Scientists sequence genome of the N2-fixing, soil-living bacterium Azotobacter vinelandii

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A collaboration of researchers, which includes scientists at the Virginia Bioinformatics Institute (VBI) and Virginia Tech, have completed the genome sequence of *Azotobacter vinelandii*, uncovering important genetic information that will contribute to a more complete understanding of the biology of this versatile, soil-living bacterium. The work will help advance research on *A. vinelandii*'s role as a model study organism for investigation of nitrogen fixation and other biochemical processes. It will also pave the way for new applications in biotechnology, including the possible use of *A. vinelandii* as a "factory" for the production of other proteins, in particular those that may be damaged by the presence of oxygen.

A. vinelandii

lives in soil and has the ability to convert nitrogen from the atmosphere into ammonia via bacterial enzymes called nitrogenases. Nitrogen fixation *is essential for life since different nitrogen-containing molecules are used to produce DNA and the amino acids that are the building blocks of proteins. For most bacteria, the nitrogenase enzymes involved in nitrogen fixation are very susceptible to destruction by oxygen. A. vinelandii is one of the few bacteria that can fix nitrogen in the presence of oxygen, using three distinct nitrogenase systems.*

The work of the *Azotobacter vinelandii* genome project team, which will be featured on the cover of the second July 2009 issue (14) of the *Journal of Bacteriology**, identifies unique features of the *A. vinelandii*

genome that explain how the bacteria is involved in oxygen-sensitive reactions such as nitrogen fixation, while maintaining strictly aerobic metabolism.

A. vinelandii has one of the highest respiratory rates of any known bacterium and has the capacity to maintain low levels of cytoplasmic oxygen through a process called respiratory protection. The sequence of the bacteria's genome allowed the team of researchers to identify the genes involved in respiration, including those responsible for respiratory protection. Genome analysis helped pinpoint the chromosomal location of the three known oxygen-sensitive nitrogenases, as well as the genes that code for other oxygen-sensitive enzymes such as carbon-monoxide dehydrogenase and a formate dehydrogenase. The sequence also provided important information on the genes that code for alginate, a polymer that further protects the organism from excess oxygen by forming a physical barrier around the bacterium.

"*A. vinelandii* is an attractive model organism for biochemical studies because of its ability to produce high yields of quality enzymes," said João Setubal, associate professor at VBI and the Department of Computer Science at Virginia Tech. "The findings in this study provide new prospects for the wider application of this bacterium as a factory for the production and characterization of oxygen-sensitive proteins through the use of genetic approaches to achieve high-level protein expression."

According to Ray Dixon, project lead in Molecular Biology at the John Innes Centre in the United Kingdom, "The international collaboration created by the Azotobacter genome project was an integral part of the discovery of these unexpected anaerobic processes in *A. vinelandii*, which confirmed the importance of this organism as a host for the expression and purification of oxygen-sensitive enzymes."

The work will also provide more information about the unique

biosynthetic pathways involved in the bacteria's ability to adapt its metabolism to diverse sources of nutrients. For example, if no carbon source is present, *A. vinelandii* will undergo a differentiation process, forming cysts that are resistant to desiccation and other chemical and physical challenges.

A. vinelandii belongs to the Pseudomonadaceae family. The completion of the *A. vinelandii* genome will serve as an essential phylogenetic anchor point for comparative genomics work with other systems. This research has also helped the *Azotobacter vinelandii* genome project expand its existing undergraduate training platform in genomics technology. Undergraduate researchers from around the world worked on many aspects of the project, including the finishing, annotation, bioinformatics, and analytical phases. This work provided critical training for these new scientists in the fields of genomics and bioinformatics.

"The key to the success of this project was an extremely effective multinational collaboration between Azotobacter biologists and our core genomics team," explained Derek Wood, associate professor in Seattle Pacific University's Department of Biology and the University of Washington's Department of Microbiology. "Numerous undergraduate researchers both in the classroom and in the laboratory worked closely with the team on annotation and analyses. This multilevel interdisciplinary collaboration led to an excellent annotation and informed analysis of this model bacterial system and provided an excellent training platform for our undergraduate partners."

According to Dennis Dean, biochemistry professor in Virginia Tech's College of Agriculture and Life Sciences and director of the Fralin Life Science Institute, "Given the long history of Virginia Tech's research involving nitrogen fixation studies, including work from J.S. Chen, Bill Newton, and others, it is particularly satisfying that the university has

contributed to this important endeavor."

Source: Virginia Tech (news : web)