was used up at 360 h

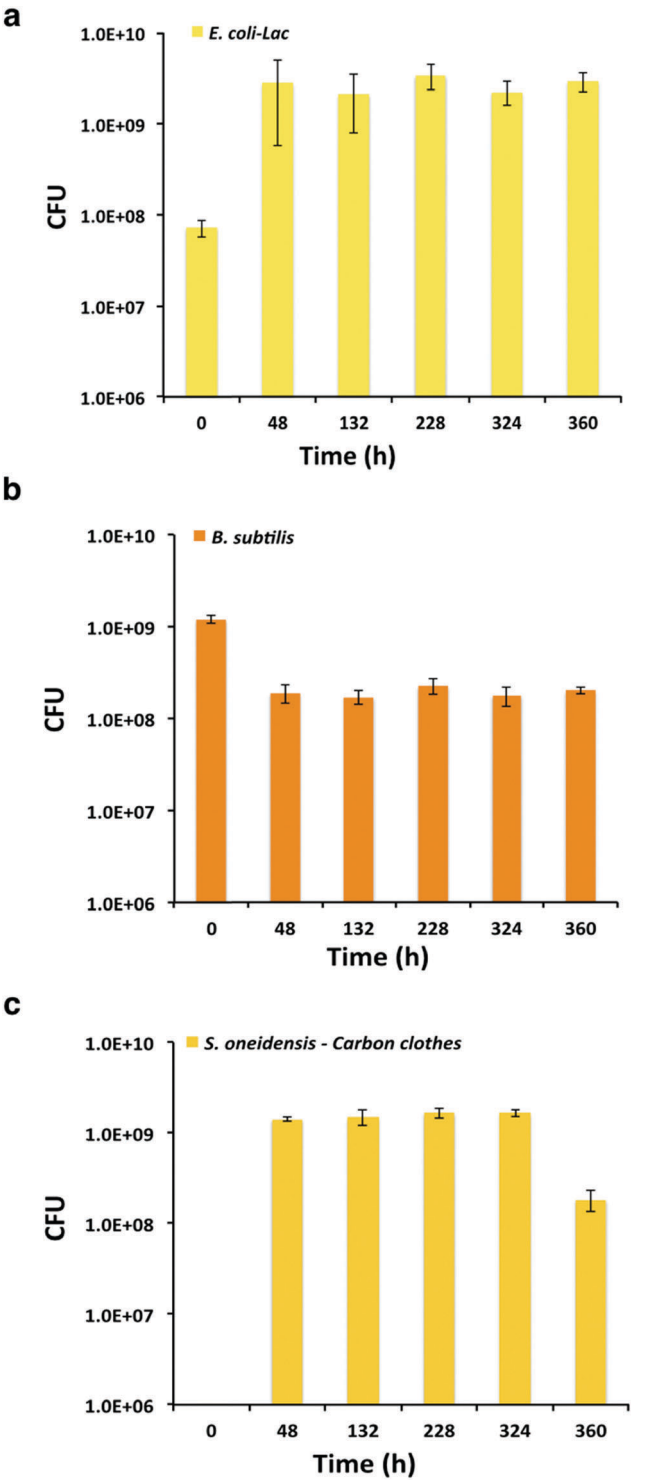

Fig. 5 Composition of the synthetic three-species microbial consortium. The composition was stable after 48 h when glucose was consumed. (a) CFU counts of *E. coli*-Lac at different times during long-term electricity output. *E. coli*-Lac increased cell mass in the initial 48 h by using glucose. (b) CFU counts of *B. subtilis* at different times of long-term electricity output. The CFU counts of *B. subtilis* decreased over 48 h and then remained stable. (c) CFU counts of *S. oneidensis* on the anode at different times during long-term electricity output. *S. oneidensis* remained attached on the anode steadily until lactate was used up at 360 h. All experiments were performed at least in triplicate; error bars indicate s.d.

(Fig. 5c). The stable composition benefited from cross-feeding based cooperation, which avoided the dominance of one species over another and supported synergistic growth in the consortium.

