Julia Vorholt investigates methylotrophic bacteria.

Systems biology of bacterial methylotrophy for biotechnological products from methanol (MetApp)

Methanol as bacteria fuel

Plastics, paints, antibiotics. These and many other everyday products use chemicals, whose biotechnological manufacture requires huge quantities of crude oil or plant-based raw materials. In future, methane or methanol could increasingly be used for this purpose. However, the bacteria in the bioreactors first need to learn how to convert this new energy source into the desired products.

It's a persuasive idea: methane could be used for the production of fine and basic chemicals. This would be a sensible way of using up this abundant, climate-damaging gas. "Methane not only occurs naturally in large quantities, but is also a by-product in oil production, sewage treatment and waste disposal sites," says Julia Vorholt, Swiss research partner on the ERASysAPP Project MetApp. "Methane is a largely untapped resource and has great potential for the production of useful chemicals without the need to sacrifice valuable agricultural land," adds the biochemist.

Only specialized organisms use methane or methanol as an energy source

However, what sounds so simple in theory is in practice a complex undertaking. Methane forms a highly explosive mixture with air. Although it can be used to power bioreactors, an alternative is to convert methane into methanol, a simple alcohol compound. Some specialized microorganisms are able to use this as a substrate. "Most microorganisms lack the necessary enzymes to do this, and some become damaged by the toxic compounds that arise throughout the metabolic cycle," explains Julia Vorholt. This is because methane and methanol are single-carbon (C1) compounds. These are organic compounds that do not contain carbon-carbon bonds, such as those found in oil or sugars like sucrose and cellulose.

There are, however, a few microorganisms that have found ways to use C1 compounds for growth, and they are known as methylotrophs. Two such bacteria, *Methylobacterium extorquens* and *Bacillus methanolicus,* are the focus of the MetApp project. Vorholt summarizes the two main objectives of the project: "First, we want to comprehensively understand and model the metabolism of methylotrophic bacteria, and second, we're looking for ways of applying this knowledge to the production of fine and basic chemicals."

Examining the bigger picture

The scientists working on the MetApp project are not scrutinizing every involved enzyme in detail. Instead, they want to understand the metabolism as a whole, from the genes all the way to the metabolic products. "We want to find out which elements are essential for the growth of microorganisms from C1 compounds," specifies Vorholt. This is why the team has decided to study *Methylobacterium extorquens* and *Bacillus methanolicus* in particular. Both of these microorganisms have decisive advantages. Firstly, they are able to use C1 compounds as well as sugars or organic acids as an energy source. "This enables us to analyze the metabolic pathways for each nutrient substrate in the same microorganism, and therefore to determine which specific cellular elements are crucial for the metabolism of methanol," explains the biochemist. Secondly, the part of the genome responsible for the C1 metabolism differs in the two organisms, and different genes mean different metabolic pathways. "The two bacteria have developed separate strategies for making use of C1 compounds," says Vorholt.

In a subproject, researchers are manipulating individual gene sequences to see how the bacteria react. But the MetApp team is going further and transplanting this genetic material into other organisms that are as yet only able to metabolize carbon-carbon compounds. Amongst these is *Escherichia coli,* an organism that has long been used in the biotechnology industry. "Thanks to experiments such as these, we're able to learn a great deal about C1 metabolic pathways, and will hopefully be able to make these established organisms available for industrial methanol conversion," enthuses Julia Vorholt.

The initial results of the MetApp project are promising. The researchers have so far managed to confirm the involvement of genes thought to be responsible for C1 metabolism. Furthermore, they have identified many new genes that are also involved. And although the precise role of these DNA pieces has not yet been clarified in detail, the findings give the scientists important clues, which they are now investigating.

Europe-wide collaboration

In order to make efficient progress, each research group involved in the MetApp project is concentrating on solving one part of the puzzle. While Trygve Brautaset's team in Norway is studying the *Bacillus methanolicus* bacteria in depth, the team based in Toulouse is constructing mathematical models. The scientists in Bielefeld, Germany, are developing the required omics methods for the experimental work and are also looking for ways to optimize the use of methylotrophic bacteria in chemical production. "Our team in Zurich is mainly studying *Methylobacterium extorquens,"* adds Vorholt, who has many years of experience working with this particular microorganism.

Since not all conventional analytical methods can be used for the investigation of methylotrophic organisms, Julia Vorholt and her team will need to develop existing technologies further – a challenge that the researcher is happy to rise to. Thanks to the Europe-wide network of research groups pursuing a shared goal, it is feasible that in the near future, bacteria will be able to utilize methanol in place of sugars to produce valuable chemicals. This project is the first step on the way to a C1 economy with tangible ecological benefits.

More information on MetApp:
www.sintef.no > projectweb > metapp
More info on ERASysAPP: **www.erasysapp.eu**

Bioreactor with *Methylebacterium extorquens.* Photo: Jethro Hemmann

MetApp at a glance

Research groups:

- Prof. Trygve Brautaset, Department of Biotechnology and Food Sciences, Norwegian University of Science and Technology, SINTEF Materials and Chemistry, Trondheim, Norway – Molecular biology, biotechnology
- Prof. Julia Vorholt, Institute of Microbiology, ETH Zurich – Microbiology, biochemistry
- Prof. Volker F. Wendisch, Center for Biotechnology (CeBiTec), Bielefeld University, Germany – Genetics, biotechnology
- Prof. Jean-Charles Portais, INSA Toulouse, France – Modelling

Total budget (2015–2018): EUR 1.5 million, including EUR 400,000 from SystemsX.ch

Project type: International Project – As a partner in the European research network ERASysAPP, SystemsX.ch has co-funded six international application-oriented projects in which Swiss consortium partners are involved.