ACS SyntheticBiology

In This Issue

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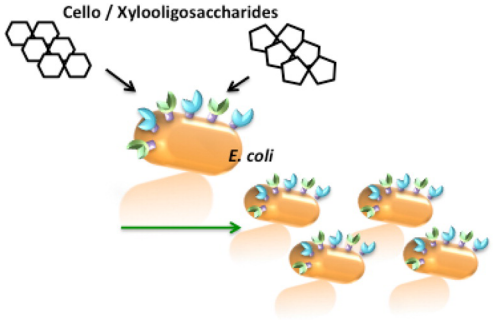
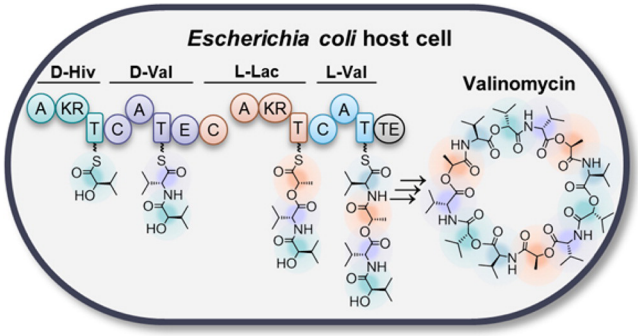
CREATION OF CELLOBIOSE AND XYLOOLIGOSACCHARIDES-COUTILIZING *E. COLI*



Industrial effluents such as lignocellulose hydrolysates are composed of a mixture of mono- and oligomeric hexoses and pentoses. Thus, coutilization of several sugars in industrial effluents is essential for economically feasible productivity of biofuels and chemicals. However, coassimilation of xylose/glucose is poor when compared to fermentation using glucose or xylose as the sole carbon sugar. In this study, Tanaka *et al.*, (DOI: 10.1021/sb400070q) now describe the construction of a cellobiose/xylooligoshaccharide coutilizing *E. coli* strain displaying both *β*-glucosidase and *β*-xylosidase on its cell surface.

The authors successfully demonstrate direct growth on a cellobiose/xylooligosaccharide mixture and also report a slightly increased cell growth rate as compared to growth rates with xylooligosaccharides or cellobiose as sole carbon sources. This approach holds promise in the production of biofuels and/or biochemicals from lignocellulosic biomass.

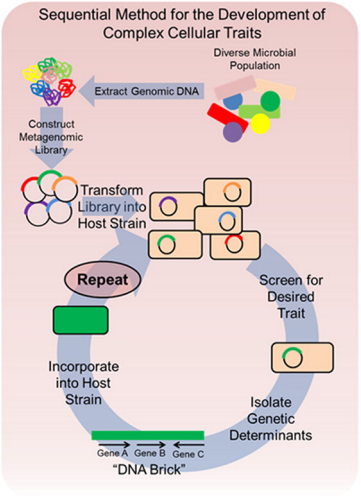
RECONSTITUTED BIOSYNTHESIS OF THE ANTIBIOTIC VALINOMYCIN



Production of complex natural products in a surrogate host that is easy to manipulate genetically and performs well under scalable fermentation conditions will promote natural product access and industrial development. Here, Jaitzig *et al.*, (DOI: 10.1021/sb400082j) report the successful heterologous production of valinomycin, a 36-membered cyclic depsipeptide of non-ribosomal origin from *Streptomyces sp.*, in *E. coli*.

In this case study, the authors discuss soluble and active expression of large and complex biosynthetic enzymes, posttranslational modification, precursor requirements, product toxicity and transportation. They also present the first biochemical evidence of a previously postulated mechanism for the biosynthesis of valinomycin. This work broadens the spectrum of non-ribosomal peptides that can be produced in *E. coli* and encourages future engineering of the valinomycin assembly line to produce structural analogs.

BUILDING STEPWISE, COMPLEX, MULTICOMPONENT TOLERANCE TO TOXIC CHEMICALS



Modern bioprocessing applications require superior cellular traits. Many of these, such as tolerance to toxic chemicals, stem from unknown genes and gene interactions. Previously, the authors reported a semisynthetic response system expressed off a plasmid heat shock protein (pHSP). Here, Zingaro *et al.*, (DOI: 10.1021/sb400156v) probe the genomic space of the solvent tolerant *Lactobacillus plantarum* to identify heterologous genetic determinants that impart complex solvent tolerance in combination with pHSP.

