ANTIBIOTIC RESEARCH

From Bacteria: A New Weapon Against Fungal Infection

When plant pathologist Gary Strobel of Montana State University (MSU) made front-page news in 1987, he seemed to be bringing to a dramatic end a line of research he had pioneered. Armed with a chain saw, a weeping Strobel was seen in news photos cutting down a grove of Dutch elms he had inoculated with a genetically altered bacterium he hoped would kill the fungus that causes Dutch elm disease. Unfortunately, Strobel had failed to obtain approval from the Environmental Protection Agency before treating the trees with the engineered bacterium, and the resulting furor, fueled by anti-biotech zealots, caused him to cut the trees down rather than fight the agency for permission.

But, in fact, the Dutch elm debacle wasn't the end of the line for Strobel's research on this particular anti-fungal. Work in his lab has since shown that the fungus-killing compounds produced by the bacteria, the pseudomycins, may turn out to be an important addition to the pitifully small armamentarium for combating human fungal infections. Although the incidence of fungal infections has increased greatly in the past 20 years, partly because of the increased number of people with immune systems compromised by AIDS, age, organ transplants, or cancer therapy, the number of anti-mycotic agents has not grown apace. And most of the current treatments merely slow fungal growth. Not the pseudomycins, however. Test-tube work suggests they kill fungi. As a result, at least five major pharmaceutical companies have contacted Strobel with a view to commercializing his findings—if these early results hold up in animal tests.

The first steps toward that goal were taken soon after the Dutch elm fiasco. Rather than tossing the pseudomycins into the freezer, Strobel decided he would "do serious biochemistry on these things." He had worked with the bacterium that produces the compounds, *Pseudomonas syringae*, for 20 years, yet the anti-fungal proteins had stubbornly resisted purification. To identify the anti-fungal agent, Strobel teamed up with David Teplow, a protein chemist at Harvard Medical School (former MSU graduate student Leslie Harrison helped with the characterization). This team found that the pseudomycins are novel lipopeptides (strings of amino acids with fatty-acid side chains attached) containing unusual amino acids such as chlorothreonine, hydroxyaspartic acid, and diaminobutyric acid. Strobel suspects that these uncommon amino acids help keep the pseudomycins active in a serum assay in which other proteins are routinely chewed up by enzymes. "These unusual amino acids are probably involved in their unique killing action," says Strobel.

He has so far identified three pseudomycins, dubbed A, B, and C. All three have the same basic structure of linked amino acids but differ in their attached fatty-acid side chains. Most of the attention so far has focused on pseudomycin A, which the bacterium makes in the largest quantities. But that "does not rule out the others," says Strobel.

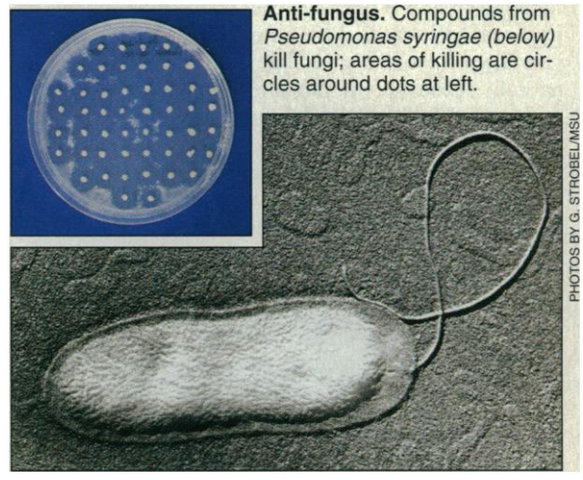
Having purified the pseudomycins, Strobel recognized they might have applications far beyond plants. Specifically, he says, he "realized that some human fungi like *Candida* were similar to plant fungi." To test his hunch, he collaborated with Michael Rinaldi, a medical mycologist at the University of Texas at San Antonio. Rinaldi reinforced his suspicion that the line between plant pathogens and human pathogens is becoming blurred. The reason, says Rinaldi, is that humans with suppressed immune systems become "human Petri plates," attacked by fungi normally associated with plants. In immunocompromised people, fungi target key organs like the brain or lungs and "eat these body organs like they would a plant leaf," says Rinaldi, causing death.

Bipolaris spicifera, for instance, once just a common plant pathogen, now kills immunocompromised humans. In the southwestern United States, the formerly benign soil fungus *Coccidioides immitis* has gone on the rampage, causing a pneumonialike epidemic that can prove fatal. "We have reached the point where there are no fungi that are not potential pathogens to humans," says Nick Pliam, director of exploratory research at Pharmagenesis, one of the companies that have contacted Strobel (the others are Ajinomoto, Novo, Miles, and Lilly).

Rinaldi set about testing the killing action of the pseudomycins against human fungal pathogens. The first results were encouraging: Six common human fungi were killed within 48 hours in a standard assay. They include *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and other fungi that cause opportunistic infections in immunocompromised individuals.

These early results were enough to kindle enthusiasm among pharmaceutical companies—largely because of the paucity of alternatives. The few drugs capable of killing fungi produce formidable side effects. Amphotericin B, the fungicidal drug that is considered the best available option, carries toxic liabilities sufficient to have earned it the nickname "Ampho the Terrible."

"We still don't have the ideal drug," concedes microbiologist William Current of the Lilly Research Laboratories. "If you had a compound that was fungicidal, but had lower toxicity than amphotericin B, then that would be very useful." Despite the enthusiasm for new options, the pseudomycins have many hurdles to clear before reaching commercial use. Their anti-fungal potency has been demonstrated only in the test tube. As far as safety goes, the data amount to a few preliminary mouse studies, which found the drugs nontoxic, even at high doses. One hopeful sign is that the pseudomycins remain biologically active when incubated with human sera for several days. "At this point one can cross their fingers," says Current, "but not hold their breath."



Anti-fungus. Compounds from *Pseudomonas syringae (below)* kill fungi; areas of killing are circles around dots at left.

PHOTOS BY G. STROBEL/MSU

"It's still too early to say that this is a great new pharmaceutical," agrees Pliam. But even if the pseudomycins never reach the pharmacy shelf, experts say the model that led to their discovery—an anti-fungal agent in association with a plant—holds great potential. The symbiotic relationship in which pseudomycin-making bacteria confer anti-fungal resistance on their host plants is probably not unique in nature; even if the pseudomycins bomb, somewhere among such relationships a useful anti-fungal drug may lurk. "That's fascinating," says Pliam, "because it means nature holds all kinds of keys" to the fight against fungal diseases, a fight that looks as if it will only become more heated as time goes on.

–Carol Potera

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