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Designer microbes: A promising venture

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Abstract
Microbes are single-cell organisms and are so small that millions of them can fit into the eye of a needle and still remain invisible to the naked human eye. In this work we discuss the procedure behind building of these microbes, possible areas where the designer microbes can work phenomenal and focus on the important and successful man made microbes. Although not an in-depth assessment, this paper will help the readers get acquainted with the miracles these microbes can pull off from fighting obesity to cleaning up of the environmental pollution. With the incorporation of a technique called multivariate-modular pathway engineering approach", production of anticancer taxol precursors in E. coli has been optimized.

Keywords: Microbes, taxadiene, polycyclic aromatic hydrocarbons (PAH)

1. Introduction
Microbes are single-cell organisms and are so small that millions of them can fit into the eye of a needle and still remain invisible to the naked human eye. Being the oldest form of life on earth, microbe fossils date back more than 3.5 billion years. Being a part and parcel of our lives these miniature living being have made significant contribution to various aspects of our wellbeing; from brewing beer to producing antibiotics and from making bread to formation of curd /yogurt. These organisms aren't kind enough all the time as they have and are continuing their decisive plan of work in causing various life threatening diseases. Drawing cues from their contribution to the food processing and pharmaceutical industries, microbes can be modified into the most reliable workhorses for the synthesis of desirable products, transform the deadliest microorganism into a disease fighting comrade and much more. No doubt these organisms grow rapidly on cheap carbon sources and have a short generation interval yet the microbial industrial biotechnology has its own fair share of drawbacks. Ranging from poor or limited output of the desirable product to the synthesis of off putting compounds the microbes of our interest need certain trimming and tweaking at the genetic level for better outputs. The successful completion of the human genome project has revolutionized the sequencing arena making it more affordable and reliable. The post genomic age has changed how we see and comprehend the microbial pathways involved in the biosynthesis of valuable products. The dawn of digitalization and advent of internet has made a large amount of microbial genome information accessible by means of databases. With all these put together along with a petite knowledge of microbial enzyme functions and the ability to synthesis DNA *in vitro* we are equipped with tools for ascertaining and designing tailor made microbial enzymes and metabolic pathways of our interest. And not to forget the developments in the fields of metabolic engineering, enzyme evolution and synthetic biology in the last decade that has helped us accomplish the unattainable feat such as the capability to engineer enzymes to carry out unnatural reactions and combining diverse enzymes in a heterologous host to produce valuable compounds.

In this work we discuss the procedure behind building of these microbes, possible areas where the designer microbes can work phenomenal and focus on the important and successful man made microbes. Although not an in-depth assessment, this paper will help the readers get acquainted with the miracles these microbes can pull off from fighting obesity to cleaning up of the environmental pollution.

2. Construction of designer microbes
The designing of microbes for biosynthesis involves a cascade of events beginning with the

selection of a suitable host. It is normal to select a microbe that can be easily cultured, with a known genome sequence, has the ability to respond to genetic manipulation and is bestowed with a well understood and studied metabolic pathways. The hands on model organisms *E. coli* and *S. cerevisiae* have met with the criteria and are considered fit for various manipulative strategies for a better and desirable output. The second criteria to be full filled is the choice of the building blocks such as enzyme and other components of the pathway that can be obtained from a vast variety of sources and grown in our host of choice. By applying a potential approach to mix and match enzymes from various metabolic pathways, renovate and construct a novel man made pathways for production of better outputs. The dawn of *clonetegration* or one step cloning and chromosomal integration of DNA has drastically decreased both the time and effort needed for integration of single or multiple DNA fragments. Already being dubbed as one of the efficient technologies along with other site directed recombination techniques *clonetegration* has the potential to help devise microbes with engineered biosynthetic pathways.

Apart from the above mentioned necessary amendments, the other important norms to be taken into consideration are the controlled gene expression and monitoring the pathway flux. Firstly, a thorough check on the gene expression that are usually mediated by promoters, which act as “on/off” switches, or as “dimmer” switches, thereby regulating the degrees of gene expression play a pivotal role in ensuring the appropriate expression of enzymes and thereby preventing any kind of an extra metabolic burden being placed on the host and augment the pathway flux. The recent effort to produce artemisinic acid, an anti-malarial drug precursor in *S. cerevisiae* with a manipulated biosynthetic pathway is an example of the success of this technique.