The authors used two targeted enrichments, one for better viability and one for better growth under ethanol stress, and identified several beneficial and specialized heterologous DNA determinants that act synergistically with pHSP. Additionally, they developed a complex composite phenotype of improved growth and survival by combining the identified *L. plantarum* genetic fragments. Thus, this study details a sequential, iterative assembly strategy for building multigenic traits by exploring the synergistic effects of genetic determinants from a much broader genomic space.

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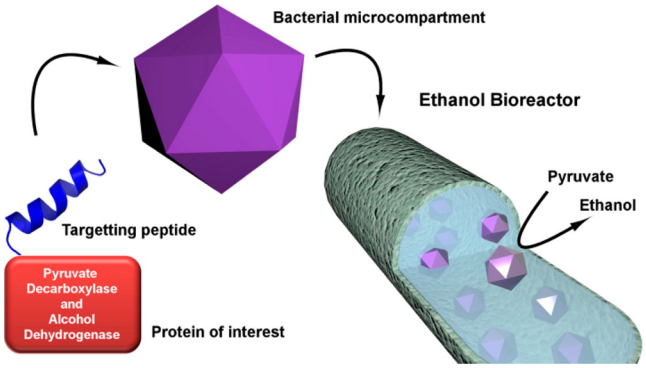
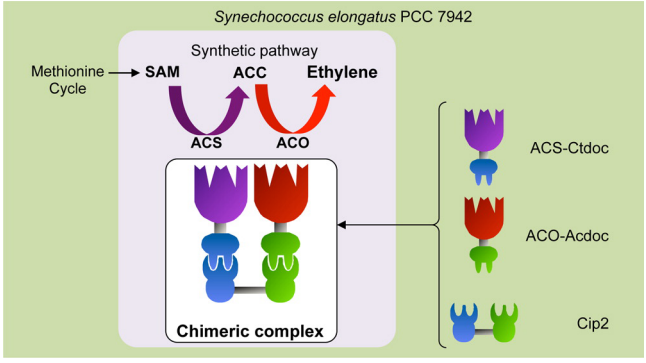
SOLUTION STRUCTURE OF A BACTERIAL MICROCOMPARTMENT TARGETING PEPTIDE



Bacterial microcompartments (BMCs) are proteinaceous organelles that are composed of an outer protein shell that encases a specific metabolic process. BMCs are attracting significant attention as they have potential to be engineered for biotechnological applications. The manipulation of BMCs is dependent upon an ability to encapsulate heterologous enzymes. Internalization of native enzymes into the BMCs is facilitated by short targeting peptides. Using NMR, Lawrence *et al.*, (DOI: 10.1021/sb4001118) have now solved the structure of an N-terminal targeting peptide and determined the region with which it interacts on the BMC shell.

Additionally, by tagging two enzymes with such targeting peptides the authors were able to direct a short, functional pathway inside an empty BMC. In doing so, the metabolic function of the BMC has been changed from propanediol catabolism to ethanol synthesis.

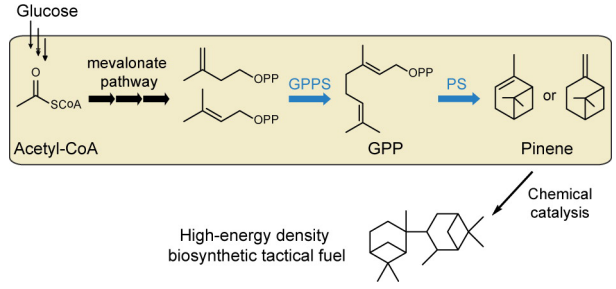
ENGINEERED PLATFORM FOR BIOETHYLENE PRODUCTION



Ethylene is an industrially important compound whose generation requires high energy, often obtained via the steam cracking process. Thus, more sustainable production methods are desirable. Cellulosomes are naturally occurring bacterial constructs that improve the efficiency of cellulose degradation by physically linking relevant enzymes. In this study, Jindou *et al.*, (DOI: 10.1021/sb400197f) capitalized on the efficiency of cellulosomes by genetically engineering a chimeric complex of two ethylene-generating enzymes from tomato plants using a scaffold of bacterial proteins.

