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**Research milestone brings goal  
closer of inexpensive antimalarial  
drug for developing world**

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**Berkeley** — Researchers striving to create a less expensive version of a life-saving antimalarial drug, artemisinin, have cleared a major hurdle, according to a new report in the journal *Nature*.

Two and a half years ago, a University of California, Berkeley, team led by Jay D. Keasling, UC Berkeley professor of chemical engineering and bioengineering, succeeded in engineering bacteria to make a chemical precursor of artemisinin – the best drug available today to cure malaria.

The team's ultimate goal was to retool the microbe's metabolism to perform as much of the drug synthesis as possible in order to sidestep the expensive laboratory synthesis needed to make artemisinin. That synthesis would have increased the drug's cost beyond the researchers' ambitious target of 25 cents per dose.

They now have nearly achieved that goal by engineering the production of artemisinic acid, one chemical alteration away from artemisinin. The fact that the researchers have not yet been able to produce artemisinin itself is not a disadvantage, they said, since drugs currently on the market – all made from extracts of the wormwood plant, *Artemisia annua* – are synthetic derivatives of both artemisinic acid and artemisinin.

"This is probably as close to artemisinin as we are going to get in microbes. The rest is going to be done by chemistry," said Keasling. His lab partnered with the San Francisco-based Institute for OneWorld Health, a nonprofit pharmaceutical company, and Emeryville, Calif.-based Amyris Biotechnologies in late 2004 on a \$43 million grant from the Bill and Melinda Gates Foundation to develop low-cost artemisinin drugs using Keasling's genetically engineered microbes.

A detailed description of the researchers' work appears in the April 13 issue of *Nature*.

Keasling noted that his team achieved its recent feat in yeast, not *E. coli* bacteria. Bacteria breed faster and are often the microbes of choice, but the ability to get the drug out of both bacteria and yeast provides flexibility in achieving the goal of complete synthesis of artemisinin within another four years, he said.

Despite its achievement, the team cautioned that a microbe-produced version of artemisinin will not be on the market soon. It added that the current method of production – extraction from the wormwood plant grown by farmers in Asia – will be essential in the next five to 10 years until production and widespread distribution of the less costly alternative becomes possible.

"While we have made a lot of progress in the past two years, there still are a lot of unknowns," Keasling said. Keasling is director of the UC Berkeley Synthetic Biology Center and of the Lawrence Berkeley National Laboratory's Synthetic Biology Department, and a UC Berkeley member of the California Institute of Quantitative Biomedical Research (QB3).

Artemisinin derivatives, in combination with other drugs, have proven nearly 100 percent effective against malaria, and thus represent a major hope for the 300-500 million people each year who become infected with malaria, and the more than 1.5 million people – largely children in Africa and Asia – who die. Even at \$2.40 per person for a cure, however, the cost is too great for most developing countries.

In 2003, Keasling and his team pieced together bacterial genes, yeast genes and genes from the

wormwood plant to create a chemical pathway – essentially a miniature factory – in bacteria to make amorphadiene, an artemisinin precursor that can be converted chemically into artemisinin.

Supported by funding from the Gates Foundation, Keasling and his team will work with Amyris to push the research towards a final goal: a microbe that can make sufficient amounts of artemisinic acid to allow scientists to produce the antimalarial drug inexpensively enough for widespread use in Africa and Asia, where malaria is endemic.

To ensure affordability, UC Berkeley has issued a royalty-free license to both OneWorld Health and Amyris to develop the technology to treat malaria. Amyris will transform the Keasling lab's research into a robust fermentation process and perform the chemistry and scale-up necessary to bring the drug to market. OneWorld Health will conduct pre-clinical studies and implement a global access strategy for the drug.

"The work coming out of the Keasling lab is world-class. We are very confident that the UC Berkeley-Amyris collaboration team will be able to build on this work to finish the development of an artemisinin production process," said Kinkead Reiling, president of Amyris.

"The team at UC Berkeley has done a great job moving this important project forward," said Victoria Hale, founder and CEO of OneWorld Health. "We still have a long way to go, but this puts us one step closer to a low-cost treatment for malaria."

The team's work accelerated after first author Dae-Kyun Ro, the UC Berkeley artemisinin project manager, identified last July the enzyme in wormwood that chemically changes amorphadiene into artemisinic acid. He plucked the gene out of wormwood after searching for candidate genes in the published genomes of *A. annua*'s relatives – lettuce and the sunflower.

The enzyme, a member of a large family of cytochrome P450 enzymes, attaches itself to internal cell structures not present in bacteria, so Keasling's team tried first to make it work in yeast, which has the required internal membranes.

Led by UC Berkeley graduate student Eric Paradise, co-first author of the *Nature* article, a large team of plant biologists, chemical engineers, organic chemists, biochemists, bacteriologists, bioengineers, bioinformatics and fermentation specialists worked together to construct in yeast a mirror of the pathway engineered earlier in bacteria. The researchers used some of the yeast's own genes, plus bacterial genes and wormwood genes inserted into the yeast genome. With the added wormwood gene for the P450 enzyme, the yeast manufactured the desired chemical, artemisinic acid.

"We reached our goal early, thanks to a number of miracles: The first gene Dae-Kyun isolated was the right one, the gene was functional in yeast, the gene's enzyme did in one step what we thought took three enzymes, and the artemisinic acid it produced didn't interfere much with the cell," Keasling said.

The yeast produces perhaps one-tenth the amount of amorphaadiene as the current version of the engineered bacteria, he noted, so its output of artemisinic acid is still relatively low. But Keasling is optimistic.

"This was our highest hurdle, what kept me up at night," he said. "Now that we've got all the parts, I feel it's just a matter of time before we have a microbe ready for scale-up to production."

The team's next goal, he said, is to try for the same result in bacteria, which grow faster and thus are preferable if the goal is to produce lots of the drug quickly and inexpensively. Ro admitted, however, that large scale production of the drug by yeast could turn out to be a superior strategy.

"Yeast is an easier host in which to express the P450 enzyme that transforms amorphaadiene to artemisinic acid," he said. "However, we plan to push forward with engineering the P450 and expressing it in the amorphaadiene-producing *E. coli* strain. For now, we are delighted to have one attractive host strain for artemisinic acid production in our hands, and we now are considering yeast as an alternative fermentation organism for the production of artemisinic acid."

UC Berkeley coauthors with Ro, Paradise and Keasling are co-project manager Karyn L.

Newman and post-doc Michelle C. Y. Chang and research assistants Mario Ouellet, Rachel A. Eachus and Kimberly A. Ho of QB3; post-docs James Kirby and Sydnor T. Withers and visiting scholar Yoichiro Shiba of the Department of Chemical Engineering; post-doc John M. Ndungu and assistant professor Richmond Sarpong of the Department of Chemistry; and graduate student Timothy S. Ham of the Department of Bioengineering. Karl J. Fisher of Amyris also coauthored the paper.

The work was supported by the Gates Foundation as well as by the Akibene Foundation, U.S. Department of Agriculture, UC Discovery Grant Program, National Science Foundation and Diversa Corp.

More information on the collaboration between the Institute for OneWorld Health, UC Berkeley and Amyris Biotechnologies to develop a low-cost malaria drug, can be found at [www.artemisininproject.org](http://www.artemisininproject.org).

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