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CURRENT STATUS OF BIOTECHNOLOGY IN CHINA

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There has been increasing interest over past decade in exploring the possibility of using new biotechnology to produce new products and to improve the old productive process. The researches and applications of genetic engineering, cell fusion, mutagenesis, cell and enzyme immobilization in enzyme, antibiotic, vitamine, steroid, amino acid, organic acid, solvent, food and brewage industries is reviewed.

Key words: genetic engineering – cell fusion – mutagenesis – immobilization

Biotechnology, as an emerging technology, has broght great impact and excitement in China. There has been increasing interest over the past decade in exploiting the application of biotechnology in medicine, industry, agriculture, and environmental protection. The research and development program of biotechnology consists of 3 levels in China: the spark program including mainly the existing and easily adoptable techniques, the 7th five-year plan being the priority areas of scientific and technical development which have important economic benefit and some key technologies of strategic importance, the high technology programme directing to the more advanced technology and targeting to the most recent world wide development.

The main research and development areas of biotechnology are: (1) breeding for high yielding, superior quality and stress-resistant cultivars and more productive animals and fishes. (2) developing new vaccines, pharmaceuticals, diagnostic reagents and gene therapy techniques. (3) reforming the traditional biotechnology industries to reduce cost, energy consumption and pollutions, and to change the unreasonable product structure.

In this decade, biotechnology is an active and successful field and genetic engineering and hybridoma are most exciting and attractive areas. A series of transgenic plants, including tobacco, tomato, rape, rice, cotton, with high yield and resistance to herbicides, virus infections and insect damages have been constructed. Some of them have been planted in the fields for 7 to 10 generations. Human growth hormone gene had been transferred to fishes and some super transgenic fishes were obtained. Transgenic rabbits expressing human growth hormone and hepatitis B surface antigen genes were also constructed.

The genetically recombinant microbial and animal strains had been constructed to produce new vaccines, pharmaceuticals, for examples, hepatitis B surface antigen, malaria vaccine, K-88, K-99 antigens, interferons, interleukins, growth hormones, insulin, tumor necrosis factor, tissue plasminogen activator, colony stimulating factors, epidermal growth factor. Among them interferon-α has been marketed and hepatitis B surface antigen, K-88, K-99 antigens, human growth hormone are being studied at pilot scale.

Hybridomas have been widely used to prepare various monoclonal antibodies in laboratories and in production. Several monoclonal antibody diagnostic kits have been marketed. The conjugates of monoclonal antibody and toxic agent have been studied in China and some toxins, antibiotics and isotopes are used to couple with monoclonal antibodies. Some exciting results have been obtained with T lymphocyte antibody-ricin conjugate and melanoma antibody-abrin conjugate.

Recombinant DNA and cell fusion techniques have widely used to improve industrial strains. For enzymes, genetic engineering and protein engineering have become the powerful tools

to increase the yield and to change the properties of enzymes. Chinese scientists have met with success in increasing penicillin acylase yield and in reducing the oxidability of subtilisin. Antibiotic production is a major target for recombinant DNA and cell fusion techniques. Some antibiotic biosynthesis genes have been cloned and the potential to improve antibiotic producing strains are being tested. Some important antibiotic transformation genes have been cloned and have been successfully used to constructed high yielding strains. Cell fusion has been applied both in improving the antibiotic producer and in exploiting new antibiotics. Recombinant DNA technique has been also used to improve the amino acid producers, the threonine, tryptophan and proline biosynthesis operons have cloned by using *in vitro* and *in vivo* recombinant DNA techniques (Wu et al., 1987; Wang et al., 1990).

