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An Age-Old Microbe May Help Cure an Age-Old Affliction

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Yeast, the tiny plantlike microbe that's been used to make bread, beer, and wine for thousands of years, may soon help tame the scourge of malaria—a disease first described in the fourth century B.C.E. by Hippocrates. A team of researchers with Berkeley Lab, the University of California at Berkeley, the California Institute of Quantitative Biomedical Research (QB3), and Amyris Biotechnologies, Inc. has genetically engineered a strain of yeast to synthesize artemisinic acid, the immediate precursor of the antimalarial superdrug, artemisinin.

The team led, by bioengineer Jay Keasling, worked under a $42.6 million grant from the Bill and Melinda Gates Foundation to the Institute for OneWorld Health, a San Francisco-based nonprofit pharmaceutical company. Having created a strain of *Saccharomyces cerevisiae* capable of producing high levels of artemisinic acid, the team will use chemical means to synthesize artemisinin at potentially far lower costs than extracting it from *Artemisia annua*, the sweet wormwood tree.

"Microbially produced artemisinic acid is a viable source of this potent family of antimalarial drugs," says Keasling. "Artemisinin combination therapies could be offered significantly below their current prices."

Keasling, director of Berkeley Lab's Physical Biosciences Division and a professor of chemical engineering and bioengineering at UC Berkeley, also directs the university's Synthetic Biology Center, is a faculty affiliate with QB3, and is founder of Amyris.

Jay Keasling, a leading authority on synthetic biology, heads research to produce affordable antimalarial drugs from microbes.

In 2003 he and his research group genetically engineered *Escherichia coli* bacteria to produce another artemisinin precursor, amorphadiene. When the wormwood plant's gene for converting amorphadiene to artemisinic acid was identified, the researchers switched their focus to yeast.

"We still plan to transfer the gene to *E. coli*," Keasling says. "Having the option of being able to work with either yeast or bacteria gives us more flexibility and a better chance of achieving our goal of complete artemisinin synthesis in the microbe."

Each year nearly 500 million people living in the tropics and subtropics become infected with malaria, suffering burning fever and severe pain. An estimated one to three million victims die, most of them children. The disease has been impossible to eradicate because of the complex life-cycle of *Plasmodium falciparum*, the parasite that carries it.

Infected *Anopheles* mosquitoes transmit the protozoa to humans, where they travel straight to the liver. They replicate thousands of times and are released back into the bloodstream in a form that invades red blood cells, where they feed on iron-rich hemoglobin and multiplies.

Injected by infected mosquitoes, malaria parasites develop first in the liver and then in red blood cells, where they accumulate iron (shown here in an x-ray image taken at Berkeley Lab's Advanced Light Source). The parasites are vulnerable to free radicals released by the powerful antimalarial drug known as artemisinin.

More than a million malaria patients have already been cured by artemisinin, which with its derivatives is nearly 100 percent effective against all known strains of malaria. The drug releases oxygen-based free radicals to destroy the parasites inside red blood cells.

Wormwood trees produce artemisinin only under a narrow set of agricultural and climatological conditions. The cost of extracting it or manufacturing it entirely through chemical synthesis is so high that the impoverished populations suffering the most cannot afford it. Treatment currently runs about $2.40 per dose. Keasling aims to cut this to about 25 cents by engineering microbes to perform as much of the chemistry as possible.

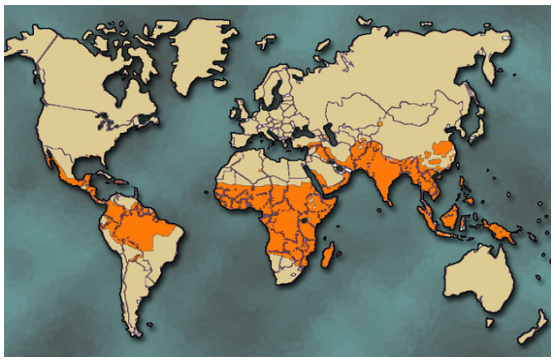
Engineering yeast was a three-step process. First the researchers created a new metabolic pathway in the microbe, similar to the one created in *E. coli* in 2003 to produce amorphadiene. Next they introduced bacterial and wormwood genes into the yeast's DNA, which interacted with the yeast's own genes to produce amorphadiene. Finally they cloned the wormwood gene that produces the enzyme P450, which the plant uses to convert amorphadiene to artemisinic acid. This was then expressed in the amorphadiene-producing yeast strain.

"The synthesized artemisinic acid can be transported out and retained on the outside of the engineered yeast," says Keasling. "This means that a simple and inexpensive purification process can be used to obtain the final product." Keasling says the research team is "on track to have a therapeutic drug out the door within two to four years."

Artemisinin from microbes has other advantages. Unlike wormwood cultivation, it won't be subject to politics or the weather. Some producers use diesel-fuel purification, which can retain toxic impurities, but extracting artemisinic acid from microbes will be environmentally benign.

With support from the Gates Foundation, Keasling and his Berkeley research group will work with collaborators at Amyris to develop processes that will yield large quantities of microbial artemisinic acid. To make sure it's affordable where antimalarial drugs are needed most, UC Berkeley has issued a royalty-free license to both Amyris and OneWorld Health to develop the technology. Amyris will take the research and scale it up to the robust fermentation and chemical processes necessary to bring the drug to market. OneWorld Health will conduct preclinical studies and implement a global access strategy for the drug.

In addition to the Gates Foundation, the work was supported by the Akibene Foundation, the U.S. Department of Agriculture, the UC Discovery Grant Program, the National Science Foundation, and the Diversa Corporation.



Each year nearly 500 million people become infected with malaria; nearly three million, mostly children, die from it. Areas facing the greatest risk (reddish brown) harbor some of the world's most impoverished people.

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