The authors transformed this chimeric complex into the cyanobacterium *Synechococcus elongatus* PCC 7942 and found that, at low protein expression levels (without IPTG), the chimeric complex produced substantially more ethylene *in vivo* than the uncomplexed enzymes, indicating that the cellulosome greatly enhanced the efficiency of ethylene production. Thus, cyanobacteria can be used to sustainably generate ethylene, and cellulosomes can be adapted to improve the production efficiency of other industrial compounds.

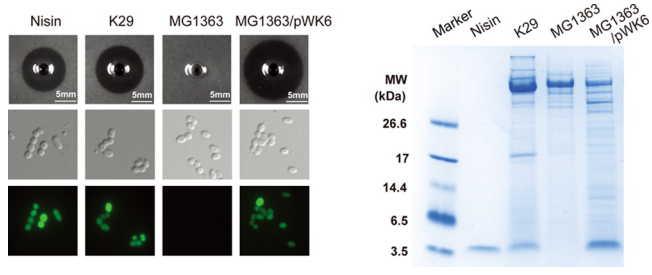
MICROBIAL SYNTHESIS OF PINENE



Advanced biofuels have properties similar to those of petroleum-based fuels. While recent progress in microbial engineering has resulted in biosynthetic alternatives to gasoline and diesel, the development of microbial platforms for the production of high energy-dense fuels has lagged behind. Pinene dimers have been shown to contain high volumetric energy similar to that found in the tactical fuel, JP-10. In this study, Sarria *et al.*, (DOI: 10.1021/sb4001382) describe the engineering of *E. coli* for the production of pinene.

The authors combinatorially expressed three pinene synthases and three geranyl diphosphate synthases from three conifers and obtained a best titer of ~28 mg/L of pinene. They also investigated the pinene isomer ratio of the microbial production platforms and whether pinene toxicity was limiting production. This manuscript puts forward the concept of ring strain as an important feature of advanced biofuels and also presents a pinene production platform with a 6.5-fold improvement as compared to previous pinene production in *E. coli*.

CLONING AND OPTIMIZATION OF A NISIN BIOSYNTHESIS PATHWAY



Nisin is an important antimicrobial peptide that is widely used in the food industry. It is effective against a broad spectrum of Gram-positive pathogens, such as *Listeria monocytogenes* and *Staphylococcus aureus*. Despite much effort, nisin overproduction has proven to be a challenge due to the complexity of the underlying biosynthesis pathway and the difficulty in genetic modification of lactic acid bacteria. Here, Kong and Lu (DOI: 10.1021/sb500225r) report the successful, systematic engineering of a nisin biosynthesis pathway (14.5 kb) for recombinant bacteriocin overproduction.

Through rational construction and optimization, the authors were able to develop synthetic lactic acid bacteria strains that produce bioactive, recombinant nisin with a yield 6 times greater than that of the wild-type strain.

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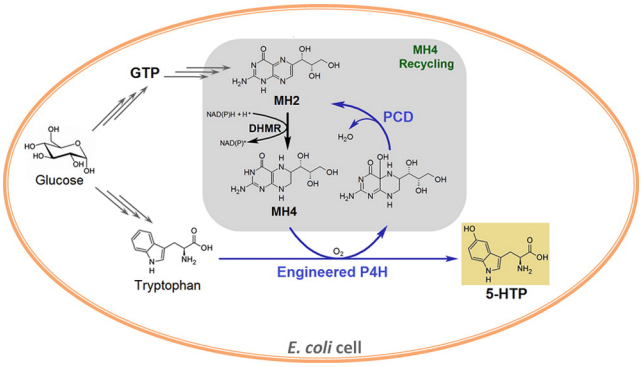
ENGINEERING BACTERIA FOR THE MICROBIAL SYNTHESIS OF 5-HYDROXYTRYPTOPHAN



5-Hydroxytryptophan (5-HTP) is a drug that is effective in treatment against several conditions, including depression, insomnia, obesity and chronic headaches. There are currently no synthetic methods available for its production—it is commercially produced by extraction from the seeds of *Griffonia simplicifolia*. Now, Lin *et al.*, (DOI: 10.1021/sb5002505) report microbial production of 5-HTP via combinatorial protein and metabolic engineering.

The authors reconstituted bacterial phenylalanine 4-hydroxylase activity in *E. coli* and used protein engineering to increase the efficiency of conversion from tryptophan to 5-hydroxytryptophan. Thus, the platform described here holds great potential for scale-up production of 5-HTP in microorganisms.

dx.doi.org/10.1021/sb5002714 | *ACS Synth. Biol.* 2014, 3, 426−428