Catalyzing Innovation

Innovating New Bioconversion Pathways

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Introduction

Microorganisms have long played a critical role in converting natural materials into something useful for humans, first dating back 1,000 years when yeast cells were harnessed to ferment beverages and foods for food preservation. Civilizations took notice and embraced the use of various natural microorganisms, until modern times when the advent of DNA science and bioengineering helped scientists pioneer new, innovative ways to genetically modify these natural microorganisms to produce food products, chemical commodities, and biofuels on an industrial scale.

Methanotrophs are one of the most recent classes of microorganisms that caught the interest of scientists over 10 years ago because of their unique potential to consume methane from natural gas and convert it into different products and applications, including liquid transportation fuel. Genetically engineered methanotrophs can also overproduce naturally occurring metabolites or non-native compounds, including molecules such as carotenoids, isoprene, 1,4 butanediol, farnesene, and lactic acid.[1,2] Some companies (like Unibio (London)) and Calysta (Menlo Park, CA) are using methanotrophic bacteria to produce highly concentrated protein products for animal and fish feed. No matter what the compound, by harnessing methanotrophs for the conversion of methane, we can reduce the release of harmful greenhouse gases and its impact on climate change.

Large sources of renewable methane have either already been captured or present the opportunity for capture. Opportunities for methane capture include landfills (38 megatons (Mt)/y), wastewater treatment (21 Mt/y), and manure management (11 Mt/y).[3] Typically, only large landfills are capable of generating sufficient methane to justify the capital and operational expenditure required for the infrastructure and equipment for methane capture and conventional conversion to electrical energy. However, the return on capital for any such investment is closely tied to the selling price of the final output of the project. In light of recent lower global energy prices, projects that utilize biomethane for electricity production or as a vehicle fuel are frequently not economical unless subsidized.

The fact that methane is the principal component of natural gas, for which there are ample reserves globally, figures into the narrative as to why methanotrophs warranted keen interest. The US Energy Information Administration calculated the reserves of US total natural gas at a record-high level of 388.8 trillion cubic feet in 2014.This is encouraging because one of the major obstacles to the production of renewable fuels is the supply of feedstock, which is often sugar-based and can negatively impact the global food supply chain, water, and arable land. The International Energy Agency estimates that the production of natural gas will continue increasing, with 25% of global energy derived from natural gas by 2035.

The methanotroph's natural ability to convert methane into microbial lipids and subsequently convert those intermediary products into liquid diesel fuel via a hydrotreating process created great interest. This conversion process demonstrated that methanotrophs could potentially serve as a viable approach to producing various energy and fuel products on a large scale. So began the investigation several years ago into this relatively new bioconversion process for liquid fuel production and its potential to deliver strong fundamental economics for affordable fuel and specialty products in a sustainable way that directly benefits the energy and chemical industries.

Biological approaches hold significant potential to efficiently yield energy products, such as biofuels, due to their favorable economics and a receptive market attitude keen on having sustainable alternatives for biofuel production. The global market's rapid access to methane, in the forms of natural gas and methane-rich biogas, has in recent years improved. This greater accessibility has significantly lowered the price of natural gas. Past attempts to produce "clean" energy have been hindered by high expense and difficulties achieving industrial-scale output with high efficiency. New bioconversion engineering approaches are helping to address and overcome this issue.

Methane is a good place to start. It represents an enormous resource and opportunity for conversion into higher value products, especially if it can be sourced from stranded natural gas deposits that are not economical to process for conventional heating, or from sustainable point sources, such as high-rate digesters or capped landfills. The World Bank estimates that 92 Mt/year is wasted by flaring or venting natural gas alone.[4]

Bioengineering Methanotrophs

The evolution of scientific inquiry involving methanotrophs has been progressing steadily over the past decade, promising and delivering a new path for bioengineered microorganisms and products (*Fig. 1*). About 20 years ago, scientists at Norferm, a joint venture between Statoil (Stavanger, Norway) and DuPont (Wilmington, DE), showed that the microorganism could be grown at industrial scale (more than 10 kiloton (kt)/y) at low cost and minimal media to produce animal feed.[5] The key benefits identified were low-cost growth parameters and minimal

