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yields are likely to become routine when researchers acquire enough experience of the relative merits of different vectors. Purities tend to be at least as good as those of equivalent proteins from more traditional sources. In short, Atkinson said he was confident that there are no insuperable technical barriers to the large-scale production of almost any protein—so long as scientists are provided with enough time and money, and regulatory and political idiocies are kept to a minimum.

One of the top priorities now is for researchers to grow their proteins in thermophilic organisms, since cooling down large culture tanks accounts for a surprisingly large part of the production cost, and thermophilic cells grow remarkably fast and produce enzyme faster too. Protein chemists think they have recently begun to understand what makes proteins thermostable; now they will be eager to test these ideas by deliberately making enzymes run hotter.

—**Anthony Durham**

STANFORD CONFERENCE

HOW TO ENGINEER BACILLI FOR PROTEIN SECRETION

STANFORD, California—Bacilli have a great track record in industry. More than a score of their secreted proteins (mostly cleaving enzymes) are successfully produced and widely employed by dozens of industries. But what about the future now that biotechnology has become something more than just an industrial buzzword? Will there still be room for old workhorses like the bacilli? Judging from the fact that nearly half the participants at the Second International Conference on Genetics and Biotechnology of Bacilli held here July 6–8 were from industry, the answer must be a resounding yes.

Thomas Parmeter, president of Syntro Corporation, which co-sponsored the conference with the Stanford Medical School, predicted that the gene expression systems of bacilli will become some of the most important tools for industry during the coming years. Many of the conference's 250 attendees agreed that bacilli may become major stars in the biotechnology show as industries increase their production of new protein products via the use of recombinant DNA technology. When compared to *Escherichia coli*, traditionally the most common host for cloned DNA, bugs such as *Bacillus subtilis* offer significant advantages. They are not pathogenic; they have been used successfully in industrial scale fermentations and are considered predictable and well-mannered organisms; and they efficiently secrete a large number of proteins directly into the growth medium.

The fact that bacilli can excrete proteins efficiently has not been exploited fully until recently. Although secretion by bacilli has long been studied, interest centered around what, and how much, was secreted. That has changed. An entire session at this conference was devoted to details of the secretion mechanism, and how that mechanism might be manipulated to allow excretion of new proteins (especially those produced from cloned DNA). Secretion is a hot topic in bacilli research, and for good reason. In theory, if there were cloning vectors that not only express foreign genes, but also facilitate secretion of the resulting proteins, the secreted proteins would be less likely to inhibit cellular metabolism and more likely to be of higher initial purity than if they remain inside the cell. These are important considerations for any industrial production process.

The conference presentations on the protein secretion mechanism owe much to previous studies on the secretion process in *E. coli* and mammalian cells. Briefly, those studies established that most secreted proteins are synthesized with an extra sequence of 15 to 30 hydrophobic amino acids attached to their amino-terminal end. This extra bit of protein, which has been dubbed the "signal sequence," plays an essential role in secretion by acting as a kind of hook, inserting the polypeptide into the membrane and facilitating translocation of the protein out of the cell before it is cleaved off.

As a first step in understanding the excretion mechanism in the bacilli, several genes encoding bacilli exoenzymes were cloned, sequenced, and examined to determine if they, too, contained DNA that would code for signal sequences. They do. The signal sequences for α-amylase from *B. amyloliquefaciens* and the β-lactamase from *B. licheniformis* are well characterized, and are similar to those of *E. coli.*

The bacilli signal sequences have now been incorporated into cloning vectors. Mervi Sibakov, representing research groups from the Recombinant DNA Laboratory and the Central Public Health Laboratory in Helsinki, Finland, and Kunio Yamane, from the University of Tsukuba in Ibarai, Japan, discussed similar methods for constructing so-called secretion vectors.

Sibakov reported on the following construction of a protein-excreting cloning vector (pKTH50) in *B. subtilis*. The α-amylase structural gene, its signal sequence, ribosome binding site, and promoter from *B. amyloliquefaciens* were cloned into the plasmid pUB110 and transformed into *B. subtilis.* Because pUB110 forms multiple copies (about 50 per cell), and because the α-amylase promoter from *B. amyloliquefaciens* is especially strong (the organism is used for industrial production of amylase), amylase constituted up to 50 percent of the total protein synthesized in cells carrying the plasmid, and virtually all of the amylase was secreted. Researchers then removed the amylase structural gene from the plasmid, and put a HindIII site in its place. This provided a unique site for insertion of foreign DNA into the plasmid, adjacent to the high level promoter and the signal sequence of the α-amylase.

Foreign DNA inserted into the HindIII site of the secretion vector pKTH50 was expressed, and the resulting proteins were secreted efficiently. Both an *E. coli* β-lactamase gene, which lacked a signal sequence, and the human interferon α_2 gene, which was previously cloned in *E. coli,* were inserted into the *B. subtilis* secretion vector. Each gene was expressed efficiently and the resulting proteins were secreted into the growth medium. Evidently the signal sequence of the α-amylase was synthesized as an attachment to the new proteins, and it successfully mediated their secretion. In both cases, the signal sequence was cleaved from the secreted protein at the correct site. The value of this vector may soon be extended because the hepatitis B core antigen and the major antigen of hoof-and-mouth disease have been cloned and expressed in *B. subtilis,* but not yet in vectors that would promote secretion.

Although these vectors seem promising, researchers must still overcome some problems. *B. subtilis* produces a potent extracellular protease that can destroy the secreted proteins. Isolating a protease-negative mutant will undoubtedly be a high priority.

Scientists will develop a more detailed understanding of the bacilli secretion process from the cloning and analysis of other bacilli exoenzymes and from molecular studies of the secretion process. The former has the added appeal that, if high levels of production and secretion occur, the clones may be of commercial value. However, researchers have already achieved advances sufficient to make cloning in bacilli an attractive alternative to other systems.

—**Charles L. Ginther**