Biological robustness is important for microbial consortia to maintain their functions despite perturbation, and thus is important when constructing more sophisticated synthetic microbial consortia.[20,22,43] Using lactate as the carbon source was crucial for *S. oneidensis* to survive and maintain electricity generation. The robustness of the synthetic three-species microbial consortium was first tested by gradually reducing the initial inoculation of *E. coli* from 5 to 100 fold up to an OD of 0.00005, because the initial population of *E. coli* was the key to producing lactate. Following the reduction of the initial *E. coli* inoculation, the consortium exhibited functional robustness with the voltage output remaining at 500 mV for at least 240 h (Fig. 6a).

Further experiments were performed by totally removing *E. coli*, an extreme form of environmental perturbation meant to destroy the completeness of the consortium. Engineered *B. subtilis* retained the function of producing lactate but at a slow rate compared to *E. coli* (total 1.1 g l^{-1} in 192 h, Fig. S1, ESI†) and played the complementary role of providing the carbon source that maintained the function of the three-species consortium. Thus, in the absence of *E. coli*, the voltage output of the *B. subtilis* and *S. oneidensis* consortium increased at 36 h and maintained electricity generation (Fig. 6b). Compared with the consortium of *S. oneidensis* and two *E. coli*, without *E. coli*-Lac, the *S. oneidensis* and *E. coli*-Rib consortium had a loss of function and rare electricity output because *E. coli*-Rib did not have the ability to produce lactate (Fig. 6b).

The effect of higher riboflavin on the system was investigated by exogenous addition of different concentrations of riboflavin (*i.e.*, 0, 30, 80, 150 and 300 μM) to the individual and the ternary cultures (Fig. 7). Adding larger concentrations of riboflavin rarely affected the respiration of *E. coli* and *B. subtilis* (Fig. 7a and b). Addition of riboflavin significantly increased the voltage output for individual *S. oneidensis* (Fig. 7c), which, however, could not further increase the bioelectricity generation of our synthetic three-species microbial consortium (Fig. 7d). The conversion efficiencies ranged from 34% to 48%, which were lower than the initial synthetic three-species microbial consortium conversion rate of 56%. The high-performance of our synthetic three-species microbial consortia mainly benefited from the "division-of-labor" based cooperation and mutualistic interactions among individuals, which could not be achieved by exogenous addition of high concentrations of riboflavin.

