A Study of Recent Advances in Systems Biology of Pseudomonas in Biotechnology

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Abstract

Engineering and changing synthetic microbial chassis is one of the most effective ways to not only understand the underlying principles of life, but also to improve applications in health, medicine, agriculture, veterinary medicine, and food. The top-down strategy (genome reduction) and the bottom-up approach (genome expansion) are the two basic strategies for developing a microbial chassis (genome synthesis). Several nations have financed research studies on this subject. The 'Minimum genome factory' (MGF) initiative began in Japan in 2001 with the purpose of creating microorganisms with reduced genomes for industrial use. The 'cell factory' project, which ran from 1998 to 2002 as part of the EU's Fifth Framework Program, aimed to get a complete understanding of microorganisms utilized in the application sector. The elucidation of proteins produced by Bacillus subtilis, known as the'secretome,' was one of the project's most notable outcomes. In the United States, the GTL (Genomes to Life) initiative began in 2002. Researchers in this programme tried to develop artificial cells both in silico and in vitro, such as John Craig Venter's group's successful design and manufacture of a minimum bacterial genome. This study gives an update on recent breakthroughs in synthetic microbial chassis engineering, modification, and application, with a focus on the importance of learning about chassis as a tool to better understand life and enhance applications.

Keywords: *Microbial chassis; Systems; Synthetic; biology.*

I. INTRODUCTION

Synthetic biology has grown dramatically in scope, expectation, and production during the last decade, thanks to discoveries in genomics and the advent of systems biology. Systems and synthetic biology speed up the creation and modification of microbial chassis for use in basic research and applications in biotechnological, pharmaceutical, biomedical, and other industries. An ideal chassis in systems biology might be an organism with a reduced genome for full functioning and a metabolic network capable of more efficiently synthesising the needed molecules. A chassis in synthetic biology is an organism that contains and maintains genetic components by providing the resources necessary for them to operate.

Top down and bottom up ways to building a synthetic microbial chassis can be characterised as complementary and alternative approaches. Top-down techniques are also used to reduce genome size by deleting superfluous cellular genes in order to better understand genome architecture and features. Comparative examination of genomes, particularly those from varied species, may frequently uncover genes that are essential for cellular life and metabolic pathways that are similar and/or significantly different, thanks to the advent of large-scale DNA analysis. Following that, deletions can be performed by plasmid and linear DNA-mediated techniques, as well as the use of site-specific recombinases, transposons, and the CRISPR/Cas system (Fig. 1). Such reductions have been studied in Escherichia coli, Streptomyces, Bacillus subtilis, and Pseudomonas putida in the laboratory. Some of these chassis had physiological qualities that were completely untouched, while others had unanticipated properties. This method, however, has limits due to the beginning organism, and it is primarily empirical and time intensive.

