Improvement of strains for industrial purposes and bioengineering of Microorganisms for industrial purposes.

Strain Improvement –
The techniques and approaches used to genetically modify strains, to increase the production of the desired product are called strain improvement or strain development.

Product yields can be increased by:-
(i) Developing a suitable medium for fermentation,
(ii) Refining the fermentation process
(iii) Improving the productivity of the strain.

Strain improvement is based on the following three approaches:

(i)Mutation
Of mutational change in the genome occurs as a natural process, though the probability is small.
Exposing a culture of a micro-organism to UV light, ionizing radiation or certain chemicals, enhances the rate of occurrence of mutations.

(ii) Recombination
Recombination is defined as any process that brings together genes from different sources.
A number of human proteins, such as insulin, human growth hormone, bone growth factor, alpha, beta and gamma interferon's, interleukin-2, tumour necrosis factor, tissue plasminogen activator, blood clotting factor VIII, epidermal growth factor, etc., are being produced through recombinant micro-organisms.

(iii) Recombinant DNA technology.
In vitro DNA technology was used to increase the number of copies of a critical pathway gene (operon), as for example the production of threonine in Escherichia coli, at rates 40 to 50 times higher than usual.

Basic steps in the production through genetic engineering could be summarized as follows :-

1-Source of Donor genetic material.
Isolation of desired gene from the DNA cell which hold the coding of the desired properties.

2- Production of hybrid DNA molecule
The isolated DNA segment which code for the desired property is integrated into a bacterial plasmids or DNA bacteriophage .
This step is done by using two kinds of enzymes :-
1- restriction endonuclease enzymes 2- ligases enzymes.

Restriction endonuclease enzymes cut the DNA at certain specific position of the nucleotide sequence so a DNA segment is produced for a specific (certain) enzyme and certain DNA.

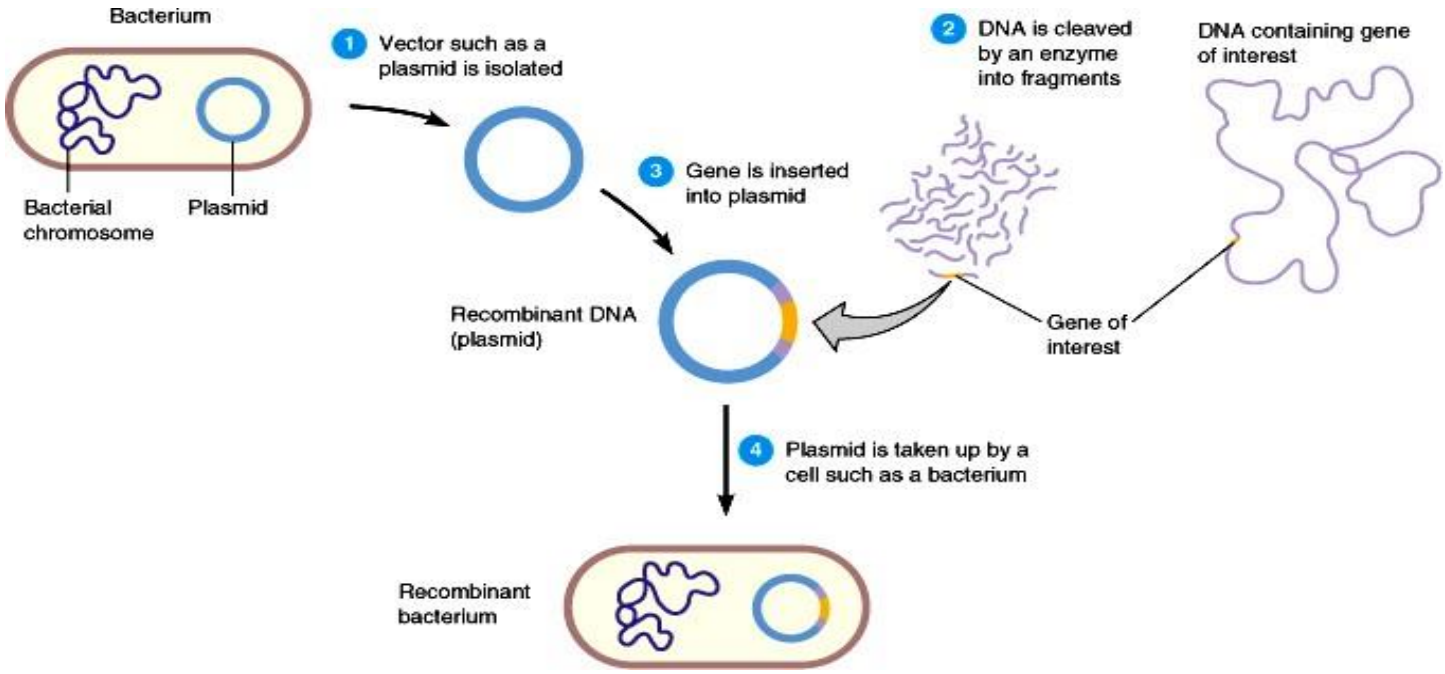
In this processes both of the donor DNA and the other DNA in which the donor DNA is integrated with, are treated with same restriction endonuclease. And these parts are linked together with the ligase enzyme .

