SYNTHE TIC BIOLOGY AND THE RATIONAL DESIGN OF MICROBIAL CHEMICAL FACTORIES

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ABSTRACT
A central feature in long-duration environmental control and life support systems (ECLSS) includes a bioregenerative core. Major components of such an ECLSS bioregenerative core system include plant/crop production systems and microbial bioreactors. Bioreactor design has most often focused on using extant species, and mixed culture communities, but there is great promise in the use of recombinant microorganisms to allow the combination of metabolic engineering with biocatalysis to expand and optimize the biosynthetic capacity of microbial bioreactors. This review will introduce current efforts in synthetic biology to build microbial chemical factories, and will also consider the design and assembly of recombinant microorganism bioreactors as small molecule production facilities to produce compounds such as antifungals and chelators needed to balance other components of ECLSS subsystems. The development of tools and methodologies for novel biosynthetic pathway design and considerations for implementation of these designs will be addressed. This review will also emphasize design principles for the production of unnatural organic compounds within the framework of the nascent field of synthetic biology.

INTRODUCTION
Current manned missions, such as the International Space Station (ISS), are not self sufficient, and require shuttle missions for resupply. Long-duration space exploration requires closed loop ECLSS capable of providing potable water, oxygen, and edible biomass for a typical crew of astronauts, while removing/transforming harmful byproducts and metabolites. Considerations in the design of ECLSS include shelf life, resupply-return logistics, crew time needed for maintenance, power requirements, launch weight and stowage volume. Physical and chemical ECLSS technologies typically have high consumables, high energy consumption, and/or produce secondary waste streams (Garland et al., 2005; Jackson et al., 2009). Thus, incorporation of bioregenerative technologies as waste preprocessors and/or for food production within ECLSS reduces overall mission costs.

Bioregenerative life support

Microbial bioreactors are one of the technologies under consideration for long-duration water reclamation and nutrient recycling in ECLSS systems, and will be a necessary subsystem for extended missions (e.g., lunar expeditions, missions to Mars). Early bioprocessors were primarily designed to promote microbial oxidation of organic carbon and ammonia (Edeen et al., 1998; Pickering et al., 1998; Campbell et al., 2003). Many technologies have been developed which meet some or all of the equivalent systems mass (ESM) requirements for improving ECLSS subsystems, such as bubble free nitrification (Tansel et al., 2005; McLamore et al., 2007; Rector et al., 2007; Chen et al., 2008), anaerobic ammonia oxidation (Smith et al., 2008), dual phase gas-liquid treatment (McLamore et al., 2008), thermophilic aerobic digestion (Staton et al., 2009), and graywater biodegradation (Rector et al., 2004; Morse et al., 2008).

Phytoremediation processes in ECLSS are often coupled with upstream/parallel microbial bioreactors, and control of microenvironments promoting synergistic degradation of rhizosphere contaminants is often difficult (e.g., nitrogen fixation by Rhizobium nodules in Glycine max roots). Phytoremediation applications have been conducted for targeted removal of acetone (Darlington and Dixon, 1990), graywater (Cook et al., 2003) and a urine/graywater/humidity condensate blend (Vairavan et al., 2007) using various plant species. Although improvements in bioreactor design (Campbell et al., 2003; Rector et al., 2003) and growth chambers (Burtress et al., 2002; Chamberlain et al., 2002) have significantly enhanced performance (leading to a reduction in ESM), the complexity of the wastestreams often limit the efficiency of metabolic conversion rates (i.e., carbon/nitrogen loading from urine, graywater, humidity condensate) (McLamore et al., 2007). Similar to research aimed at engineering plants for space flight (Bugbee, 1999; Salisbury, 1999), there is a growing need to utilize metabolic engineering in microbial ECLSS systems for reuse of gas, liquid, and solid phase waste.

Bioregenerative product production

Due to systems mass restrictions, extended duration space exploration will be heavily dependent on bioregenerative production of crew consumables (e.g., edible biomass, antifungal agents). In addition to biomimetic products for promoting wound healing (Krishen et al., 2009), recent research has investigated the use of extant microbes for the production of useable products in ECLSS. Examples include a gram-negative facultative proteobacteria (Chromobacterium violaceum) which has recently been sequenced. C. violaceum produces a number of natural

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Gravitational and Space Biology 23(2) August 2010 49
antibiotics, including violacein (Melo et al., 2009), aztreonam (Sader et al., 2003), and aerocavin (Vasconcellos Antônio and Creczynski-Pasa, 2004). Microbial bioreactors are also a simple source of chelating agents used by crew members for a multitude of purposes.

Many studies have been conducted regarding hydroponic production of edible crops for space exploration, including soybeans, tomatoes, lettuce (Wheeler et al., 2008), potatoes (Mackowiak et al., 1997), sweetpotatoes (Mortley et al., 1991), chives (Vairavan et al., 2007), and wheat (Bugbee and Koerner, 1997). Research has been conducted regarding inedible crop biomass degradation by edible fungi, and subsequent use of mycelium in downstream Tilapia harvesting systems (Nyochembeng et al., 2008). In addition to advancements in lighting systems (Massa et al., 2006) and environmental control systems (Levine et al., 2001), genetic engineering has played a major role in improved crop production for extraterrestrial exploration (Shands et al., 1992).