3. Ability to produce cancer fighting agents

With the incorporation of a technique called multivariate-modular pathway engineering approach”, production of anticancer taxol precursors in E. coli has been optimized. A metabolic flux enhancement approach wherein the isoprenoid biosynthetic pathways are separated into two modules-a native upstream precursor supply module, and a heterologous downstream isoprenoid producing module and a perfect pathway balance is stroke. When the genes specific for the production of precursors are cloned together in an operon and were placed under an inducible promoter, resulting in increased yields from the native MEP pathway; the precursors are channelized and a yield of upto 1020 mg/L of taxadiene was recorded from a well fed batch of cultivation.

4. Production of Biofuel

The manipulation of the carbon storage regulator system (Csr) of *E. coli* has shown to improve the yield from microbes. These microbial factories with engineered pathways to overexpress the central carbon metabolism regulatory element Csr B paved way for the production of biofuels such as n-butanol and amorphadiene. When the expression levels of the Csr B, which binds to and disrupts the translation inhibit or protein Csr A were altered in the *E. coli* cells, they were shown to accumulate glycolytic and TCA cycle intermediates, and used carbon more efficiently. Apart from this researchers have engineered a novel biosynthetic pathway in E. coli to make the second generation biofuel pentanol. By using a multi-level modular approach, a three module precursor supply pathway was designed which resulted in the which resulted in conversion of glucose or glycerol to pentanol which made use of precursors such as propionyl-CoA and acetyl-CoA and five carbon molecule 3-hydroxyvalerate from the first and second modules.

5. Bioremediation of Pollutants

One major concern that torments every citizen of planet earth is to save it from further insults in the form of pollutants especially the non-biodegradable and the radioactive wastes. Bioremediation is defines as the treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances. And one such bioremediation technique is known as mycoremediation wherein fungal mycelia are used to cleanse the area. With this framework, the ideal would be creating a perfect microbial factory that shall secrete mycelia without the need to alter or optimize already existing systems. The successful construction of these microbes shall help us breakdown lignin and cellulose. And a further modification shall degrade nerve gas, sarin and polycyclic aromatic hydrocarbons (PAH).

Ability to fight obesity

One microbe that can be comfortably used to kick the extra pounds can be the *E. Coli* prescribed as a digestive probiotic in many European countries. The engineered bacteria can be made capable to produce an appetite-suppressing compound that’s normally secreted by the intestines in response to food intake, and satisfies one’s appetite. Further sophisticated genetic engineering tools can be used to insert a genetic “kill switch” triggered by the same compound yet be harmless to human tissues as well as the members of the natural microbiome. This research is still in the initial stages and a very little of information has been made available.

At present all the experiments using these engineered microbes are carried out on a “trial and error” basis. The advent of computer-aided design (CAD) tools has widened our horizons to construct the strategic and logic design of biosynthetic pathways. On the other hand, recent developments in genome sequencing and our ability to manipulate large pieces of DNA have contributed to the successful implementation of metabolic engineering and synthetic biology design principles in creating microbes for biosynthesis. In the days to come may the researches be equipped with the techniques to circumvent the bottlenecks and lacunae and design idealized biocatalysts for the production of target molecules

6. References

1. Lan EI, Liao JC. Microbial synthesis of n-butanol, isobutanol, and other higher alcohols from diverse resources. Bioresour Technol. 2013; 135:339-49.
2. Giddings LA, Newman DJ. Microbial natural products: molecular blueprints for antitumor drugs. J. Ind Microbiol Biotechnol. 2013; 40(11):1181-210.
3. Quin MB, Schmidt-Dannert C. Designer microbes for biosynthesis. Curr Opin Biotechnol, 2014, 55-61.
4. Husain Q. Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile andother synthetic dyes from polluted water. Critical Reviews in Biotechnology, 2006; 26(4):201-221