SYNTHETIC BIOLOGY

Engineering bacteria in yeast

Bacterial genomes shuttled into yeast can be easily altered before transplantation back into bacteria.

With a few prominent exceptions, most bacteria are not easy to work with. John Glass and Sanjay Vashee from the J. Craig Venter Institute in Rockville, Maryland, USA aimed to change that. Their goal, as Glass describes it, was "to create a tool that would allow people to take their organism, clone its genome, manipulate its genome, then boot it up."

As direct manipulation of a bacterial genome is often not possible, the researchers sought to do the manipulation in *Saccharomyces cerevisiae,* a host that is amenable to genetic engineering.

In previous work scientists from the J. Craig Venter Institute have shown that they can transplant a genome from one *Mycoplasma* species to another. Now they cloned an *M. mycoides* genome into a yeast artificial chromosome, genetically manipulated it, and transplanted it into *M. capricolum*.

What sounds straightforward was not without challenges. One concern was that yeast would modify bacterial DNA and hinder transplantation back into bacteria. To the scientists' relief, this did not occur. Instead, problems arose from the bacteria's 'immune system': restriction endonucleases that destroy foreign DNA, thus preventing a successful transplantation.

The researchers found two solutions. First, they eliminated the endonuclease in *M. capricolum*, and second, they methylated the donor genome before transplantation, protecting it against digestion.

It was an important goal for Glass and Vashee to create a universal tool for bacterial genetic engineering, but *Mycoplasma* may not be the ideal bacterial representative. *Mycoplasma* do not have a cell wall, and their genome is small and very (A+T)-rich, which ensures proper replication in yeast. Bacteria with high G+C content may have a harder time getting replicated in yeast. Also, *Mycoplasma* have a nonstandard genetic code, thus preventing expression of potentially toxic proteins in yeast. How much of a challenge these features will be for other bacteria the researchers cannot presently say.

Scientists at the J. Craig Venter Institute are currently applying this technology to produce a synthetic cell based on the *M. genitalium* genome. "Then our research will diverge in two directions," Glass anticipates, "to find the minimal microbial genome and to apply the technology to conventional bacteria to solve human needs in medicine, the environment and industry."

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RESEARCH PAPERS

Lartigue, C. *et al.* Creating bacterial strains from genomes that have been cloned and engineered in yeast. *Science* advance online publication (20 August 2009).