

Microorganisms

Microorganisms can be identified today using DNA analysis.

One of the world's most useful collections of microorganisms is classified, stored, and maintained at the Northern Regional Research Center in Peoria. It contains more than 80,000 strains of microbes, including some 10,000 bacteria, the simplest one-celled forms of life. About 60,000 additional strains make up the fungi collection, comprising the molds and yeasts. These are nature's wrecking crews; they decompose practically everything in the organic world, permitting the elemental constituents to be used again. Housed in an ordinary refrigerator, there is also a collection of 10,000 actinomycetes, filament-like organisms that are intermediate between bacteria and the more complex fungi. These are the source of such important antibiotics as streptomycin and aureomycin. All the Peoria collections, which keep growing year after year, contain microorganisms of use or potential use to industry and agriculture.

There is much more to collecting microbes than labeling them and keeping them alive. Each has to be correctly identified to guarantee the purity of many foods and beverages that use microorganisms in their manufacture. Identification is also necessary to ensure quality control in making food supplements, vitamins, antibiotics, and other products, and to prevent contamination. Another area in which differentiating among strains of a particular microorganism is vital is in settlement of patent disputes. The ARS Culture Collection in Peoria is one of only two in the United States officially recognized by the U.S. Patent Office as a depository for cultures used in patents.

Identification of microorganisms is more accurate today than ever before because it is based on analysis of the DNA, or genetic material, peculiar to each species. Variants in genes, found in all microorganisms, are also observed and classified. This research provides the groundwork for developing molecular probes. A probe provides a researcher with a fast, simple way to recognize specific microbes and to control their presence—whether welcome or unwelcome—in agricultural products and manufacturing processes.

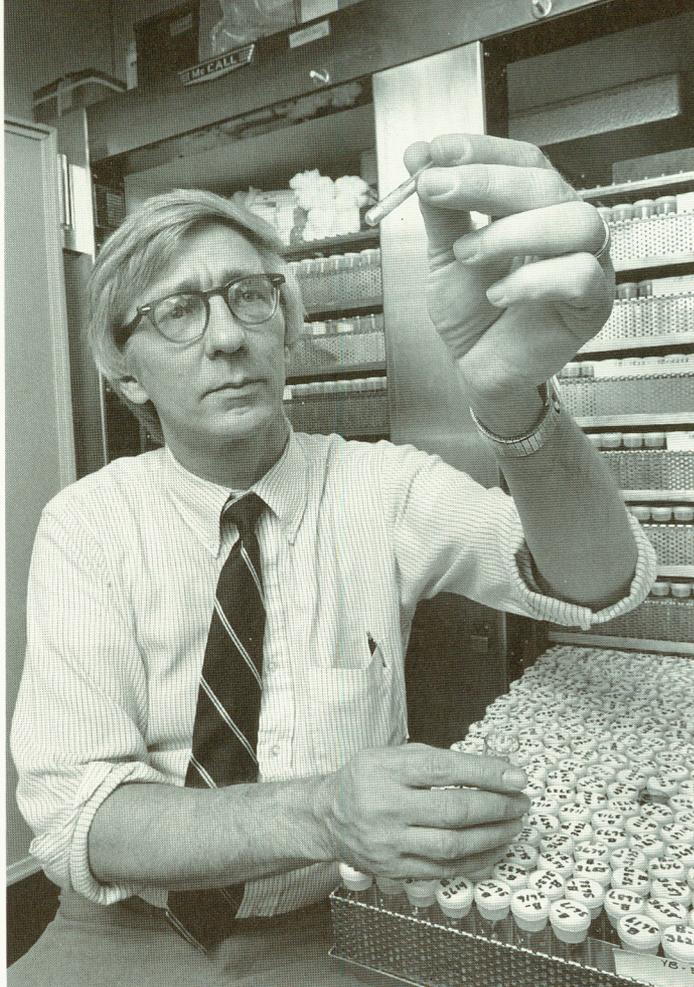
The use of species and strains from the NRRC collection has resulted over the years in accomplishments so important as to defy economic evaluation. A strain of the mold *Penicillium chrysogenum*, originally found on a Peoria cantaloupe, came from the collection and made large-scale wartime production of penicillin possible. In 1943, a variant observed by an NRRC scientist in a culture of the mold *Ashbya gossypii* was found to produce riboflavin, or vitamin B2. A bacterium, *Streptomyces olivaceus*, proved capable of producing vitamin B12 in a fermentation process, as did several other *Streptomyces* species. The fermentation process made possible production of the vitamin as a feed supplement for poultry and swine.

The collection of molds in Peoria is probably the largest in the world and unquestionably the largest that is accessible to the public. It was the source of improved strains of *Aspergillus awamori* that produced fungal amylase, used in the fermentation of starch. The process, now used worldwide, replaced the less efficient and more expensive production of amylase from barley malt. Today the ARS collection is the world reference collection for *Aspergillus*, *Fusarium*, and other important molds.

As described in an earlier chapter, Dextran, a blood extender, and xanthan gum, a valuable industrial product, were both developed from bacteria found in the Peoria collection. And *Blakeslea trispora*, a fungus first found on pumpkin, squash, and cucumber blossoms, produces beta-carotene, a precursor of vitamin A, in a fermentation process.