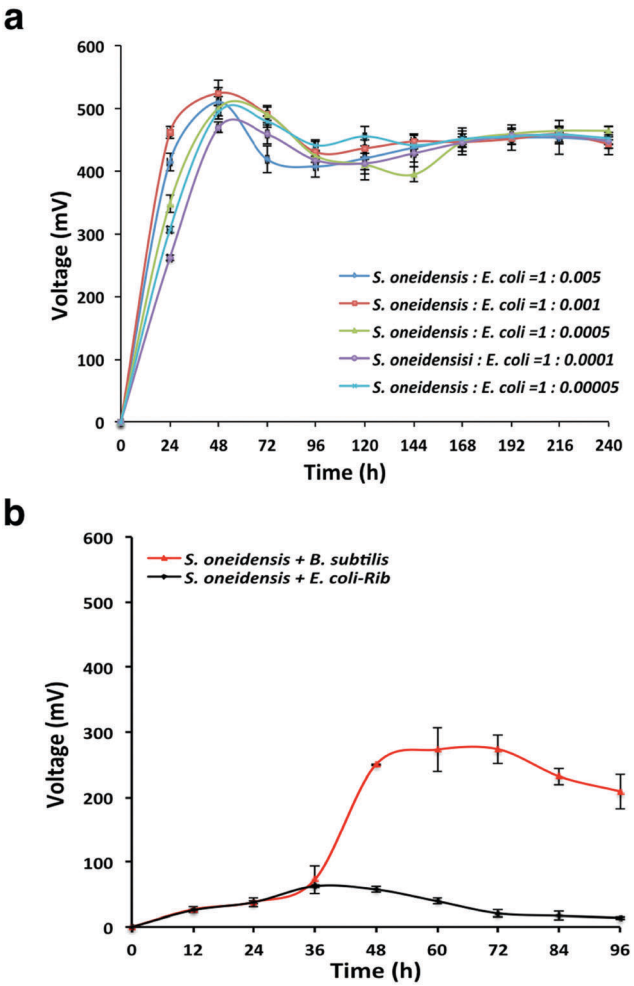

Fig. 6 Robustness of the synthetic three-species microbial consortium. The robustness of the synthetic three-species microbial consortium was tested by gradually reducing the initial inoculation of *E. coli* to zero, a form of extreme environmental perturbation that eliminated one of the paths for biosynthesis-labor of lactate conversion. (a) The robustness of the synthetic three-species microbial consortium was first tested by gradually reducing the initial inoculation of *E. coli* from 5 to 100 fold up to an OD_{600} of 0.00005. The consortium exhibited functional robustness, and the voltage output remained at 500 mV for at least 240 h. (b) In the absence of *E. coli*-Lac, the voltage output of the *B. subtilis* and *S. oneidensis* consortium began to increase up to 36 h and maintained electricity generation, because *B. subtilis* was engineered to retain functional redundancy by producing lactate but at a slow rate. Without *E. coli*-Lac, the *S. oneidensis* and *E. coli*-Rib consortium had a loss of function and rare electricity output. All experiments were performed at least in triplicate; error bars indicate s.d.

Materials and methods

Plasmid construction and transformation

The synthesized L-lactate dehydrogenase gene (*ldhA*) from *Lactobacillus* was inserted into the vector pSB1C3 (iGEM) to form the expression plasmid *ldhA*-pSB1C3. *E. coli*-Lac was constructed by knockout of the *pflB* gene and transforming the *ldhA*-pSB1C3 plasmid. *E. coli*-Lac–Rib was constructed by transforming the EC10 plasmid containing *rib ABDEC* gene cluster into *E. coli*-Lac. *E. coli*-Rib and *B. subtilis* were stored in our lab.

Culture condition

E. coli-Lac with 100 μg ml^{-1} ampicillin, *E. coli*-Rib with 34 μg ml^{-1} chloramphenicol, *E. coli*-Lac–Rib with 100 μg ml^{-1} ampicillin and 34 μg ml^{-1} chloramphenicol were inoculated into LB broth shaking at 37 °C for 12 h. Engineered *B. subtilis* was inoculated into LB broth supplemented with 30 μg ml^{-1} spectinomycin shaking at 37 °C for 12 h. *S. oneidensis* was inoculated into LB broth shaking at 30 °C for 12 h.

