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TRANSGENIC MICROBES

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Nowadays, the problem of transgenic microbes is one of the most burning, because the population of the earth by 2050 should achieve 10 billion people and scientists are concerned about the problems of the lack of food.

In generally, genetically modified organisms (TMG) - are living organisms whose genotype has been modified by genetic engineering manipulations to form a new properties of organisms.

Thus genetic engineering technologies are based on obtaining recombinant DNA molecule and introducing it into a bacterial host cell.

The date of genetic engineering occurrence is considered to be XXth century 1972 when P.Berh together with the colleagues first constructed a recombinant DNAinvitro, consisting ofDNA virus SV40 andbacteriophage A.

A recombinant DNA experiment often follows such a format as:

- The DNA from the donor organism is extracted, enzymatically cleaved andjoined to another DNA entity to form a new, recombined DNA molecule.
- This cloning DNA construct is transffered into and maintained within a host cell.
- Those host cells that take up the DNA construct (transformed cells) are identified and selected from those that do not.

Today it is known that the main tool of genetic engineering manipulation is natural enzymes. Recombinant DNA technology would not exist without the availability of enzymes that recognize specific double-stranded DNA sequences and cleave the DNA in both strands at these sequences (restriction endonucleases)

The basis of all genetic engineering advances is availability of small plasmids, which are under seared cells control. Thank's to restriction enzymes,

the plasmid is cuted in a strictly defined place with the formation of unpaired "sticky ends". Using the same restriction enzymes scientists extract the DNA from the donor organism which carries the desired gene, such as an antibiotic resistance gene.

Then this extracted DNA is joined complementary to the multicopy R-plasmid by unpaired "sticky ends" and thus the desired gene is included to the plasmid, so the new recombinant DNA is formed. Then the other enzymes, DNA-ligase, covalently sews up breaks in the DNA chains.

The next step in a recombinant DNA experiment requires the uptake by the bacterial cell of the cloned DNA. The process of introducing purified DNA into a bacterial cell is called transformation and a cell that is capable of taking up DNA is said to be competent.

The competence can be induced by various special treatments which enhance the acquisition of DNA by the cell. Transformation is usually carried out in solution calcium chloride or by electroporation. It is the process of the effect on the cell membranes of electric current to increase their permeability.

After the transformation step, it is necessary to identify those cells that contain plasmid-cloned DNA constructs. The cells from transformation mixture are plated onto medium that contains the antibiotic. Only those cells that have antibiotic resistance gene can grow under these conditions. The nontransformed cells are sensitive to antibiotic. So the antibiotic treatment allows to leave only transformed cells which multiply and form many thousands of colonies. The cloned plasmids are isolated from the bacterial culture and are treated at the same restriction endonucleases used in the manufacturing of recombinant DNA. It allows to cut out a fragment of DNA from the vector.

Today transgenic microbes are widely used in medicine to produce proteins (insuline), hormones (somatotropin), enzymes, vitamins and other different antibiotics.

They are also need to produce vaccine, different sourdough, milk products.

More often *E. coli, Bacillus subtilis, Saccharomyces cerevisiae* are producers of recombinant proteins.

The methods of genetic engineering are actively used to develop strains-degraders able to degrade petroleum and a lot of xenobiotics.

Using the cyanobacterium Synechoccus elongatus, the researchers from Arizona genetically increased the number of RuBisCO enzyme, that captures the carbon dioxide. As a result the modified bacteria produce liquid fuel — isobutanol. How this process is carried out? The surface of bioreactor captures light and using carbon dioxide and nutrients to produce fuel in the process of photosynthesis. The separator separates waste products, selecting diesel fuel and returning the water back into the system.