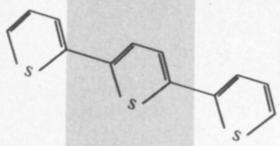
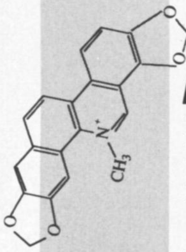
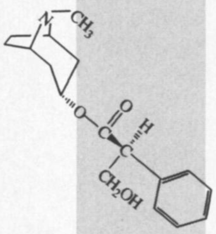
Hairy Harvest

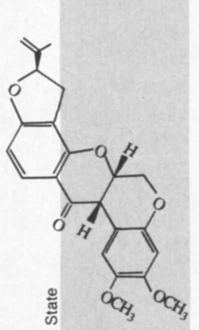
Bacteria turn roots into chemical factories



By ELIZABETH PENNISI







Complex chemicals that plants know best how to make.

Flores/Penn State

No chemist or genetic engineer can make what the rosy periwinkle produces as part of its daily routine. A native of Madagascar, this tiny flowering plant (*Catharanthus roseus*) churns out 75 different alkaloids — complex, usually bitter compounds that include caffeine, nicotine and two substances used to treat leukemia and other cancers in children.

Researchers can culture periwinkle cells in bioreactors for fast production and high yields of some alkaloids. But right now, drug companies must harvest the anticancer substances, vinblastine and vincristine, from the plant itself.

Like other plant medicines, these compounds result from complex chemical processes involving several enzymes. Thus altering bacteria through genetic engineering to produce these substances could require the transfer of many genes, which must then turn on in the proper sequence. Such a task lies beyond biotechnology at this point, says Jacqueline V. Shanks, a chemical engineer at Rice University in Houston. And the chemical complexity of these compounds makes them difficult to synthesize in the laboratory.

So scientists have devised a different way to harvest these and other important chemicals. By infecting seedlings with certain bacteria, researchers cause the cells at the infection site to transform into a state of perpetual growth. The cells form rootlets that grow and branch into "hairy roots," so called because very fine hairs protrude along the entire length of the rootlet, not just at the end, as in regular roots.

Scientists first succeeded in culturing roots from snipped-off root tips about 60 years ago. But in the past decade, they discovered they could use bacteria to make hairy root systems, which develop much faster than root cuttings. To set up a culture, researchers cut off a piece of hairy root, disinfect it, then place it in a liquid growing medium. The cutting still contains root-inducing genetic material transferred to it by the bacteria, so it can increase its biomass several thousand-fold in a month, says Hector E. Flores, a plant physiologist at Pennsylvania State University in University Park. Researchers then extract chemicals that ooze from the roots into the culture medium or accumulate in the roots themselves.

Actually, the technique is not quite that straightforward, says Shanks. Only about two-thirds of the plants transform and sprout roots, and not all of those grow well in liquid. The hairy roots that do thrive produce varying amounts of the desired compounds, even when they come from the same variety of periwinkle. "So source material is pretty important," she adds.

As chemical engineers, Shanks and her students want to maximize production of the valuable compounds. They evaluated four *C. roseus* cultivars as sources for hairy roots, then infected 200 plants, of which 60 percent developed fine hairs. The Rice group checked the clones' production rates by analyzing the chemical contents of root and media samples.

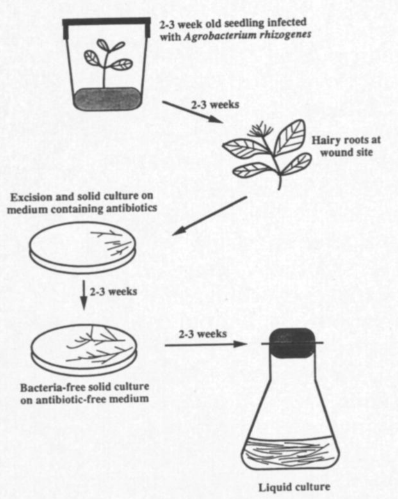
The analyses indicate that three cultivars — Snow Carpet, Little Bright Eye and Little Linda — produce vindolin, a chemical that does not appear in other types of plant cell cultures, Shanks reported in April at the American Chemical Society's spring meeting, held in San Francisco. Chemists can easily convert vindolin into vinblastine and vincristine, leading Shanks to hope that hairy roots will become a new source for these anticancer compounds. She and her co-workers are now experimenting with new types of culture media and growing conditions to improve yield.