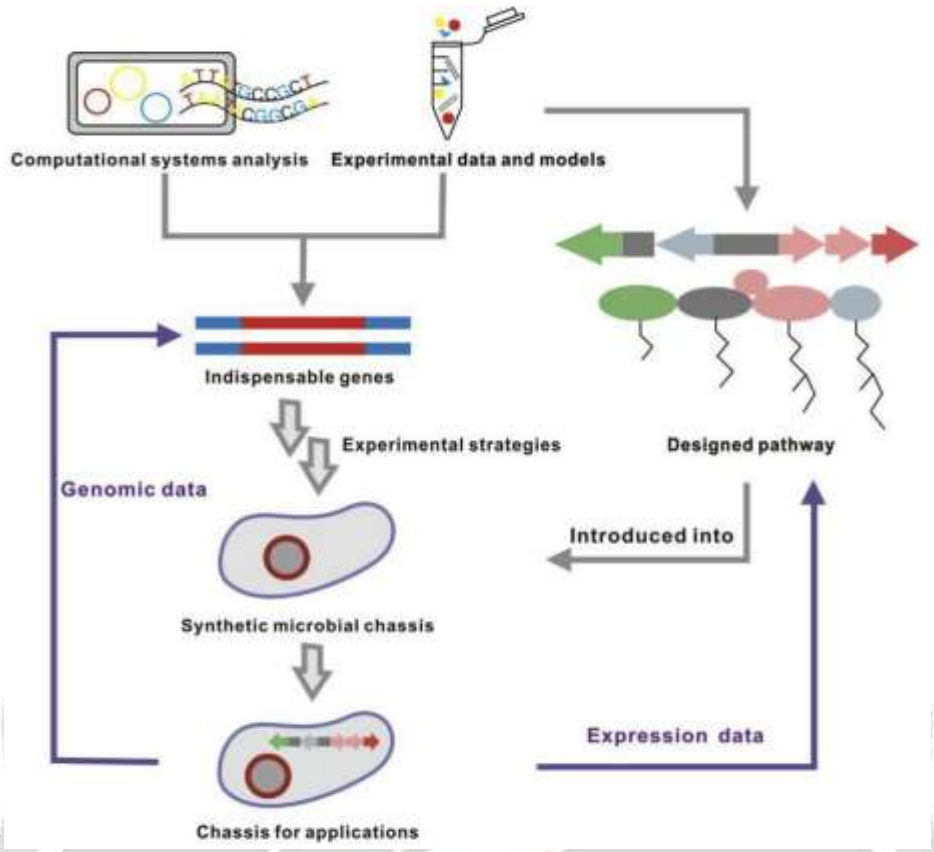

Figure 1: Schematic illustration of engineering and modification of synthetic microbial chassis using a top-down strategy

Bottom-up techniques, on the other hand, aim to build chassis from the ground up that might credibly self-assemble into artificial chassis. Advances in DNA synthesis, sequencing technology, and transplanting have enabled the de novo creation of lengthy DNA sequences with complex gene compositions. The use of polymerase chain reaction (PCR) technology to construct pools of overlapping short oligonucleotides is crucial to de novo synthesis methods for large DNA molecules and even the entire genome (Fig. 2). These methods enabled the entire restoration of a genome as well as the creation of a new synthetic microbial chassis.

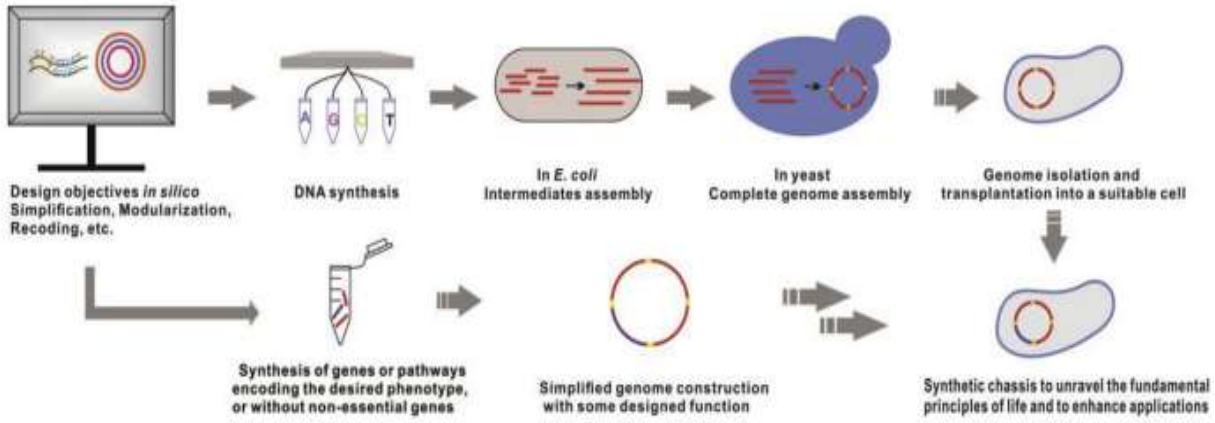

Figure 2: Construction of synthetic microbial chassis by a bottom-up strategy

II. TOP-DOWN APPROACH IN SYSTEMS AND SYNTHETIC BIOLOGY

2.1 Escherichia coli

E. coli is an essential host for engineering chassis because of its unique qualities, such as its clear genetic background, simplicity of handling, and potential for industrial and medicinal applications. E. coli K-12 is one of the most extensively studied organisms and is frequently used in genetic, biochemical, and metabolic studies. The majority of E. coli chassis were generated from MG1655 and W3110, two closely related K-12 strains. The deleted genome size has increased from 5.6 to 38.9% since the first report of a genome-reduced E. coli strain was published in 2002.

A quick and easy approach was established to create MDS strain 12. By red-type homologous recombination, a PCR-generated DNA fragment was introduced into the genome, followed by a DSB-stimulated recombinational repair process, resulting in a scarless deletion.

