

# Novel experiment documents evolution of genome in near-real time

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A team led by bioengineering researchers at UC San Diego report in the November issue of *Nature Genetics* rapid evolutionary changes in a bacterial genome, observed in near-real time over a few days. Scientists have previously published static "snapshots" of the genome sequences of more than 100 bacterial species, from the harmless to those that cause plague, but this new report shows how these genomes are moving targets.

"Paleontologists look at the fossil record to study how evolution of dinosaurs and other animals occurred over millions of years, but in the case of the *E. coli* bacterium, new technology has given us the ability to observe evolution as it is occurring over a matter of days," said Bernhard. Palsson, the senior author of the study and professor of bioengineering at UCSD. "The published genomic sequences of bacteria are like a fossil record and our experiments confirm that these genomes can change quickly as bacteria adapt to new conditions."

Because of past technical limitations, biologists have historically made inferences about rapid bacterial evolution by carefully observing changes in a handful of genes at a time or by monitoring the visible characteristics, or phenotypes, as the organisms adapt. Palsson's team used comparative genome sequencing technology developed by NimbleGen Systems Inc., a Madison, WI-based biotech firm, to identify changes that occurred during the experiment in the bacterium's complete set of genes.

In a paper scheduled for online publication Nov. 5 on *Nature Genetics's*

Website, the researchers report that they grew *E. coli* in an environment that favored the emergence of mutants: the organism was fed a poorly metabolized carbon and energy source called glycerol. The researchers removed samples of cells from the culture and sequenced their entire genomes as a way to find mutations that enabled faster growth.

After six days of growth, mutations appeared in the gene for an enzyme that initiates the process of enzymatically breaking down glycerol. Cells with mutations in the so-called glycerol kinase gene grew 20 to 60 percent faster than those without the mutation.

Mutations also appeared in a second, unrelated gene for an enzyme called RNA polymerase. "That was a surprise to almost everybody because RNA polymerase is involved in one of the core processes of any cell," said Palsson. "You wouldn't expect that gene to change because a wide variety of cellular process would be affected; it's like replacing the wiring system in a building when a light bulb burns out. But we repeated the experiment more than 50 times and mutations in the RNA polymerase gene appeared again and again."

The researchers report that mutations in both genes appeared together in several cases after six to eight days in the glycerol-based cultures, and *E. coli* cells containing both mutated genes grew 150 percent faster than the starting strain of *E. coli*. To confirm that the mutants were indeed responsible for the faster growth, the researchers substituted the two mutant genes into the original strain and duplicated the faster growth rate. "We expected to find many mutated genes and we thought our results would be very difficult to understand, but neither was the case," said Christopher D. Herring, a co-author of the paper and a former member of Palsson's team who is now a research scientist at Mascoma Corp. in Lebanon, NH.

All the mutants arose in the experiments presumably as the result of

naturally occurring errors in copying DNA into daughter cells during cell division. The precise changes in the sequence of DNA subunits were determined with comparative genomic sequencing technology from NimbleGen Systems, and further analyzed with technology from Sequenom Inc., a San Diego-based biotech company.

"This straightforward approach to the study of experimental evolution can be used as a tool for discovery and analysis, and could even be used to discover bacterial capabilities that would benefit humankind in a variety of ways," said Herring. "There may be a number of biotech companies that want to use this new approach to help design bacteria to do useful jobs."

UCSD's office of Technology Transfer and Intellectual Property Services has filed U.S. patent applications related to the experimental evolution approach. The approach combines computer modeling techniques with evolutionary design processes to optimize bacteria to perform commercially important processes. Aside from its commercial potential, experimental evolution of bacterial strains is also useful for refining the theory of evolution and the related process of natural selection of individuals with traits that convey a selective survival advantage. "Opinion surveys indicate that many people don't believe that evolution occurs," said Herring, "but if the skeptics could witness evolution actually occurring, as we did, I think they'd be more likely to believe that it's not just a theory."

Source: University of California - San Diego

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