MEETING REPORT

RESEARCHERS CULTIVATE NEW USES FOR BACILLI

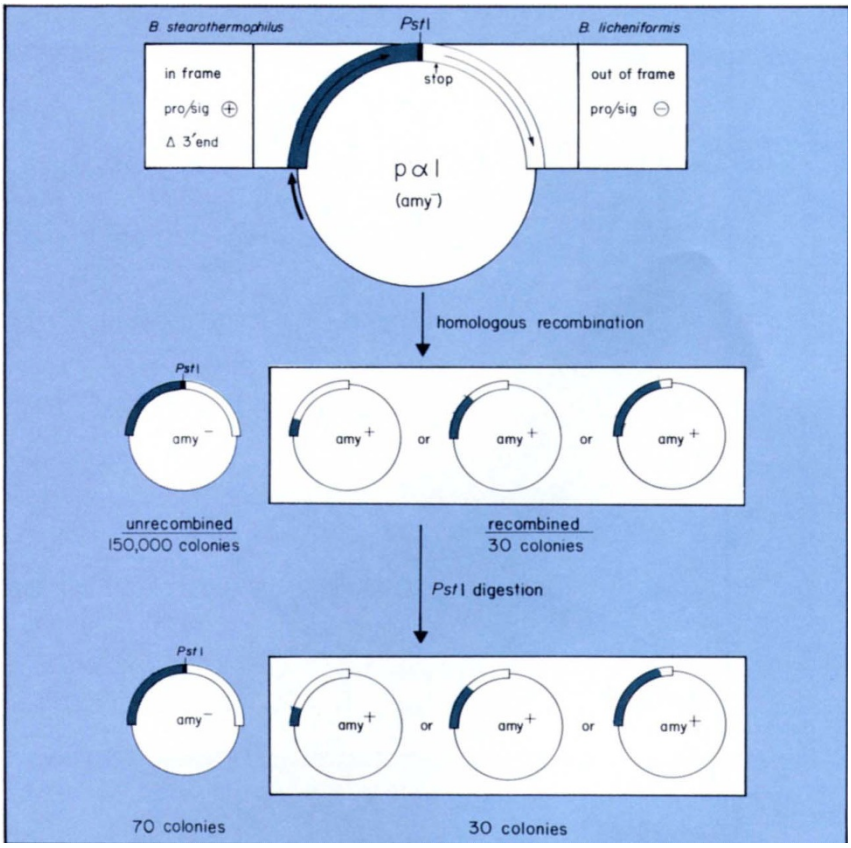
STANFORD, Calif.—Bacilli definitely have useful properties to offer basic and applied researchers alike. That was the clear message from the Third International Conference on Genetics and Biotechnology of Bacilli, held July 15–17 and sponsored by Syntro Corp. (San Diego, CA) and Stanford University Medical School. Despite the diversity of presentations, attendees were most interested in new technologies for increasing gene expression and protein secretion.

Mark Ruppen (Genentech, South San Francisco, CA) described his use of the inducible, hybrid *spac* promoter system in an asporogenous host grown to high cell density to obtain elevated intracellular expression of a human growth hormone (HGH) gene in *Bacillus subtilis*. Serving as a model system to study variables ranging from promoter regulation and plasmid stability to host mutations and product quality, an optimized process was achieved yielding 1.5 g/l of undegraded HGH.

Stabilization of mRNA could explain the role of a positive retroregulator that enhances the expression of upstream gene(s), said Shing Chang (Cetus Corp., Emeryville, CA). When a 78 bp fragment containing the transcriptional terminator of the crystal protein from *B. thuringiensis* vs. *Kurstaki* HD-1 was fused to the distal ends of either the penicillinase gene of *B. licheniformis* or interleukin-2 cDNA from the human Jurkat cell line, the half-life of the resulting mRNA was increased three-fold in both *Escherichia coli* and *B. subtilis*. A significant increase in synthesis of the corresponding polypeptides was also observed, and such an enhancement was apparently independent of the orientation of the retroregulator.

Gregory Gray (Genencor, South San Francisco, CA) presented a novel scheme whereby cloned overlapping sequences which exhibit partial homology were allowed to recombine in *E. coli* or *B. subtilis*, generating a variety of chimeric forms, depending upon the points of crossover. Two related amylases were treated, and, by enrichment, several thousand distinct recombinants could be generated in a single experiment; understandably, these chimeric enzymes show new properties.

Prokaryotic secretion systems look particularly attractive in the case of Bacilli because of the benefits conferred by only one cell membrane. Approaches include those based upon sequences derived from proteases, α-amylase, and alkaline phosphatase.



Genecor's Gregory Gray generated hybrid α-amylase genes in the following way: Plasmid pα1 contains a 3′ deleted transcriptionally active *B. stearothermophilus* amylase gene fragment fused out of frame to the codons for the mature form of the *B. licheniformis* amylase. *E. coli*/pα1 transformants are amylase negative. Low frequency recombination results in a plasmid population containing a small minority with recombined amylase genes. These are detected by their ability to give rise to amylase positive colonies upon retransformation. Since the unique *PstI* site of pα1 is always deleted by recombination, it is possible to enrich for recombinant plasmids by *PstI* digestion prior to retransformation.

By utilizing the promoter and pre-pro-peptide coding region of *B. amyloliquefaciens* neutral protease, Masaru Honjo (Mitsui Toatsu Chemicals, Japan) and coworkers have produced 10^9 units of human beta-interferon per liter of *B. subtilis* culture, where more than 80 percent of the total activity was secreted. Similarly, N. Vasantha (Genex, Rockville, MD) described the creation of a series of secretion vectors for *B. subtilis* which use the signal sequences of either alkaline or neutral protease from *B. amyloliquefaciens*, and permitted the secretion of Protein A.

Systems based on *B. amyloliquefaciens* α-amylase can increase the yield of certain naturally occurring secreted proteins at least 100-fold, said Ilkka Palva (University of Helsinki, Finland). This is apparently due to the lack of a strong link between the production rate and copy number of the clone over a wide range of copies. In related work, Kunio Yamane (University of Tsukuba, Japan) detailed the development of another α-amylase secretion vector for *B. subtilis* which, under special culture conditions, enabled stable hyperproduction of *E. coli* β-lactamase (but not of mouse beta-interferon).

The attractive alternative of adapting *B. licheniformis* alkaline phosphatase sequences, with its high level of secretion as the major protein moiety, is another avenue, this one being explored by F. Marion Hulett (University of Illinois, Chicago).

Although subsequent research and experience should dictate which host and sequence combinations offer the best conditions for efficient expression and secretion, *B. subtilis* was certainly the favorite host for most of the work described. **—Anthony S. Weiss**

COURTESY GREGORY GRAY (GENENCOR) DRAWN BY CAROL MORA (GENENTECH)