Module – 08: The Improvement of Yielding Potential of Industrially Important Microbes

Introduction

Industrial Products produced by the wild strains isolated from nature. They selected based on particular product produced by strain. E.g. Bacillus is isolated from soil sample for the production of amylase. (Amylase can act on starch to give glucose). These naturally isolated organisms usually produce commercially important products in very low concentrations and therefore it is essential to increase the productivity of the selected organism. Yield of the desired product, can be increased by optimizing medium and growth conditions. Medium and growth optimization is having limited effect on increase in the product due to organism's maximum ability to synthesize the product, which is control by its genome. Thus if one want to increase the product then one should modify the genome. After the creation of desired genome for the increased desired product, then cultural requirements of the modified organisms is tested. Modified genome further modified forimprovement. Thus the process of strain improvement require continuous changing of genes of given organism with changes in the medium components.

Genetic modification achieved by

1. **Mutation**
2. **Recombination**

Only yield increase is not the criteria for the improvement of microbes but their stability, resistance to infection, medium components, non-foaming capabilities, tolerance to low oxygen tension, no undesirable product formation is also taken into consideration.

Mutation

Mutation can be spontaneous or induced. Spontaneous mutation can lead to the change in the genetic make-up of the organisms but chances are there that culture may have problem of yield degeneration because variants are usually inferior producers. Sometime natural variants can also give better yield but it is not reliable one so other techniques are employinglike induced mutation and recombination.

Induced Mutation

Variants or mutants obtain giving better yield by inducing mutation to the wild strain. Mutation Induced by various physical and chemical agents, known as mutagens.

Mutation can creates so many mutants of which some are superior producers and some are inferior producers of the product. It is not easy to select them because of one or two criteria but superior mutant is selected using more than one criterion for the formation of product.

Selection of Mutants giving better primary metabolites

Any metabolite is produce by particular microorganisms via certain pathway. When concentration of particular metabolite increases then organisms have regulatory mechanism to control over the production of those metabolites. If such control is block by mutation, than improvement in product is possible. Example

Corynebacterium glutamicumproduces glutamic acid; this product increased by mutation. Kinoshita isolated mutant of *C glutamicum*, which is deficient in the production of biotin as well as in the synthesis of enzyme α-ketogluterate dehydrogenase. Biotin deficient strain do not produce proper membrane and thus deficient in selective permeability. While defect in the production of enzyme α-ketogluterate dehydrogenase will not allow the formation of succinic acid from the α-ketoglutaric acid, and α-ketogluteratediverted to glutamic acid synthesis.

Organisms used for the commercial production of primary metabolites rarely modified at only one genetic site, sometime it is necessary to alter several control sites to produce desire product in high quantity.

Selection of mutants producing improved levels of secondary metabolites

The design of producers for the isolation of mutants overproducing secondary metabolites is more difficult due to the fact that far less information is available on the control of production and, also, that the end products of secondary metabolism are not required for the growth.

Screening achieves considerable success in selecting mutant for the production of secondary metabolites.

Several workers have obtained improved secondary metabolite producing strains by isolating auxotrophic mutants.

In many cases, there is no correlation between the compound and the secondary metabolites produced. Possible explanation for this may be that they are double mutants and their auxotrophy not directly related to the improved productivity.

Organisms exploited by using Nitrosoguanidine (NTG) as a mutagen. NTG causes clusters of mutations around the replication fork of the bacterial chromosome. Thus if one of the mutations were selectable it may be possible to isolate a strain containing the selectable mutation which is close by, for this one should require the accurate knowledge about the positions of the genes important in secondary metabolites.

The technique of selecting mutants resistant to inhibitory analogues has found some application in the selection of secondary metabolites overproducers.

For example,***Elander et al.*** (1971) isolated tryptophan analogue resistant mutant of ***Pseudomonas aureofaciens***, which overproduced antibiotic pyrrolnitrin. Tryptophan is precursor for this pyrrolnitrin and resistant mutant can produce more of this limiting precursor.

Recombination

Recombination is process of creating new combination of genes in given organisms. Recombination process is not that much successful as the use of induced mutant and selection, this is due to success of mutation programme.

Now recombination technique used for the strain improvement after the different techniques available, which help us to use this technique more conveniently.

Recombination process carried out **naturally or artificially**. Recombination that is occurring naturally is applicable to few organisms, due to limitation of genetic exchange between these organisms. With artificial recombination,insertion of any gene is possible in any cell.

Natural recombination by parasexual cycle in some fungi

In Fungi, imperfect nuclear fusion and gene segregation could take place outside the sexual organ. This process is parasexual cycle. For this process genetically unlike nuclei must be present in one of the fungi. After the fusion of genetic material of two different organisms, heterokaryon is produce. These heterokaryons contain genetic information of two different organisms or recombinant gene. However, this technique is not useful in strain improvement is the establishment of heterokaryons.

Natural recombination by Conjugation

Conjugation is the process in bacteria whereby genetic information is transfer from one cell to another by cell-to-cell contact. The chromosome of the 'donor' cell mobilizes by the integration of a normally extrachromosomal DNA particle into the recipient. This technique used in the preparation of strain producing particular compound in excess, or producing compound, which is not previously present in recipient organism.

Conjugation demonstrated in Streptomyces, which have enormous industrial significance. However, the disadvantage of the conjugation is that considerable genetic knowledge of the organism is required to perform the cross effectively.

Natural recombination by Protoplast Fusion

Protoplasts are the cells devoid of cell wall. It can be prepared by subjecting organisms to wall degrading enzymes. Cell fusion, followed by nuclear fusion will occur between protoplasts that would not otherwise fuse and resulting in fused protoplast may generate cell wall and grow into mutant cell. In this technique whole genome of one fused with genome of other organism thus chances of mutations are more in this technique compared to conjugation. Fusion of two protoplast result in the formation of heterokaryons, where the limitation of one cell with different genome of traditional parasexual cycle is over in this technique.

In vitro recombinant DNA technology

In this technique,Host's chromosomal or extrachromosomal DNA cut and the desired small DNA inserted into it, this lead to the recombination of the Host DNA.

The majority of the recombinant DNA prepared by this technique is use for the improvement of organisms producing primary metabolites.

The efficiency of the organisms used in the single cell protein process, ***Methylophilusmethylotrophus***, improved by the incorporation of a plasmid containing the glutamate dehydrogenase from ***E coli***.

Techniques of genetic manipulation improveproduction of commercially important enzyme. Enzyme yield increases by incorporating the chromosomal gene coding for the enzyme into a plasmid, which then introduced into the original strain and maintained at high copy number.

This technique is also available for bacteria, yeast, and fungi. However, it is limited to improvement of organism for primary metabolites, due to lack of information regarding basic genetics of secondary metabolites production.

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