3- Incorporation of hybrid DNA molecule into host cell.

Incorporation of plasmid hybrid DNA molecule into bacterial host cell is done through transformation while Incorporation of phage hybrid DNA molecule into bacterial host cell is done through transfection.

The common technique used in transformation is based on the treatment of host bacterial cell with calcium chloride to make the membrane permeable for the DNA.

The host bacterial cell (Recipient bacterial cell) is able to recipe the recombinant DNA on the basis of one molecule for each bacterium.



Pathways used by microorganisms.

Four pathways for the catabolism of carbohydrates up to pyruvic acid are known.
All four pathways exist in bacteria, actinomycets and fungi.
The four pathways are the:-

1. Embden-Meyerhof-Parnas,
2. Pentose Phosphate Pathways,
3. Entner Duodoroff pathway
4. Phosphoketolase.

Although these pathways are for the breakdown of glucose. Other carbohydrates easily fit into the cycles.

(1) The Embden-Meyerhof-Parnas (EMP Pathways):
The net effect of this pathway is to reduce glucose (C6) to pyruvate (C3) (Fig. 5.2). The system can operate under both aerobic and anaerobic conditions. Under aerobic conditions it usually functions with the tricarboxylic acid cycle which can oxidize pyruvate to CO_2 and H_2O. Under anaerobic conditions, pyruvate is fermented to a wide range of fermentation products, many of which are of industrial importance (Fig. 5.3).

(2) The pentose Phosphate Pathway (PP):
This is also known as the Hexose Monophosphate Pathway (HMP) or the phosphogluconate pathway. While the EMP pathway provides pyruvate, a C3 compound, as its end product, there is no end product in the PP pathway. Instead it provides a pool of triose (C3) pentose (C5), hexose (C6) and heptose (C7) phosphates. The primary purpose of the PP pathway, however, appears to be to generate energy in the form of $NADPA_2$ for biosynthetic and other purposes and pentose phosphates for nucleotide synthesis.

(3) The Entner-Duodoroff Pathway (ED):

The pathway is restricted to a few bacteria especially Pseudomonas, but it is also carried out by some fungi. It is used by some organisms in the anaerobic breakdown of glucose and by others only in gluconate metabolism.

(4) The Phosphoketolase Pathway:
In some bacteria glucose fermentation yields lactic acid, ethanol and CO_2. Pentoses are also fermented to lactic acid and acetic acid.
An example is Leuconostoc mesenteroides.

Pathways used by microorganisms
The two major pathways used by microorganisms for carbohydrate metabolism are the EMP and the PP pathways.
Microorganisms differ in respect of their use of the two pathways.
Thus Saccharomyces cerevisae under aerobic conditions uses mainly the EMP pathway; under anaerobic conditions only about 30% of glucose is catabolized by this pathway.

In Penicillium chrysogenum, however, about 66% of the glucose is utilized via the PP pathway.

The PP pathway is also used by Acetobacter, the acetic acid bacteria. Homofermentative bacteria utilize the EMP pathway for glucose breakdown.

The ED pathway is especially used by Pseudomonas.