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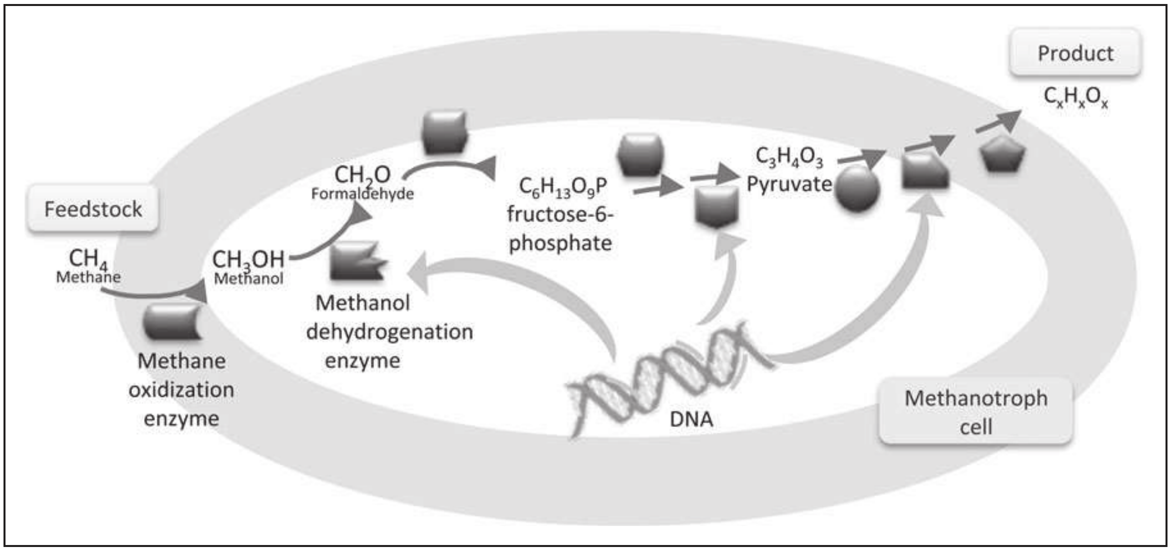

Fig. 1. Methane bioconversion platform.

media, but challenges were also recognized such as a poorly understood metabolic pathway and the need for more research and innovation.

In 2013, Intrexon's (Germantown, MD) scientists began to work with methanotrophs, developing a biocatalyst and process that enables the production of value-added molecules at commercial scale from natural gas. At that time, methanotroph research was in its early phases and scientists had few genetic and screening tools at their disposal, in comparison to commonly studied microorganisms like *E. coli* and yeast. Scientists began to fill the gap in their study of methanotrophs with a variety of tools to enable efficient genetic manipulation and strain evaluation: DNA transformation, DNA integration, high throughput screening (HTS), vectors, promoters, mutagenesis, and regulatory elements, just to name a few. Building these tools made sense, as methanotrophs research had produced promising results including superior yields of target molecules.

Innovative Bioengineering and Methanotrophs

This did not happen overnight. Scientists went to work on methanotroph science, tackling it on several fronts, including exploring DNA construction using in-house-developed software for creating semi-automated construction designs. Scientists also established workflows for the rapid assembly of large complex designs of methanotrophs. The scientists focused on improvements such as increasing throughput, reducing cycle time, and generating robust diversity-generation workflows. Early strains were bioengineered to increase the titer and output of the microorganisms over the course of a year. The results were encouraging and revealed a steady overall improvement in product titer from the engineered methanotrophs.