Antibiotic industry is a large enterprise in China. The annual output amounts to 11,600 tons in 1985. More than 35 kinds of antibiotics have been marketed and some new antibiotics and physiologically active substances have been in development. The urgent target is to change the structure of the antibiotic industry which is rather unreasonable. Tetracyclines and streptomycin account for more than 70% of the total antibiotic output and the semisynthetic penicillin and cephalosporin only account for 0.75% in 1985. One of the main efforts is to construct strains with high specific acitivity for pinicillin acylase and cephalosporin acylase production. The penicillin G acylase gene was cloned and expressed in *Escherichia coli*, then the recombinant plasmid was stabilized by *in vivo* recombination and used to transform suitable *E. coli* host. The penicillin acylase yield of resultant engineered strain was twenty fold higher than the original one (Yang et al., 1987). The immobilized cells was used to transform penicillin G in pilot scale production and the yield of the crystal 6-APA was over 85% (Yang & Wu, 1990). GL-cephalosporin-acylase gene was cloned to *E. coli* from *pseudomonas* chromosome. After mutagenesis, the expression level of GL-cepharosporin acylase gene increased markedly. Another antibiotic transformation gene was cloned and used to transform kanamycin to amikacin. Medicamycin and spiramycin biosynthetic genes were cloned from *Streptomyces mycarofaciens* and *S. ambofaciens* respectively by using polyketide biosynthetic gene as probe. The cloned genes could restore the ability of the block mutants to produce antibiotics (Wang, 1989). Lincomycin biosynthesis genes were also cloned from *S. linocolnesis* and expressed in *S. lividans*.

Protoplast fusion was used to improve industrial antibiotic producers and to exploit new antibiotics. In some interspecies recombinant fusants, new antibiotic spots were detected on chromatographs. For examples, the fusant from *S. fradia* and *S. lividans; S. lincolnesis* and *Micromonospora* Sp. SIPI 4812; *S. qinfengmyceticus* and *S. hygroscopicus var. jinggangensis* (Zheng et al., 1985). The nature of these new antibiotics are being studied (Xu et al., 1985). These approaches implies that interspecific protoplast fusion is a promissing technique to exploit new antibiotics including the non-naturally occurred antibiotics. The ability of the fusants to synthesis new antibiotics might arise from the activation of the silent genes or the fusion and complement of different metabolic pathways due to the gene recombination.

Besides these new techniques, the conventional breeding and screening are still the useful and important technique and widely used in the improvement of various industrial strains. For examples, a pair strains of *Gluconobacterium oxydans* and *Bacillus cereus* were screened for the production of 2-keto-*L*-gulonic acid, the precursor of vitamine C, from sorbose and the co-immobilized cells were used in this conversion (Yuan et al., 1990). High yielding strains for glutamic acid, lysine and arginine have been bred by using mutagenesis (Gong et al. 1988; Gong & Chen, 1987). The yield of glutamic acid and lysine reaches 10% and 8% respectively. A mutant of *Aspergillus niger*, which is resistant to heavy metals and has the ability to saccharify raw starchy materials, has been used in the industrial production of citric acid. Using this mutant citric acid could be produced directly from potato powder without presaccharification.

Immobilized enzymes and cells received wide attention in China. Immobilized enzymes that have been used in commercial scaleinclude penicillin G acylase which converts penicillin G to 6-APA, glucosephosphate isomerase which is used to produce fructose syrup, glutamate decarboxylase, which converts glutamate to 4-aminobutyrate, phosphodiesterase, which hydrolyses ribonucleic acids to nucleotides.

Cell immobilization was developed after enzyme immobilization, but it had rapidly developed and came into industrial application. Immobilized *E. coli* cells has been first used in industrial production of 6-APA in 1960's. Recently combining recombinant DNA and membrane techniques a new process with high efficiency was developed. A hollow fiber reactor was used to immobilize the genetically recombinant cells, the yield of 6-APA reached 90%, and the specific acitivity of the reactor was comparative to immobilized enzyme reactor (Yang & Wu, 1990). It is a successful example combining genetic engineering and cell immobilization technology to increase the efficiency of immobilized cell reactor.

The immobilized bacterial, yeast and filamentous fungal cells have been used to produce various kinds of products, such as *L*-aspartic acid, steroid hormones, itaconic acid, *L*-alanine. and ethanol (Ju, 1990; Li & Yan, 1988). A remarkable process has been developed in the production of beer by the immobilized growing yeast cells, the production of beer on a scale of 6.4 tons per batch was successful. As compared with the traditional process, the new peocess significantly shortens the fermentational period from 21 days to 12 days, and the beer produced by this new process meets all chemical and physical specifications (Ju, 1990). The immobilized living cells have been also used in the production of steroid hormones and enzymes such as amylase, glucoamylase, pectinase and phosphotase.

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