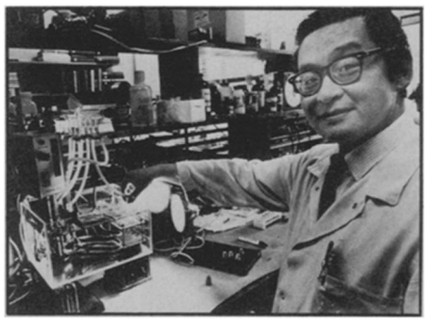
ENGINEERING ANTIBIOTICS WITH ISOLATED ENZYMES



Daniel I. C. Wang

Isolated enzymes show promise in large-scale antibiotic manufacture

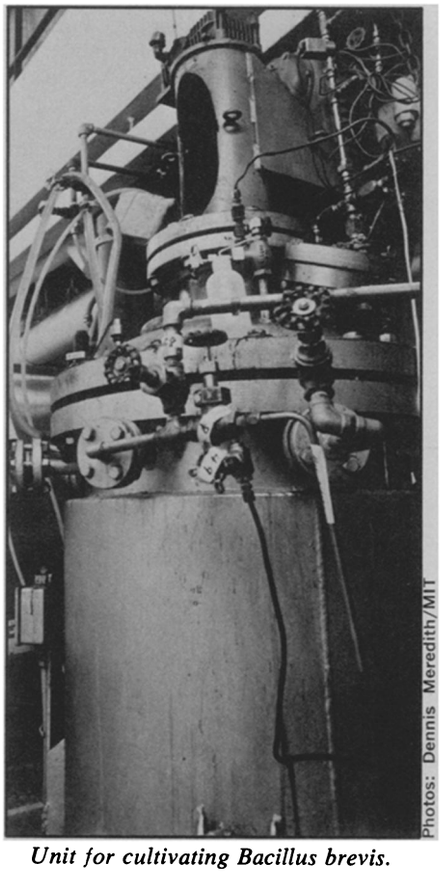
by Joan Arehart-Treichel

Antibiotics became clinically available in the early 1940's. Since then, they have saved millions of people from tuberculosis, influenza, scarlet fever, meningitis, typhoid fever, cholera, whooping cough, syphilis and a host of other deadly infectious diseases. Antibiotics are *the* miracle drugs of the 20th century. They have shaped, and continue to shape, our history.

Today, some hundred different antibiotics are marketed by American drug companies. A common method of manufacture consists of putting fungi or bacteria into flasks that contain nutrients. The microorganisms use the nutrients to grow and to multiply. As they increase their numbers, they are moved to seed tanks, where they reproduce some more. Then they are transferred to fermentation tanks, where the nutrients and environmental conditions encourage them not to just grow and multiply but to use their enzymes to make antibiotics. These fermentation tanks are enormous, some of them five stories high. There, an astronomical number of bacteria or fungi bubble and seethe as they churn out 125,000 gallon batches of antibiotics.

Harvesting antibiotics by fermentation has its drawbacks, however. Fermentation takes weeks to accomplish and is not very efficient because the microorganisms use nutrients not just for making antibiotics but for growth, reproduction and other metabolic activities. Then the antibiotics have to be separated out from the nutrients and other metabolic products.

A team of engineers, food scientists, microbiologists and chemists at the Massachusetts Institute of Technology, led by Daniel I. C. Wang, has now developed a new way to make antibiotics that may prove to be more efficient and economical than fermentation. It consists of using isolated enzymes from microorganisms, rather than whole microorganisms, to make antibiotics.



Photos: Dennis Meredith/MIT

Unit for cultivating Bacillus brevis.

Two years ago the team received a grant from the National Science Foundation's program for Research Applied to National Needs (RANN), to come up with some new kind of enzyme technology that would benefit society. Enzymes are already being used to make antibiotics, wine, cheese. They are used in laundry detergents, the processing of foods and the tanning of leather. But the NSF believes that because of recent advances in molecular biology, chemical engineering and related sciences, enzymes could make far more contribution to useful products, especially if used in isolated form. And it is supporting research toward that aim—say, the use of enzymes to synthesize nutrients, dispose of wastes or to catalyze industrial reactions.

So the MIT team asked: If enzymes are to be used, what can they do that organic chemistry synthesis cannot do? What can we as an interdisciplinary team do with enzyme technology that we could not do as individual scientists? What can we devise that industry can quickly capitalize on? After six months of brainstorming, the group decided on using enzymes to synthesize polypeptide antibiotics. It is something that chemical synthesis can achieve, but only with difficulty; something that requires an interdisciplinary team, and something that the drug industry should be able to use, at least in the manufacture of certain antibiotics.

They chose a polypeptide antibiotic called gramicidin S as their model system—for several reasons. The amino acid sequence of the antibiotic had

Continued on page 284

. . . Antibiotics

been worked out by various research groups. So had the means by which the bacterium, *Bacillus brevis,* makes the antibiotic. The antibiotic is made by a bacterial enzyme, gramicidin synthetase, rather than by the usual ribosomal peptide synthesis route. So they theorized that if they could isolate the enzyme from the bacterium, the enzyme might be used to make gramicidin S outside the bacterium.

They worked at isolating enough of the enzyme in sufficiently pure form so that it could be used to make gramicidin S in preparative scale amounts. While meeting this challenge, they had to keep economic production for industry in mind. An ultrapure enzyme solution would do a fine job of making the antibiotic, but it is also much more expensive to make than a partly crude enzyme solution. They then worked at getting the enzymes to remain stable, and hence functional, for days at a time, instead of the usual minutes or several hours. They figured out how production of the enzyme could be scaled up 250 times—from one-fiftieth of a gallon (laboratory flask size) to 50-gallon vats. They determined how much ATP, the natural energy-releasing molecules of the cell, had to be added to the vats for the enzymes to make antibiotic, and how this ATP could be economically regenerated from its breakdown products—AMP or ADP.

"We can now produce gramicidin S in large batches, and we can get it very efficiently," Wang reports. This means they can get a preparative batch of antibiotic within hours. The antibiotic is easier to separate from the enzyme reactor than from the fermentation system. So it looks as if this means of antibiotic production might turn out to be cheaper than fermentation.

The MIT scientists and engineers are now trying to find economic ways of purifying the enzymes further. They are trying to produce bacterial mutants that make the gramicidin synthetases in even larger quantities than normal. They are looking for ways to recover the enzymes from antibiotic production so that they can be used again.

Although gramicidin S is not a commercially important antibiotic, there is an antibiotic that is vital commercially and made by similar enzyme production. That antibiotic is bacitracin. Bacitracin is put in livestock feed around the world to promote livestock growth. The MIT scientists are now exploring ways of adapting their antibiotic production technology to bacitracin, in hopes that the technology might be of use eventually to the manufacturers of this antibiotic.

Their ultimate aim is that the technology might be used by many drug companies in the manufacture of various antibiotics. □