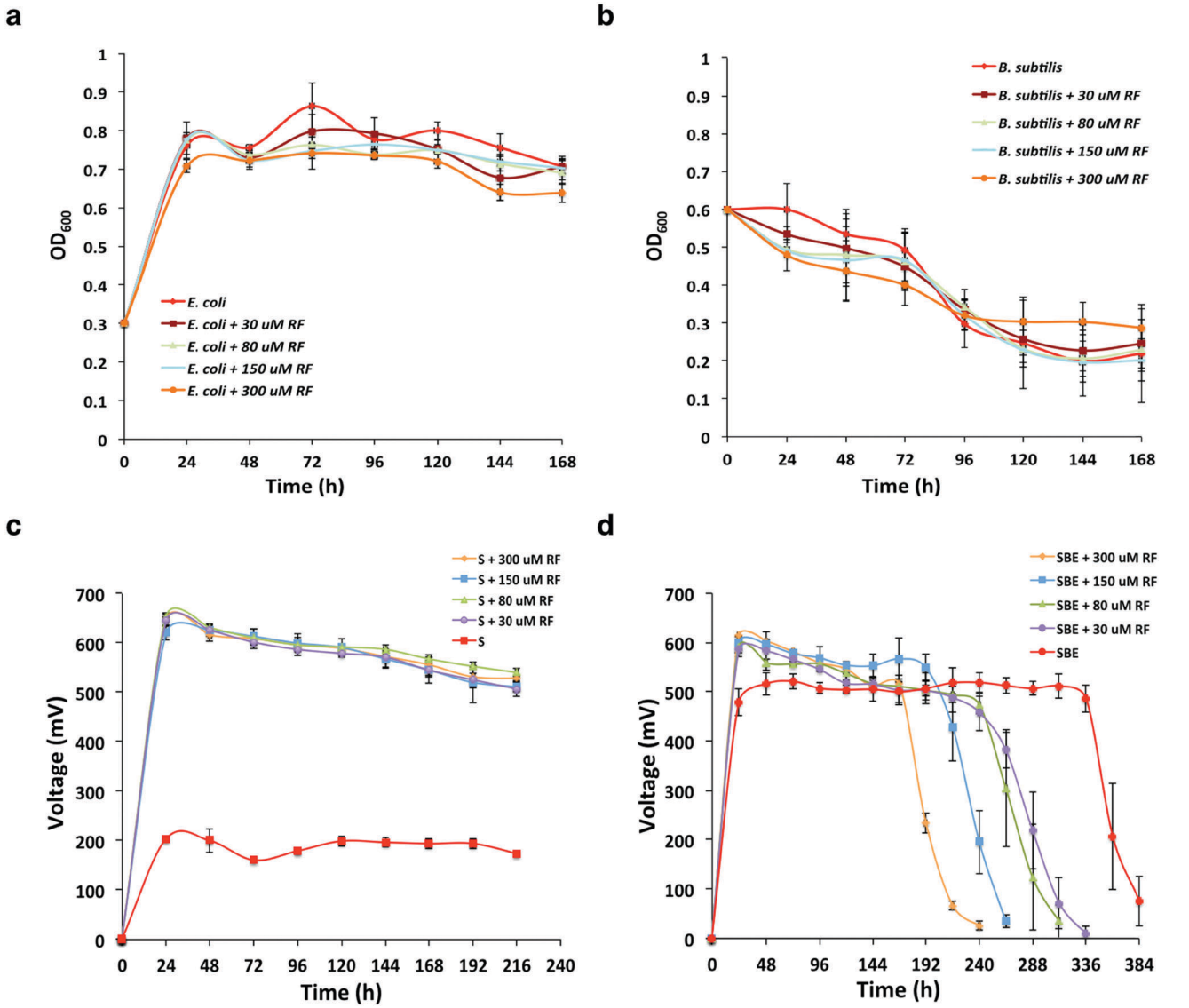

Fig. 7 Exploring the effect of increasing riboflavin concentration on individuals and the synthetic three-species microbial consortium. Different concentrations of riboflavin (0, 30, 80, 150 and 300 μM) were added to individuals and the synthetic three-species microbial consortium. (a) Cell growth of *E. coli*. (b) Cell growth of *B. subtilis*. (c) Voltage output for individual *S. oneidensis*. (d) Voltage output for individual species in the synthetic three-species microbial consortium. All experiments were performed at least in triplicate; error bars indicate s.d.

Co-culture setup

The dual-chamber bioreactors (140 ml for both anode and cathode chambers) were separated by Nafion 117, and carbon cloth was used as the working electrode (2.5 cm × 2.5 cm) and the counter electrode (2.5 cm × 3.0 cm). Carbon cloth was treated with acetone and 1 M HCl before use. The cathodic electrolyte was made of 50 mM $K_3[Fe(CN)_6]$, 50 mM K_2HPO_4 and 50 mM KH_2PO_4. The anode medium consisted of M9 minimal medium supplemented with 5% LB broth, 7.2 g l^{-1} HEPES buffer and 2 g l^{-1} glucose (11 mM). Each co-culture system was triplicated. The bacteria culture was harvested and washed with fresh M9 medium. The ratio of inoculated cell densities of *E. coli*, *B. subtilis* and *S. oneidensis* was 10 : 1 : 100. The co-culture solutions were dispersed into three anode chambers for parallel experiments. The anodes were purged with nitrogen gas using a 0.2 μm filter to remove oxygen. The bio-reactors were incubated at 30 °C. The anode and cathode were connected by a 2 kΩ external resistor. The voltage across the external resistor was recorded using a multimeter (Pro's Kit MT1820, Taiwan).

Electrochemical analysis

Cyclic voltammetry with a low scan rate (1 mV s^{-1}) was conducted on a three-electrode configuration with an Ag/AgCl (KCl saturated) reference electrode (CH Instrument, Shanghai, China) using a CHI600E electrochemical workstation (CH Instrument, Shanghai, China). Linear sweep voltammetry (LSV) with a slow scan rate (0.1 mV s^{-1}) was applied to obtain the polarization curves and the potential decreased from open circuit potential (OCP) to ~−0.4 V controlled by CHI600E.

