

RESEARCHERS SCRUTINIZE BROWN TIDE GENES

Is it something in the genetic makeup of the tiny brown alga, *Aureococcus anophagefferens*, that triggers the brown tide blooms that sporadically darken the waters of some of bays, causing declines in bay scallop and other shellfish populations as well as the decrease of eelgrass beds that serve as shellfish nurseries? Researchers of the self-assembled *Aureococcus* Genome Consortium (AGC) believe that the organism's genetic makeup or genome holds the key.

In the summer of 2007, the Office of Science within the US Department of Energy's Joint Genome Institute (JGI) announced that the *Aureococcus* genome sequencing was complete, and that 11,500 predicted genes exist within its 56 "megabase" genome. After the release of this information, members of the AGC sought to examine gene sequences and pathways, looking for genetic causes of brown tide formation. According to **Dr. Christopher Gobler** of Stony Brook University's School of Marine and Atmospheric Sciences and investigator on numerous NYSG brown tide research projects, "The AGC used an approach of comparative genomics whereby the genome of *Aureococcus* is being directly compared to other phytoplankton species which it may compete with to form blooms."

By October 2007, AGC members came together to share the preliminary results of their comparative analyses in a two-day New York Sea Grant-sponsored *Aureococcus anophagefferens* Genome Jamboree held in Southampton, NY.

The Jamboree kicked off with an overview of the history and ecology of *Aureococcus anophagefferens* by Dr. Gobler. He presented the foundation for the comparative genomics approach, explaining how the *Aureococcus* clone in this current sequencing project was isolated from the Great South Bay of LI, which is also home to the diatom *Thalassiosira* and the green alga *Ostreococcus*, making them excellent comparative choices. Dr. Gobler showed a comparison of the ecology of these species, highlighting their differential bloom dynamics, and usage of nutrients and light as the framework for identifying critical genes which may or may not be present and absent in each species.



Left to right: **Dianna Berry, Christopher Gobler, Theresa Hattenrath, Adam Kustka, Jackie Collier, Steven Wilhelm, Kathryn Coyne, Sonya Dyhrman, Astrid Terry, Louie Wurch, Erin Bertrand and Peter Countway.** Photo courtesy of Chris Gobler

Dr. Astrid Terry, a genome analyst from the Joint Genome Institute in Walnut Creek, CA, described how the *Aureococcus* genome with its 11,500 genes has a gene count larger than all eukaryotic algae sequenced to date and the nuclear genome of *Aureococcus* is surprisingly most similar to that of another phytoplankton, *Ostreococcus*. *Aureococcus* has a large proportion of genes related to amino acid metabolism and synthesis and has many unique classes of genes not found in similar organisms. Dr. Terry also provided advanced training related to the *Aureococcus* genome for members of the Consortium.

Also providing training was **Dr. Dianna Berry** of Stony Brook University, who acquainted attendees with internet-based tools that allow members to make protein and nucleic acid sequence comparisons, search JGI genome databases for sequences of interest, and to search databases for similar genes by protein domain structure.

During a work session, Consortium members were able to present their gene models to Drs. Berry and Terry for assistance. Members also presented gene models which showed discrepancies between computer models and known gene models in other species.

During the second day, **Dr. Steven Wilhelm** of the University of Tennessee, working with **Dr. George Bullerjhan** of Bowling Green State University, presented his analysis of the chloroplast genome. In this organellar genome, he found the *Aureococcus* chloroplast genome was smaller than most other chloroplasts which have been sequenced to date and is most similar to several diatoms and the coccolithophore, *Emiliania huxleyi*.

WHAT IS A JAMBOREE?

According to the US Department of Energy's Office of Science Joint Genome Institute (JGI), "A jamboree is a scientific meeting at which members of a scientific community gather to discuss the genome of an organism of common interest. Participants have the opportunity to annotate the genome in advance of the meeting. The focus can be a single organism or a family of organisms. Similar creatures are also typically studied in order to draw comparisons and contrasts. Attendance is by invitation."



... for more brown tide and its causes

Dr. Jackie Collier (Stony Brook University) presented her findings of Light Harvesting Complex (LHC) genes in the *Aureococcus* nuclear genome which assist in the proper aligning and functioning of chlorophyll. In contrast to the small chloroplast genome, Dr. Collier found 62 light harvesting genes in *Aureococcus* which is, on average, double the number found in other organisms. Moreover, there are 25 LHC genes which are unique to *Aureococcus*. These genes may convey a competitive advantage to *Aureococcus* during periods of low and/or variable light levels found during blooms.

Research findings have shown that *Aureococcus* has a series of unique nitrogen metabolism genes which would allow for its usage of alternate forms of nutrients (as shown by experimental data over the years). In lab experiments conducted by Drs. Gobler, Berry and **Kathryn Coyne** (University of Delaware), *Aureococcus* has been shown to be able to utilize chitobiose, an abundant polysaccharide. Genes for the chitobiase enzyme are found in *Aureococcus* but not in other phytoplankton species. The genome also contains genes for metabolizing proteins, amino acids, nitriles, amides, urea, and all the standard nitrogenous nutrients (nitrate, nitrite, ammonium). These findings confirm that *Aureococcus* is suited for dominating under a variety of nutrient conditions.

Dr. Sonya Dhyrman (Woods Hole Oceanographic Institution) related her experiments on phosphorus uptake and metabolism genes in *Aureococcus*. Her work shows that *Aureococcus* has six ortho-phosphate transporters while co-occurring picoplankton have only two. *Aureococcus* also has more than 20 genes which can be used to synthesize enzymes for the degradation of organic phosphorus compounds, demonstrating it can access organic matter as a source of phosphorus.

Graduate student **Louie Wurch** of the MIT/Woods Hole Joint Program has investigated nitrogen transporter genes in *Aureococcus*. Transporter genes are located at the interface of the cell and its geochemical environment. *Aureococcus* has more transporters for urea, ammonium, and amino acids than all competing species. However, it has only a single nitrate transporter, a finding consistent with its inability to dominate when nitrate levels are high.



Dr. Jackie Collier of Stony Brook University examines a DNA sequence. Dr. Collier found that *Aureococcus* has approximately double the number of light harvesting genes of other organisms which may convey to brown tide a competitive advantage during periods of low light found during blooms.

Photo by Barbara A. Branca

Another MIT/Woods Hole Joint Program grad student, **Erin Bertrand**, presented that *Aureococcus* is a vitamin B12 auxotroph, meaning it lacks the genes needed to grow without B12. It also is likely a B12 scavenger, meaning it can salvage degraded vitamin B12 molecules to reconstruct the molecule.

Dr. Adam Kustka of Rutgers University focused on iron use and transport in *Aureococcus*, identifying three genes responsible for iron transport. By contrast, one or none were present in other species. However, he did not find any genes for enzymes that could be used to reduce or store iron.

Each successive presentation helped to further connect aspects of brown tide blooms with specific *Aureococcus* genes. While the researchers continue to confirm the presence of similar genes in the comparative organisms as well as manually annotate genes of interest, Consortium members are currently refining the details of a joint manuscript that will be submitted to a peer reviewed journal in the near future.

— **Dianna Berry, Chris Gobler and Barbara A. Branca**