One of the Northern lab's earliest discoveries was a way to ferment carbohydrates with different strains of the mold *Aspergillus terreus* to produce itaconic acid. The NRRC method is still used today to produce the acid for use in manufacturing plastics, lubricants, and other chemicals. In 1950, NRRC researchers developed a fermentation process in which the mold *Aspergillus niger* is used for the direct production of sodium gluconate from corn sugar. The method not only uses surplus corn, but it brought down the price of sodium gluconate by more than 50 percent when it was commercialized in 1951. The chemical is used as a sequestering agent in glass-washing and aluminum-etching compounds. The value of this discovery, which cost relatively little in research funds, is estimated in the

At the Peoria center, microbiologist Cletus Kurtzman, curator of an ARS Culture Collection containing more than 80,000 strains of microbes, selects yeasts that can grow on sugars and other organic compounds.



millions of dollars. Another use for microorganisms is to produce plant growth hormones. One of them, gibberellic acid, speeds up plant development, including the formation of blossoms and seeds.

The curator of the ARS Culture Collection is responsible, among other things, for learning all he can about a yeast collection of 600 species and more than 14,000 different strains. "We're not just looking at them under a microscope," he explains. "We are isolating and mapping their molecular genetic materials, like DNA. The most spectacular uses of yeast in the future will be for genetic engineering, using recombinant DNA technology."

Genetic transfer is already possible using yeast as a vehicle, but better methods are needed for transferring genes from one yeast cell to another. Among other things, Peoria scientists envision using yeasts to produce citric acid more cheaply. Citric acid is an ingredient of carbonated beverages, syrup, food, and pharmaceuticals. Scientists also speculate that yeast-manufactured hormones might be developed to regulate human metabolism for weight control and that yeasts could be engineered to impart specific flavors to food, such as making a potato taste like Cheddar cheese.

But such uses as these for yeast lie in the future. Many new uses have already been discovered at NRRC. As early as the 1950's, basic research on yeasts led to a better understanding of the mechanism of yeast reproduction. These discoveries, in turn, made it possible to create improved yeasts for industry. A team of Japanese scientists then used the knowledge obtained in Peoria to breed hybrids of a yeast used in fermenting shoyu, a soy sauce made from soybeans and wheat. The hybrids not only increased the rate of shoyu production but also improved its flavor.

Brightly colored strains of a yeast called *Aureobasidium pullulans* were found to excel at releasing xylose, a sugar in plants, from xylan, a hitherto resistant component of plant fiber. Xylose, a 5-carbon sugar, is prevalent in brans milled from grains and in food processing wastes. It makes up 25 percent of the 500 million tons of crop residues produced each year in the United States. It is also found in paper-milling wastes. (See also "Starch Facts," p. 121.)

Another yeast completes the picture. Some 30 years ago, French scientists found what they called a "remarkable" yeast in tanning liquor derived from the wood of chestnut trees. They sent a sample to Peoria. It was *Pachysolen tannophilus*, a yeast like no other with a thick-walled tube that grows from a vegetative cell. The walls refract light and glow under a light microscope, making *Pachysolen* unusually easy to identify. Years later, in the 1980's, researchers found that the yeast can ferment xylose into ethanol, or ethyl alcohol. There is reason to hope that these two yeasts will enable industry to make alcohol and other products from mountains of unused crop residues.

Other NRRC researchers are using yeasts to produce organic acids and to break down fats. After screening 175 strains of yeast, scientists also pinpointed 4 that provide control of fungal decay in stored apples and pears. And a recent study showed that *A. pullulans*, the yeast that can break down xylan in plant fiber, contains an enzyme that may also be useful in processing fruit juices and in treating wood pulp to make fibers for paper and rayon.

Coloring certain foods to make them more appetizing—and perhaps more healthful—may also be possible with yeast. Many foods owe their natural color to the presence of carotenoid pigments, harmless substances that improve the color of foods such as salmon, shellfish, and sea trout. One pigmented yeast, for example, *Phaffia rhodozyma*, can synthesize a carotenoid that imparts a red color to the flesh of trout. There is considerable commercial interest today in the use of such pigments in animal feeds and food dyes. Carotenoids are not only safe, but some of them, including beta-carotene, are thought to inhibit the growth of certain types of cancer cells.

Enzymes in bacteria have also been put to work in the Northern lab. One bacterium found in the culture collection, *Aerobacter aerogenes*, produces a useful chemical called BHPA from glycerol, or glycerine. BHPA, which stands for beta-hydroxypropionic acid, is easily converted to acrylic acid, presently made from petrochemicals and used in acrylic fabrics, carpets, upholstery, and a long list of other products.

Another bacterium, *Clostridium thermoaceticus*, yields 45 percent more acetic acid from corn sugar than does the conventional vinegar fermentation process. Acetic acid is the expensive part of a compound called CMA, or calcium magnesium acetate. Nonpolluting CMA is an effective de-icer at lower temperatures than salt and will not corrode metal. The chemical is recommended by the Federal Highway Administration as an alternative to rock salt as a highway de-icer. NRRC researchers hope to bring down the cost of CMA until it is more nearly competitive in price with salt, which annually causes more than \$5 billion in damage to roads, bridges, and vehicles.