Her group's success represents just a promising beginning. "One of the big problems is trying to scale up," says Shanks. The roots form such a dense, interlocked mat that nutrients dissolved in the liquid growth medium have trouble reaching all the hairs. Also, researchers need to determine how stirring a medium to improve the transport of nutrients affects the growth and chemical productivity of the roots. Finally, like regular roots, hairy roots grow every which way, clogging ports and strangling probes or sensors in the growth chambers.

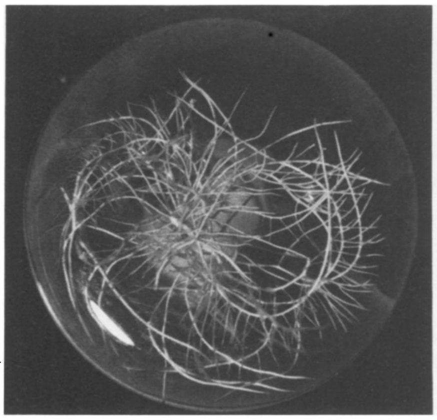
Flores expects the use of hairy roots as chemical factories to mushroom once researchers work out these technicalities. Other types of plant cultures tend to frustrate commercialization efforts because the cells eventually mutate. These genetic changes alter the biochemistry of the cells and, consequently, the amount and types of chemicals produced. "Hairy roots are genetically and biochemically more stable," says Flores. For example, his group still gets hyscyamine and scopolamine — antidotes for motion sickness and nerve gas poisoning — from 9-year-old cultures of henbane.

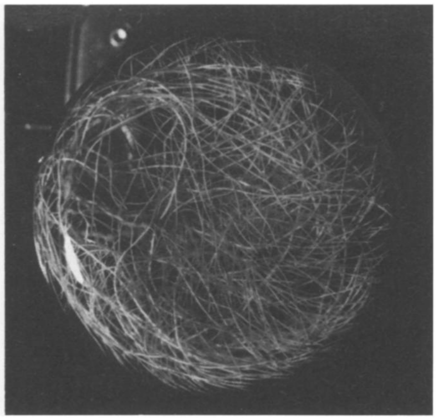
Flores and his co-workers have also created hairy root cultures of plants from the composite and gourd families, expanding the range of potentially useful pharmaceutical and agricultural chemicals produced by this technique. They find that adding pieces of fungal cell walls to some cultures stimulates hairy roots to up their yields and sometimes to make chemicals they hadn't before, Flores reported at the American Chemical Society meeting. In addition, his group has discovered that several hairy root systems will, under the right conditions, turn green, use photosynthesis to become self-sustaining and start producing increased quantities of useful substances.

Schematic of Hairy Root Development



Shanks/Rice Univ.





Shanks/Rice Univ.

Bottom view of hairy root system after 15 and 19 days.

With their various hairy root cultures, the Penn State researchers have begun to tease apart the complex biochemical pathways that yield these compounds. They are learning which enzymes play a role in the marigold's production of polyacetylenes, compounds potentially useful against fungal and nematode infections in crops. This knowledge has helped them figure out ways to modify the marigold's biochemistry to get more polyacetylenes, says Flores.

Other scientists have focused on understanding why yields vary over time. "We believe a feedback inhibition is taking effect," says Phillip L. Gomez III, a chemical engineering graduate student at Lehigh University in Bethlehem, Pa. His group set up an experimental system with hairy roots from tobacco plants and monitored the release of nicotine. They found the roots first release quite a lot of nicotine and then less and less over time. This observation makes them think nicotine oozes quickly from the fine hairs and then seeps much more slowly from a storage vesicle in the root itself.

Japanese scientists are combining genetic engineering with hairy root culture, says Shanks. By inserting genes into the hairy root cells, they hope to modify the metabolic pathways and increase the yields of desired compounds.

The Penn State group and other researchers keep finding new ways to use these roots, too. Until recently, most hairy root products were relatively small, though complex, molecules. But Flores and his colleagues have now created hairy roots from the Chinese cucumber that produce good yields of much bigger molecules: ribosome-inactivating proteins that other scientists have demonstrated will inhibit replication of the HIV virus in laboratory tests. Researchers usually extract these proteins from seeds and roots of intact plants.

Flores also started hairy root cultures of two relatives of the Chinese cucumber. He discovered that each culture produces a specific repertoire of proteins. One species makes two proteins that seem especially active as antifungal compounds. "Our results indicate that root cultures may be able to produce a wide spectrum of bioactive proteins with potential pharmaceutical use," he says.

"It's not as major a technique as mammalian or bacterial cell cultures, but it has its own niche," adds Shanks. □