Quantification of lactate, acetate and riboflavin

Filtered samples (0.2 μm nitrocellulose filter, Millipore, Billerica, MA, USA) were analyzed for organic acids using high-performance liquid chromatography (HPLC) with a refractive index detector (Waters, Corp) and with an organic acid column (Aminex HPX-87H Column, Bio-Rad). 5 mM sulfuric acid was employed as the mobile phase flowing at 0.6 ml min^{-1} and the column was incubated at 65 °C. The retention times of lactate and acetate were 12.9 min and 14.3 min, respectively.

Strains were cultivated under anaerobic conditions and riboflavin was measured by using HPLC with a UV detector (Waters, Corp). All standard solutions and sample supernatants were filtered and assayed by a reverse-phase C18 column (100 mm × 2.1 mm, 2.6 μm, Thermo scientific). The initial mobile phase was composed of methanol and 0.05 M ammonium acetate (pH = 6.0, 15 : 85 v/v). Chromatographic separation was done using a gradient eluent curve of 15 : 85 to 30 : 70 from 0.5 min to 1.5 min, followed by 30 : 70 to 85 : 15 v/v from 1.5 min to 4 min, isocratic 85 : 15 v/v to 8 min, and gradient eluent curve 85 : 15 to 15 : 85 v/v to 9.5 min, isocratic 15 : 85 v/v to 16 min at a flow rate of 0.1 ml min^{-1} at 30 °C with 20 μL injection volume.

Community composition analysis

The anode was suspended and spread onto LB agar plates with special resistance after a series of dilutions at 30 °C for 24 h before counting CFU. The colonies of *S. oneidensis* were dark orange in color. *E. coli*-Lac can grow on the plate with 34 μg ml^{-1} chloramphenicol, *E. coli*-Rib can grow on 100 μg ml^{-1} ampicillin and *B. subtilis* can grow on 30 μg ml^{-1} spectinomycin. The cell optical density was detected using a SpectraMax M2 microplate reader.

Conclusion

We designed and constructed a synthetic three-species microbial consortium consisting of engineered *E. coli*, *B. subtilis* and *S. oneidensis* for long-term bioelectricity generation. This work explored the mechanism of manipulating the distribution of carbon metabolism and electricity generation by gradual "division of labor" to maximize the energy conversion efficiency in BESs. Through such optimization of the consortium guided by the "division-of-labor" principle, the three species in the synthetic microbial consortium thus performed "better together" in terms of the efficiency of power generation, stability, and robustness to environmental perturbations. The optimized three-species consortium converted 11 mM glucose to a stable and efficient electricity output for over 15 days. Our study is of great help in understanding the operation mechanisms of complicated naturally occurring microbial communities, and also serves as a model system for developing more complex synthetic microbial consortia for many energy and environmental applications.