Researchers applied a methodology based on an iterative cycle of Designing, Building, Testing, and Learning (DBTL) to maximize their gains with the bioengineered methanotroph strains.[6] The basic toolbox for methanotroph research was readied, which helped accelerate the early strain engineering of the microorganism. All of this benefited from the design-build team's efforts to create DNA strains, which included building libraries that were then screened by researchers to ultimately identify better methanotroph strains. The DBTL approach accelerated the process as scientists realized increased throughput and reduced cycle times and generated robust diversity-generation workflows. Scientists were able to iterate on model-driven designs using the small-scale screening process, then performing fermentation and various ''omics'' analyses to select high-performance methanotroph microorganisms. Applying a combination of rational strain engineering and shorter cycle times than standard approaches, the scientists saved time in finding the right strains. The data output helped the scientists differentiate the right strains from those they needed to abandon because they did not provide the desired yield results. Over the course of three years, from 2014 to 2016, scientists were able to significantly boost the number of unique transformations per cycle.

Scientists also accelerated the process of discovering better strains by using HTS, which allowed for rapid strain improvement. The diversity libraries created went through three rounds of screening to determine the microorganisms that had the highest efficiency and hence represented the most promising strains for converting methane into liquid fuel. This advanced platform enables scientists to apply combinatorial libraries, data mining, and machine-learning technology to accelerate strain improvement.

By capitalizing on this innovative DBTL process, scientists were able to open up a new, highly efficient, and low-cost way to convert potentially environmentally harmful methane into liquid fuels for the transportation industry and specialty chemicals (i.e., 2,3 butanediol, Isobutyraldehyde) for industrial and consumer products such as synthetic rubber, acrylics, resins, and other fibers. By harnessing methanotrophs for the conversion of methane from a potentially harmful atmospheric source into a useful fuel, global methane output is reduced and its impact on climate change minimized.

Recently, Intrexon scientists scaled up its fermentation pilot plant in South San Francisco, CA and produced at pilot scale isobutanol, 2,3 butanediol (BDO), and isobutyraldehyde products. They discovered that these three products can be produced at high yields. For example, unlike the case in other industrial hosts, they were able to achieve 2,3-BDO stoichiometric yields of 93.6% of grams of product to grams of methane feed versus the traditional yeast yield of 50%. Researchers at Intrexon have expanded their work on a portfolio of target molecules over the past year.

Intrexon's methanotroph platform shows promising potential for achieving more economical, higher yields and viability for industrial use with the production of isobutanol and terpenes. This is important because natural gas is the cheapest source of carbon after coal. Intrexon's genetic toolbox introduces a disruptive technology for industrial fermentation and energy production. With the advanced platform and its characteristic ability for high throughput of methanotroph strain improvement for industrial products, there are undoubtedly more surprises ahead that will work to benefit industry and the environment.

Conclusions

Looking back, one has to consider that Liquefied Natural Gas (LNG) was first put into production 50 years ago from Algeria. It provided an outlet for excess natural gas resources and an additional revenue stream, and was a liquid fuel that could be transported. Looking forward, the global economy faces a markedly different set of circumstances. Natural gas prices are hovering around an all-time low and worldwide production continues to grow. That does not mean that we are without challenges. Natural gas has become commoditized given factors such as Henry Hub, liquefaction, and global transport, which have accelerated the downward pressure on producer economics. It does not take an economics degree to realize that this is an equation that could make it difficult for producers to realize a profit. However, just as LNG was innovative in the 1960s in processes such as bioconversion, we are starting to see innovation for today's energy landscape.

With the world facing an abundance of methane and the need to keep it from escaping as a potent greenhouse gas, coupled with the scientific progress made in bioengineering methanotrophs to produce an array of different products from methane, great progress has been made in this area of industrial biotechnology. With new innovations, the efficiencies and production levels have been improved significantly, reaching commercial levels in many cases. There are clear business benefits of engineering biology to deliver superior and environmentally responsible solutions to the commercial marketplace and to enable products in the health, energy, food, consumer, and environmental industries. Bioengineering has opened the doors of possibility for end-use applications of natural gas and to solve problems in our world.

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