2.2. Streptomyces

The capacity of Actinomycetes, particularly Streptomycetes, to develop innovative natural compounds with proven usefulness in human and animal medicine and agriculture is a significant trait of these organisms. The massive (Meta) genomic data obtained during the postgenomic period reveals that Actinomycetes possesses huge genomes that contain many secondary metabolite biosynthetic gene clusters, the majority of which are still enigmatic. The majority of these strains, however, have shown to be resistant to genetic tinkering. As a result, a flexible model chassis must be developed as hosts for heterologous secondary metabolite pathways to be expressed, particularly for the activation of cryptic gene clusters to aid in drug discovery. Without duplicate secondary gene clusters, such hosts were projected to eliminate competing carbon and nitrogen sinks, improving precursor availability and heterologous cloned gene cluster production.

Streptomyces avermitilis has a linear chromosome of 9.02 Mb and produces the anthelmintic avermectin. A comparison of three published Streptomyces genomes, Streptomyces coelicolor A3, Streptomyces griseus, and Streptomyces avermitilis, found a 6.28–6.50 Mb conserved core area where the genes are required for growth. Strain-specific genes, genes encoding secondary metabolite production, and no essential genes are found in the left and right subtelomeric sections, which are 2 Mb and 0.5 Mb in length, respectively. Using generic homologous recombination or site-specific recombination (Cre-loxP) procedures, more than 1.4 Mb of subtelomeric areas were deleted, yielding a series of multiple-deletion mutants with chromosomes that equate to 83.12–81.46 percent of the wild-type chromosome.

2.3 Other prokaryotes

Many of the conditions for the production of synthetic biology chassis are satisfied by Pseudomonas putida, a common gram-negative soil bacterium with metabolic diversity, resilience, and simplicity of manipulation. Rhamnolipids, terpenoids, polyketides, nonribosomal peptides, and other amino acid-derived substances are among the secondary metabolites produced spontaneously by Pseudomonas species. Pseudomonas putida KT2440 is the best-studied saprophytic laboratory Pseudomonas, with its whole chromosomal sequence accessible since 2002. Using a combinatorial technique, four double-deletion mutants were created based on this strain, with up to 7.4 percent of a single genome erased across two cycles. The FLP-FRT site specific recombination technology and modified mini-Tn5 transposons are used in this reusable three-step excision procedure. In LB media, strains 407.1-Δ 2 and 407.3- Δ2 grew as well as the wild-type strain, with up to 1.4-fold greater final cell densities than TEC1.

2.4 Eukaryotes

Fungi account for 38% of all known microbial bioactive chemicals (22,500). As a result, innovative yeast chassis must be developed for the synthesis of high-value secondary metabolites that are employed as antibiotics, other medications, poisons, insecticides, and animal and plant growth factors.

The fission yeast Schizosaccharomyces pombe is distinguished from other yeasts by its capacity to reproduce by fission and by having numerous molecular, genetic, and biochemical traits with higher eukaryotes such as plants and animals. S. pombe has progressively grown in popularity as a host for the expression of a growing variety of membrane, secretory, and cytoplasmic proteins. However, in S. pombe, the degradation of recombinant gene products by host-specific proteases has a considerable influence on heterologous protein output and secretion efficiency. The use of numerous protease-deficient chassis to prevent these issues is a good way to go.

III. BOTTOM-UP APPROACH IN SYSTEMS AND SYNTHETIC BIOLOGY

Synthetic biology has seen a surge in attempts to apply rational engineering ideas and processes to build and programme large-scale user-defined pathways and even complete organisms in recent years. The development of quick and affordable DNA synthesis and assembly techniques, as well as revolutionary low and high-throughput genome-engineering approaches, has opened the way for the manufacture and modification of synthetic microbial chassis. As a result, some of the world's most urgent issues, including as healthcare, food production, renewable, superior, and cleaner energy generation, and biomaterial creation, may be addressed.

3.1. Strategies for chassis in prokaryotes

Fast and efficient DNA synthesis and assembly methods like Gibson assembly, Golden Gate assembly, and DNA assembly created a desire for entire genome de novo synthesis. The biggest piece of man-made DNA, a continuous 32-kb polyketide synthase gene cluster, was manufactured in 2004 using PCR technique to build pools of overlapping short oligonucleotides. Although there were certain drawbacks, such as high error rates on a genomic scale during the PCR processes and costly oligonucleotide synthesis, this breakthrough opened the door to building artificial cells. Venter and colleagues synthesised the first full genome of an organism, a 582,970-bp Mycoplasma genitalium genome, in early 2008, a milestone dubbed "the dawn of synthetic genomics." The genes in the resultant synthetic genome, M. genitalium JCVI1.0, are almost equivalent to those of M. genitalium G37 wild type. They are distinguished by a single gene, MG408, which was damaged by an antibiotic marker to prevent pathogenicity and allow selection.