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References

1 J. K. Fredrickson, *Science*, 2015, **348**, 1425–1427.
2 K. Brenner, L. You and F. H. Arnold, *Trends Biotechnol.*, 2008, **26**, 483–489.
3 F. E. Nielsen, C. E. Reimers, H. K. White, S. Sharma and P. R. Girguis, *Energy Environ. Sci.*, 2008, **1**, 584–593.
4 Y. Gong, S. E. Radachowsky, M. Wolf, M. E. Nielsen, P. R. Girguis and C. E. Reimers, *Environ. Sci. Technol.*, 2011, **45**, 5047–5053.
5 U. Schröder, F. Harnisch and L. T. Angenent, *Environ. Sci. Technol.*, 2015, **8**, 513–519.
6 X. W. Liu, W. W. Li and H. Q. Yu, *Chem. Soc. Rev.*, 2014, **43**, 7718–7745.
7 W. W. Li, H. Q. Yu and Z. He, *Energy Environ. Sci.*, 2014, **7**, 911–924.
8 B. Min, J. Kim, S. Oh, J. M. Regan and B. E. Logan, *Water Res.*, 2005, **39**, 4961–4968.
9 B. E. Logan and K. Rabaey, *Science*, 2012, **337**, 686–690.
10 Y. Wu, B. Z. Li, M. Zhao, L. A. Mitchell, Z. X. Xie, Q. H. Lin, X. Wang, W. H. Xiao, Y. Wang, X. Zhou, H. Liu, X. Li, M. Z. Ding, D. Liu, L. Zhang, B. L. Liu, X. L. Wu, F. F. Li, X. T. Dong, B. Jia, W. Z. Zhang, G. Z. Jiang, Y. Liu, X. Bai, T. Q. Song, Y. Chen, S. J. Zhou, R. Y. Zhu, F. Gao, Z. Kuang, X. Wang, M. Shen, K. Yang, G. Stracquadanio, S. M. Richardson, Y. Lin, L. Wang, R. Walker, Y. Luo, P. S. Ma, H. Yang, Y. Cai, J. Dai, J. S. Bader, J. D. Boeke and Y. J. Yuan, *Science*, 2017, **355**, eaaf4706.
11 Z. X. Xie, B. Z. Li, L. A. Mitchell, Y. Wu, X. Qi, Z. Jin, B. Jia, X. Wang, B. X. Zeng, H. M. Liu, X. L. Wu, Q. Feng, W. Z. Zhang, W. Liu, M. Z. Ding, X. Li, G. R. Zhao, J. J. Qiao, J. S. Cheng, M. Zhao, Z. Kuang, X. Wang, J. A. Martin, G. Stracquadanio, K. Yang, X. Bai, J. Zhao, M. L. Hu, Q. H. Lin, W. Q. Zhang, M. H. Shen, S. Chen, W. Su, E. X. Wang, R. Guo, F. Zhai, X. J. Guo, H. X. Du, J. Q. Zhu, T. Q. Song, J. J. Dai, F. F. Li, G. Z. Jiang, S. L. Han, S. Y. Liu, Z. C. Yu, X. N. Yang, K. Chen, C. Hu, D. S. Li, N. Jia, Y. Liu, L. T. Wang, S. Wang, X. T. Wei, M. Q. Fu, L. M. Qu, S. Y. Xin, T. Liu, K. R. Tian, X. N. Li, J. H. Zhang, L. X. Song, J. G. Liu, J. F. Lv, H. Xu, R. Tao, Y. Wang, T. T. Zhang, Y. X. Deng, Y. R. Wang, T. Li, G. X. Ye, X. R. Xu, Z. B. Xia, W. Zhang, S. L. Yang, Y. L. Liu, W. Q. Ding, Z. N. Liu, J. Q. Zhu, N. Z. Liu, R. Walker, Y. Luo, Y. Wang, Y. Shen, H. Yang, Y. Cai, P. S. Ma, C. T. Zhang, J. S. Bader, J. D. Boeke and Y. J. Yuan, *Science*, 2017, **355**, eaaf4704.
12 P. E. Purnick and R. Weiss, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 410–422.
13 D. E. Cameron, C. J. Bashor and J. J. Collins, *Nat. Rev. Microbiol.*, 2014, **12**, 381–390.
14 J. C. Way, J. J. Collins, J. D. Keasling and P. A. Silver, *Cell*, 2014, **157**, 151–161.
15 Z. M. Summers, H. E. Fogarty, C. Leang, A. E. Franks, N. S. Malvankar and D. R. Lovley, *Science*, 2010, **330**, 1413–1415.
16 A. Venkataraman, M. A. Rosenbaum, S. D. Perkins, J. J. Werner and L. T. Angenent, *Energy Environ. Sci.*, 2011, **4**, 4550.
17 M. A. Rosenbaum, H. Y. Bar, Q. K. Beg, D. Segre, J. Booth, M. A. Cotta and L. T. Angenent, *Bioresour. Technol.*, 2011, **102**, 2623–2628.

