STEPHEN AUSMUS (D2505-5)

Beneficial Yeast

A New Weapon for Keeping Tree Nuts Safe To Eat

Tomorrow's orchards of almonds, pistachios, and walnuts might be sprayed with fine mists of a beneficial yeast, *Pichia anomala*. Studies led by Agricultural Research Service plant physiologist Sui-Sheng T. (Sylvia) Hua have shown that this yeast can undermine a troublesome mold, *Aspergillus flavus*. The mold is of concern because it produces aflatoxin, a natural carcinogen.

Federal food safety standards and quality-control procedures at U.S. packinghouses help ensure that these crunchy, healthful tree nuts remain safe to eat. Nonetheless, growers and processors have a continuing interest in new, environmentally friendly ways to combat the mold.

Hua is one of several scientists at ARS's Western Regional Research Center in Albany, California, who are investigating new strategies for thwarting *A. flavus*.

The idea of developing a practical, affordable way for growers to use a yeast to fight a mold isn't new. But Hua's tree-nut-focused investigations of *P. anomala* may be among the most extensive of their kind to date.

Her research has included exploring the yeast's talents as a biocontrol candidate in a series of laboratory tests at Albany and in a field test at a California pistachio orchard. The orchard study, documented in a patent issued to Hua in 2009, indicated that the yeast was responsible for a 96-percent reduction in the number of mold spores.

For ongoing laboratory research, Hua has selected, refined, and applied several analytical procedures to discover precisely how the yeast disables the mold. "If we understand the underlying mechanisms," she says, "we may be able to use that knowledge to increase the yeast's effectiveness."

In a collaborative experiment with Albany coinvestigators Bradley J. Hernlem, a chemical engineer; and Maria T. Brandl, a microbiologist, the mold was exposed to the yeast and later to several different compounds that fluoresce red or green when evidence of specific changes in the mold's cells is detected.

Results of these assays, documented in a peer-reviewed article in the scientific journal *Mycopathologia,* suggest that the yeast interfered with the mold's energy-generating ATP (adenosine triphosphate) system, vital for the mold's survival. The findings also suggest that the yeast damaged mold cell walls and cell membranes. Walls and membranes perform the essential role of protecting cell contents.

The team used a different analytical procedure—quantitative reverse transcriptase PCR (polymerase chain reaction) assays—to analyze the activity of certain *P. anomala* genes in the presence of the mold. Preliminary findings, which Hua reported at the annual national meeting of the American Society for Microbiology in 2010, suggest that exposing the yeast to the mold may have triggered the yeast to turn on genes that code for production of two enzymes—PaEXG1 and PaEXG2.

"These enzymes are capable of degrading the mold's cell walls and causing damage to membranes," Hua notes.

Though further studies are needed, Hua says these early, PCR-based findings point to "gene-controlled mechanisms that may be involved in the cell wall and cell membrane damage observed in the fluorescence assays."—By **Marcia Wood,** ARS.

This research supports the USDA priority of ensuring food safety and is part of Food Safety, an ARS national program (#108) described at www.nps.ars.usda.gov.

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Above: Plant physiologist Sylvia Hua (right) and technician Siov Sarreal display petri dishes showing the effectiveness of a biocontrol yeast against *Aspergillus flavus*. On the left, a mutant *A. flavus* turns the agar orange, signifying aflatoxin production. On the right, when the same *A. flavus* was inoculated between two streaks of yeast, growth was inhibited and no aflatoxin was produced.