Effects of the microgravity environment on long-term habitation

Many studies have been conducted regarding the effects of long term exposure to microgravity in humans (Lynch and Matin, 2005) and plants (Zhou et al., 2002). In addition to causing immunosuppression in humans (Yamauchi et al., 2002), long term exposure of pathogens to microgravity (e.g., Salmonella spp.) causes an increase in virulence and resistance to antibiotics (Wilson et al., 2007). Thus, much future research is required if microbial systems are to be considered for use in ECLSS. Recent technological advancements have enabled scientists and engineers to develop low-cost, rapidly developed, biological payloads (microsatellites) for understanding the effects of long term microgravity at the cellular level. Microsatellites have recently been deployed and investigated the effect of microgravity on Escherichia coli and yeast physiology using optical techniques (Kitts et al., 2007; Kitts et al., 2008; Parra et al., 200).

Recombinant Microorganisms for Microbial Bioreactors

As demonstrated in the examples above, nature has provided a vast diversity of microbes capable of performing useful functions, whether in a terrestrial setting or not. At the same time, advances in genetic engineering have led to the creation and advancement of fields such as metabolic engineering and systems biology. Collectively, these disciplines seek to provide and implement tools that lead to the understanding and improvement of natural biological systems for useful functions. Metabolic engineering was defined nearly twenty years ago as “the improvement of cellular activities by manipulation of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology” (Bailey, 1991). Since that time, there have been a number of success stories with respect to the development of highly productive organisms, especially microbes. Initially, metabolic engineering efforts were primarily focused on improving the productivity of naturally-occurring metabolites in the target organisms, as is consistent with Bailey’s original definition. Classical examples of such efforts including the engineering of microbes for high-level production of antibiotics (Li, 2006; Rokem, 2007) and amino acids (Lee et al., 2007; Luette-Eversloeh et al., 2007; Sprenger, 2007) in native hosts.

More recently, efforts have focused on engineering non-native hosts to produce products of interest (Nielsen, 2001). In this group of examples, we include products of biological origin, i.e., “natural products;” however, the host organism is not a natural producer of the compound(s) in question. Examples of such products include polyketides, with anti-infective, anti-tumor, and cholesterol-lowering properties (Pfeifer et al., 2001; Pfeifer et al., 2003); and isoprenoids, a class of compounds with uses that range from pigments (Mijts and Schmidt-Dannert, 2003) to the treatment of malaria (Ro et al., 2006). An excellent example of this kind of work is the development of a recombinant strain of Escherichia coli that is capable of producing the compound 1,3-propanediol (1,3-PDO) from glucose (Nakamura and Whited 2003). Construction of the production strain required the inclusion and over-expression of genes encoding enzymes in the biosynthetic pathway that were taken from Saccharomyces cervisiae yeast, the Klebsiella pneumoniae bacterium, and the native E. coli host, as well as several other genetic modifications. The resulting strain was employed in a fermentation process to produce PDO at titers that exceed 125 g/L. This example illustrates the feasibility of using recombinant microbes as robust microbial chemical factories in bioreactors.

The Synthetic Biology Approach

In many ways, the synthetic biology approach builds upon the principles established by metabolic engineering. In particular, metabolic engineering is often distinguished from genetic engineering in its use of mathematical formulas and computational approaches to describe and then improve upon the functions of biological systems (Stephanopoulos, 1999). The use of mathematical descriptions for physical functions is a hallmark of engineering disciplines and is critical for effective design practices. Similarly, synthetic biology is concerned with the application of engineering principles to biological systems, with the goal of “making biology easier to engineer.” Synthetic biology is still a relatively new discipline and the field is composed of a diverse group of practitioners, including those trained as biologists, chemists, physicists, chemical engineers, electrical engineers, computer scientists, and even civil engineers. As a result of this diversity, multiple definitions and approaches currently exist under the umbrella of synthetic biology. The following definition is most useful for the current discussion (taken from www.syntheticbiology.org):
• the design and construction of new biological parts, devices, and systems, and
• the re-design of existing, natural biological systems for useful purposes.

In this framework, synthetic biology serves to expand the range of possibilities for engineering biological systems by considering biology to be a “substrate” upon which new systems can be built (Leonard et al., 2008). A combination of “top-down” and “bottom-up” approaches are currently begin employed, where top-down usually refers to the simplification of a complex system through a more reductionist approach, and bottom-up typically means the step-wise assembly of complex systems in a hierarchical fashion from well-defined “parts.” As a practical matter, the ultimate goal of synthetic biology is to enable the design and construction of self-replicating and sustaining biological systems in a predictable and robust manner.