18 Y. Yang, Y. Wu, Y. Hu, Y. Cao, C. L. Poh, B. Cao and H. Song, *ACS Catal.*, 2015, **5**, 6937–6945.
19 W. R. Harcombe, W. J. Riehl, I. Dukovski, B. R. Granger, A. Betts, A. H. Lang, G. Bonilla, A. Kar, N. Leiby, P. Mehta, C. J. Marx and D. Segre, *Cell Rep.*, 2014, **7**, 1104–1115.
20 S. R. Lindemann, H. C. Bernstein, H. S. Song, J. K. Fredrickson, M. W. Fields, W. Shou, D. R. Johnson and A. S. Beliaev, *ISME J.*, 2016, **10**, 2077–2084.
21 N. I. Johns, T. Blazejewski, A. L. Gomes and H. H. Wang, *Curr. Opin. Microbiol.*, 2016, **31**, 146–153.
22 A. Konopka, S. Lindemann and J. Fredrickson, *ISME J.*, 2015, **9**, 1488–1495.
23 J. L. Zilles, L. F. Rodriguez, N. A. Bartolerio and A. D. Kent, *ISME J.*, 2016, **10**, 1809–1814.
24 M. T. Mee and H. H. Wang, *Mol. BioSyst.*, 2012, **8**, 2470–2483.
25 H. Song, M. Z. Ding, X. Q. Jia, Q. Ma and Y. J. Yuan, *Chem. Soc. Rev.*, 2014, **43**, 6954–6981.
26 B. P. Teague and R. Weiss, *Science*, 2015, **349**, 924–925.
27 C. M. Agapakis, P. M. Boyle and P. A. Silver, *Nat. Chem. Biol.*, 2012, **8**, 527–535.
28 S. G. Hays, W. G. Patrick, M. Ziesack, N. Oxman and P. A. Silver, *Curr. Opin. Biotechnol.*, 2015, **36**, 40–49.
29 J. J. Minty, M. E. Singer, S. A. Scholz, C. Bae, J. Ahn, C. E. Foster, J. J. Liao and X. N. Lin, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 14592–14597.
30 K. Zhou, K. Qiao, S. Edgar and G. Stephanopoulos, *Nat. Biotechnol.*, 2015, **33**, 377–383.
31 Z. Lin, Z. Xu, Y. Li, Z. Wang, T. Chen and X. Zhao, *Microb. Cell Fact.*, 2014, **13**, 104.
32 S. Shi, T. Chen, Z. Zhang, X. Chen and X. Zhao, *Metab. Eng.*, 2009, **11**, 243–252.
33 S. Pande, S. Shitut, L. Freund, M. Westermann, F. Bertels, C. Colesie, I. B. Bischofs and C. Kost, *Nat. Commun.*, 2015, **6**, 6238.
34 M. T. Mee, J. J. Collins, G. M. Church and H. H. Wang, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, E2149–E2156.
35 S. Pande, H. Merker, K. Bohl, M. Reichelt, S. Schuster, L. F. de Figueiredo, C. Kaleta and C. Kost, *ISME J.*, 2014, **8**, 953–962.
36 S. Pande, F. Kaftan, S. Lang, A. Svatos, S. Germerodt and C. Kost, *ISME J.*, 2016, **10**, 1413–1423.
37 S. Widder, R. J. Allen, T. Pfeiffer, T. P. Curtis, C. Wiuf, W. T. Sloan, O. X. Cordero, S. P. Brown, B. Momeni, W. Shou, H. Kettle, H. J. Flint, A. F. Haas, B. Laroche, J. U. Kreft, P. B. Rainey, S. Freilich, S. Schuster, K. Milferstedt, J. R. van der Meer, T. Grobetakopf, J. Huisman, A. Free, C. Picioreanu, C. Quince, I. Klapper, S. Labarthe, B. F. Smets, H. Wang, F. Isaac Newton Institute and O. S. Soyer, *ISME J.*, 2016, **10**, 2557–2568.
38 A. Kouzuma, T. Kasai, A. Hirose and K. Watanabe, *Front. Microbiol.*, 2015, **6**, 609.
39 Y. J. Tang, A. L. Meadows and J. D. Keasling, *Biotechnol. Bioeng.*, 2007, **96**, 125–133.
40 T. Liu, Y. Yu, X. Deng, C. K. Ng, B. Cao, J. Wang, S. A. Rice, S. Kjelleberg and H. Song, *Biotechnol. Bioeng.*, 2015, **112**, 398–421.
41 B. E. Logan, *Nat. Rev. Microbiol.*, 2009, **7**, 2051–2059.
42 Y. Yang, Y. Ding, Y. Hu, B. Cao, S. A. Rice, S. Kjelleberg and H. Song, *ACS Synth. Biol.*, 2015, **4**, 815–823.
43 B. Stenuit and S. N. Agathos, *Curr. Opin. Biotechnol.*, 2015, **33**, 305–317.