

Better sludge through metagenomics

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Few stop to consider the consequences of their daily ablutions, the washing of clothes, the watering of lawns, and the flush of a toilet. However, wastewater treatment--one of the cornerstones of modern civilization--is the largest microbially-mediated biotechnology process on the planet. When it works, it is a microbial symphony in tune with humanity. When it fails, the consequences can be dire. Researchers from the U.S. Department of Energy Joint Genome Institute (DOE JGI) and collaborators at the University of Wisconsin-Madison, and the Advanced Wastewater Management Centre, University of Queensland, Australia, have published the first metagenomic study of an activated sludge wastewater treatment process.

The research appeared online in the September 24 edition of the journal *Nature Biotechnology*.

The metagenomic strategy entails generating DNA sequence information directly from samples of sewage sludge to provide a blueprint of the genes and hence the metabolic possibilities of the wastewater environment, with a view to understanding how the system works and predicting and averting failures or crashes.

"This is a first step in a much broader strategy employing a systems biology approach to the study of microbial communities with the goal of designing predictive models to understand how these communities function," said Hector Garcia Martin, lead author of the study and post-doctoral fellow in the DOE JGI's Microbial Ecology Program. "With this information now available, there are opportunities to bioengineer the

process to make it more reliable."

Removing excess phosphorus from wastewater can be most economically accomplished by environmentally friendly biological means in a process known as enhanced biological phosphorus removal (EBPR). The researchers were able to obtain a nearly complete genetic blueprint for a key player in this process, the bacterial species *Accumulibacter phosphatis*.

Activated sludge wastewater treatment processes are used throughout the world to purify trillions of gallons of sewage annually. Many treatment plants employ specialized bacteria to remove the nutrient phosphorus, in an effort to protect lakes and rivers from eutrophication, a deterioration of water quality characterized by excessive algae blooms.

Accumulibacter play a vital role in wastewater management, accumulating massive amounts of phosphorus inside their cells.

"Engineers and microbiologists have been trying for 35 years to grow this microbe, with no success," said Trina McMahon, Assistant Professor, Department of Civil and Environmental Engineering, University of Wisconsin, Madison, and one of the study's authors.

"Remarkably, through metagenomic techniques, we were able to isolate and acquire the genome sequence of *Accumulibacter phosphatis* without a pure culture of the organism, which, like most microbes, eludes laboratory culture. We expect that clues hidden in the genome will lead to domestication of this mysterious organism, enabling further studies of its habits and lifestyle.

"The genome sequence will also enable biologists and engineers to understand why and how these organisms accumulate phosphorus, and it will lead to major advances in optimizing and controlling the EBPR wastewater treatment process," McMahon said. "In particular, it makes possible further research into why some wastewater treatment plants

occasionally fail. These failures often result in serious pollution of lakes, rivers, and estuaries."

When things go wrong, the environment can be inundated with untreated phosphorous, carbon, and nitrogen--the detritus of human activities--necessitating costly and environmentally taxing remedies and exposing the public to potential disease hazards. The scale is daunting--more than 31 billion gallons of wastewater are treated daily in the U.S. alone. Even a marginal improvement in the process would translate into huge savings and spell relief for environmental engineers.

David Jenkins is Professor Emeritus of Environmental Engineering at the University of California at Berkeley. His research spans some forty years of international professional practice in water and wastewater chemistry and wastewater treatment for government, municipalities, and industry. He has specialized in the chemical precipitation of phosphate from wastewater and sludges, the causes and control of activated sludge bulking and foaming, and biological nutrient removal.

"The findings and tools described in this landmark paper will allow the resolution of many of the questions that have arisen concerning the mechanism by which the enhanced removal of phosphate from wastewater occurs," said Jenkins. "Understanding these mechanisms will undoubtedly lead to more efficient operation of the process and to the development of more robust designs."

Microorganisms are well equipped to do the job, but activated sludge is a black box, at least for those engineers who are dependent on the microbial aspect of the equation. To shed some light on the challenge, the team compared sludge samples from wastewater plants in Madison, Wisconsin, and Brisbane, Australia, that they maintained in laboratory-scale bioreactors to control and monitor the status of the sludge microbial communities.

"We found functions that didn't make sense for the current lifestyle of the organism," said Phil Hugenholtz, head of the JGI's Microbial Ecology Program. "Accumulibacter has all the genes necessary to fix carbon and nitrogen, which it would be compelled to do in a nutrient-poor environment like freshwater, but it presumably wouldn't have much use for in nutrient-rich EBPR sludge. So it got us thinking that these bacteria must be living in natural habitats and that they have become opportunistically adapted to this manmade process, wastewater treatment." It would appear, Hugenholtz went on, that Accumulibacter has been following in humanity's environmental footprints. "The genomes of the bacteria from the two sites were surprisingly similar--practically identical in parts--from samples separated by nearly 9,000 miles."

On the Net: [www.nature.com/nbt/journal/vao ... ent/abs/nbt1247.html](http://www.nature.com/nbt/journal/vao...ent/abs/nbt1247.html)

Source: Joint Genome Institute

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