Microbial Chemical Factories and Synthetic Biology

The construction of novel microbial factories sits directly at the intersection of metabolic engineering and synthetic biology. The focus on construction of novel systems in synthetic biology is complemented by the traditional emphasis on analysis and optimization in metabolic engineering. Additionally, many of the experimental tools and “devices” built to improve productivity of microbial systems have been developed under the principles of modularity that lead to the creation of well-characterized standard biological parts (Pfleger et al., 2006; Dueber et al., 2009). Two recent reviews describe the application of metabolic engineering and synthetic biology principles to microbial chemical factories, including the de novo design of biosynthetic pathways towards novel compounds (Prather and Martin, 2008; Martin et al., 2009).

Tools and Methodologies for Biosynthetic Pathway Design

In the case of pathway design, the concept of well-characterized biological parts is considered in the context of enzymes, i.e., the specialized proteins encoded by DNA and designed to carry out chemical conversions. The question then becomes, how can one select the best combination of enzymes to perform the desired conversions and comprise the designed biosynthetic pathway? To answer this question, one first needs to consider the needs for the pathway (adapted from Martin and Prather, 2008):

• Engineering bypass routes – A bypass is desired when a natural pathway exists, but is not sufficiently productive. New enzymes may be introduced to allow a bypass between two existing metabolites, i.e., two chemicals already present in cellular metabolism.

• Composing hybrid pathways – Hybrid pathways can be composed to connect distinct pathways from two separate organisms that share a common intermediate. This strategy was employed in the previously described case of 1,3-PDO biosynthesis (Nakamura and Whited, 2003).

• Creating new pathways and products – This strategy is necessary when the desired compound does not have an elucidated and tractable biosynthetic route (e.g., Moon et al., 2009) or when the creation of new products is desired (e.g., Liao, et al., 2007). Enzymes may have to be engineered in this approach.

In the case of hybrid pathway construction, the choice of enzymes is typically limited to, at most, a handful of proteins with demonstrated activity against the targets of interest. In the other cases, engineered enzymes could be used, which greatly expands the number of theoretical possibilities. Computational tools have been developed to help address the pathway specification problem. One of the oldest algorithms exists within a set of tools and resources known as the University of Minnesota Biocatalysis and Biodegradation Database (UM-BBD; http://umbbd.msi.umn.edu/). The UM-BBD has a pathway prediction system that has been developed to predict the degradation fate of compounds if exposed to the environment (Hou et al., 2003; Hou et al., 2004; Ellis et al., 2006). An alternative algorithm, the Biochemical Network Integrated Computational Explorer (BNICE), has been developed to specifically address the problem of biosynthesis for the production of microbial chemical factories (Li et al., 2004; Hatzimanikatis et al., 2005), although a recent paper described the modification of the algorithm to predict degradation routes as well (Finley et al., 2009). A third web-based application, the Retro-Biosynthesis Tool (ReBiT) is more focused on individual enzyme transformations rather than whole pathways (Martin and Prather, 2008; http://www.retrobiosynthesis.com).

Challenges for the Implementation of Novel Biosynthetic Pathways

The development of new tools, techniques, methods, and methodologies within metabolic engineering and synthetic biology has led to an increasing interest in the concept of rational design of microbial chemical factories (see Martin and Prather, 2009 for several examples). However, several challenges of large-scale implementation of designed pathways remain unsolved. First among these is the development of robust tools for pathway design. The BNICE algorithm is the first to tackle this problem in a concerted effort; however, it suffers from an inability to predict which pathways are most likely to be successfully implemented experimentally. Variations on the algorithm have more recently included thermodynamic analyses to eliminate unfavorable pathways (Finley et al., 2009), but an experimentally intractable number of theoretical routes still remains after excluding the energetically unfavorable routes. Second, the diversity of biology results in a wide
range of sources for enzymes, including bacteria, simple eukaryotes such as yeast, and complex eukaryotes such as mammals. However, enzymes taken from one environment may not function effectively in the heterologous host (Chang et al., 2007). Some protein engineering may be required to achieve sufficient activities from these enzymes. Third, the potential for pathway design can only be fully realized as methods for protein engineering are improved. In this way, enzymes can be reliably engineered to accept a broader range of substrates, validating the pathway design methodologies. Finally, even a pathway that is “functionally assembled” in a cell, meaning that the enzymes are expressed and the desired target compound is produced, will still need optimization. Fortunately, this last challenge can be addressed using existing and yet-to-be discovered tools of metabolic engineering and synthetic biology.

SUMMARY AND CONCLUSIONS

Synthetic biology provides an excellent framework for the development of microbial chemical factories that could prove useful in ECLSS. Microbes can be engineered to produce compounds of interest, including anti-infectives and other molecules. As understanding of the effects of microgravity on biological systems increases, synthetic biology approaches could also be used to engineer increased compatibility and robustness of the systems of interest. Engineered biological systems may also be useful for the creation of novel remediation and recycle systems, especially given the development of at least two algorithms designed to address this problem. “Smart” microbes could form a stable consortium whose exact composition could change in response to fluctuations in feed composition. Such complex systems represent a “second wave” of synthetic biology that has yet to reach its full potential (Purnick and Weiss, 2009).

REFERENCES


