Abstract

Microbes have been good to us. They have given us thousands of valuable products with novel structures and activities. In nature, they only produce tiny amounts of these secondary metabolic products as a matter of survival. Thus, these metabolites are not overproduced in nature, but they must be overproduced in the pharmaceutical industry. Genetic manipulations are used in industry to obtain strains that produce hundreds or thousands of times more than that produced by the originally isolated strain. These strain improvement programs traditionally employ mutagenesis followed by screening or selection; this is known as 'brute-force' technology. Today, they are supplemented by modern strategic technologies developed *via* advances in molecular biology, recombinant DNA technology, and genetics. The progress in strain improvement has increased fermentation productivity and decreased costs tremendously. These genetic programs also serve other goals such as the elimination of undesirable products or analogs, discovery of new antibiotics, and deciphering of biosynthetic pathways.

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ing microbial strains and the application of recombinant DNA technology.

Strain improvement encompasses creation of strains with (i) efficient assimilation of inexpensive and complex raw materials; (ii) alteration of product ratios and elimination of byproducts; (iii) product excretion; (iv) tolerance to high product concentrations; (v) short fermentation times; and (vi) overproduction of native products or foreign products after genetic recombination [1].

The contributions of microbial genetics to industrial microbiology began in the 1940s when the fermentative production of penicillin became an international necessity. The early studies in basic genetics concentrated heavily on the production of mutants and their properties. The ease with which 'permanent' characteristics of microorganisms could be changed by mutation and the simplicity of the mutation techniques had tremendous

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appeal to microbiologists. Mutation has been the major factor involved in the hundred to thousand-fold increases obtained in production of microbial metabolites. The ability to modify genetically a microbial culture to higher productivity has been the most important factor in keeping the fermentation industry in a healthy state.

2 Mutagenesis

Microorganisms generate new genetic characters ('genotypes') by two means: (i) mutation and (ii) genetic recombination techniques such as protoplast fusion, transformation, conjugation and recombinant DNA technology, including metabolic engineering.

In mutagenesis, a gene is modified either unintentionally ('spontaneous mutation') or intentionally ('induced mutation'). Although the change is usually detrimental and eliminated by selection, some mutations are beneficial to the microorganism. Even if not beneficial to the organism, but beneficial to humans, the mutation can be detected by screening and preserved indefinitely. Mutation has been mainly used to improve the productivity of industrial cultures [2, 3], although it has also been used to shift the proportion of metabolites produced in a fermentation broth to a more favorable distribution, elucidate the pathways of secondary metabolism, yield new compounds, and other functions.

The most useful mutagens include nitrosoguanidine (NTG), 4-nitroquinolone-1-oxide, methylmethane sulfonate (MMS), ethylmethane sulfonate (EMS), hydroxylamine (HA) and ultraviolet light (UV). The most common method used to obtain high yielding mutants is that of treating a population with a mutagenic agent until a certain 'desired' kill is

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2.1 Increasing metabolite production

Genetics has led to tremendous increases in fermentation productivity and decreased costs mainly by mutagenesis and screening for higher producing microbial strains. Overproduction of microbial metabolites is effected by (i) increasing precursor pools, (ii) adding, modifying or deleting regulatory genes, (iii) altering promoter, terminator and/or regulatory sequences, (iv) increasing copy number of genes encoding enzymes catalyzing bottleneck reactions, or (v) removing competing unnecessary pathways [13].

The first superior penicillin-producing mutant, *Penicillium chrysogenum* X-1612, was isolated afer X-ray mutagenesis in the mid-20th Century. This heralded the beginning of a long and successful relationship between mutational genetics and industrial microbiology [14]. Improvement of penicillin production by conventional strain improvement resulted both from enhanced gene expression and from gene amplification [15, 16]. Increased levels of mRNA corresponding to the three enzymes of penicillin G biosynthesis have been found in high-penicillin producing strains of *P. chrysogenum* as compared to wild-type strains [17]. High-producing strains contain an amplified region which is at least 35 kb. A 106 kb region amplified 5 to 6 times as tandem repeats was detected in a high-producing

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