The construction of a bacterial cell controlled by a chemically manufactured genome became conceivable because to enhanced technologies of whole-synthetic-genome assembly and genome transplanting. Based on computer-generated genome sequences, the 1.08-Mb genome of Mycoplasma mycoides JCVI-syn1.0 was created, synthesised, assembled, and then transplanted into Mycoplasma capricolum recipient cells to establish new M. mycoides cells controlled only by the synthetic chromosome. The synthetic M. mycoides genome was put together using a mix of in vitro enzymatic techniques and in vivo yeast recombination. The restriction barrier was breached to transplant the synthetic genome by methylating the donor DNA or simply breaking the recipient cell's restriction mechanism. JCVI-syn1.0 possesses the desired phenotypic features and can self-replicate indefinitely.

3.2 Strategies for the chassis in eukaryotes

The Synthetic Yeast Genome Effort, often known as Sc2.0, is a large-scale project aimed at creating a synthetic genome for a eukaryotic model organism that will be used as a chassis for genetic research of eukaryotic chromosomes. Several synthetic chromosomes have been demonstrated to work in yeast, including the synthetic yeast chromosome arm (synIXR) and the completely synthetic chromosome (synII, synIII, synV, synVI, synX, synXII).

The first Sc2.0 synthetic chromosome, synIII, is based on the 316,617-bp natural Saccharomyces cerevisiae chromosome III and has a functioning 272,871-bp chromosome. The "designer" sequence was created in silico by modifying the native sequence according to fitness, genomic stability, and genetic flexibility principles. SynIII was then built using three primary steps: building block (BB) synthesis, minichunk assembly, and direct placement of natural yeast chromosome III with pools of synthetic minichunks. Subtelomeric regions, transfer RNAs, introns, transposons, and quiet mating sites were removed from the synIII genome to make it smaller. TAG/TAA stop codon replacements enable for the inclusion of non-natural amino acids utilising the liberated TAG codon, as well as the introduction of loxPsym sites and PCR Tags, which allow for facile directed evolution alterations of the synthetic yeast genome. Saccharomyces cerevisiae fitness, transcriptome, and replication were unaffected by these alterations.

IV. CONCLUSIONS

Numerous research groups around the world have worked to develop highly efficient strategies for low- and high-throughput genome engineering, whole-genome synthesis, assembly, and "booting-up" methods in order to engineer and modify synthetic microbial chassis for industrial application and understanding the universal principles of life. From both a top-down and bottom-up perspective, biological design provides a suitable framework for achieving this aim.

Non-essential gene identification is critical for designing and changing microbial chassis, and it has aided in determining which genes are required for cellular features and how many genes are required to maintain cellular life. To begin, a number of computer techniques were used to identify the key necessary genes for maintaining life. Basic metabolism, cell wall metabolism, cell division, and DNA metabolism are all regulated by essential genes. Non-essential genes, such as cryptic prophages, phage remnants, insertion sequences [ISs], some unknown genes, and several metabolic pathways, were later found or eliminated using a variety of experimental methodologies. Finally, analysing experimental data can aid in the knowledge of unknown genes or pathways, allowing for further development. Some of the chassis built by decreasing redundant genomes have demonstrated improved genome stability, greater industrial product output, or both.

The top-down strategy for building and engineering chassis is based on integrating computational system analysis, experimental data, and models like as metabolic, regulatory, and signal networks to discover the essential genes. Then, using various methodologies, a chassis is built and customised by eliminating nonessential genes. An appropriate biosynthesis pathway is devised and inserted into the chassis for industrial use after the interaction between the biosynthesis route and the genome in the chassis is examined. More information on the genomic identification and storage of natural biological components can enable synthetic biology applications progress dramatically (Fig. 1). Advances in genome fusion technologies have recently enabled not only a better method for constructing adaptable chassis for research and applications, but also significant progress.

Genetic circuits were simply created by computer automation, produced by low-cost DNA synthesis, assembled, and then transplanted to form a bacterial cell controlled by a chemically generated genome using the bottom-up technique. Sc2.0 synthesis costs are currently at US$0.10 per base pair, thanks to additional advancements in both software and DNA synthesis technology. The entire cost of the Sc2.0 project, including direct and indirect expenditures, will, of course, be far greater. The chassis research has already yielded a wealth of knowledge on gene networks and interactions. These findings will hasten the development of customized industrial chassis for the manufacture of valuable medicines and chemicals, as well as bioremediation of environmental contaminants and the development of renewable energy sources.

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