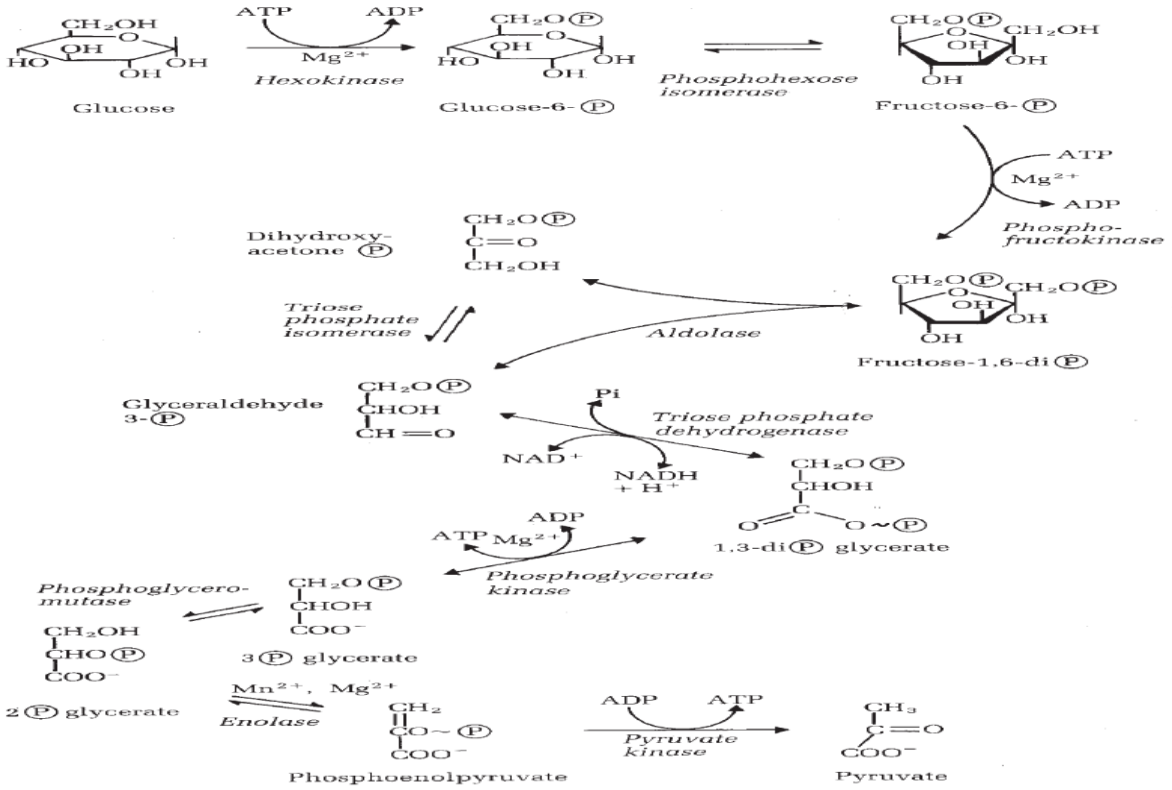
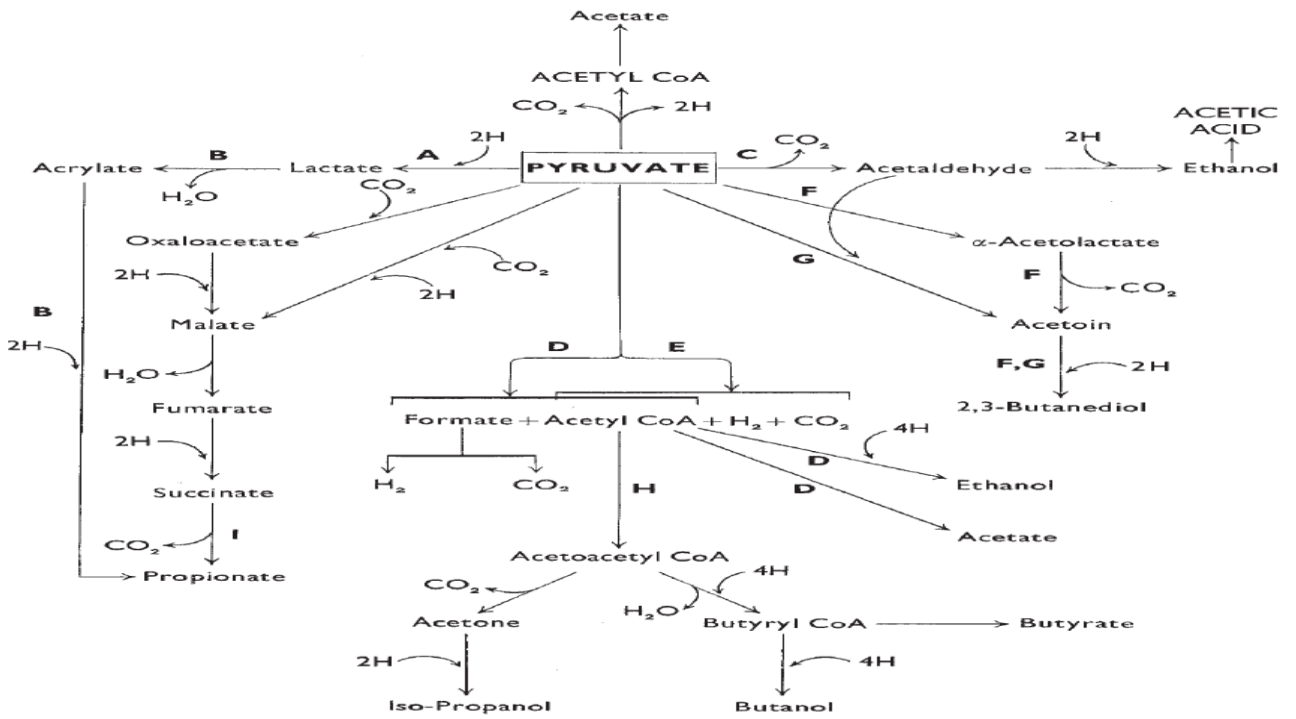


Fig. 5.2 The Embden-Meyerhof – Parnas Pathway



A	(End product, Lactate)	Lactic acid bacteria
B	(End product, Acrylate)	*Clostridium propinicum*
C	(End product, Ethanol)	Yeasts, *Acetobacter, Zymomonas*
D	(Formic acid, H_2, CO_2, Ethanol)	*Enterobacteriaceae*
E	(H_2, CO_2, Ethanol)	Clostridia
F	(Acetoin, 2-3 Butanediol)	Aerobacter
G	(Acetoin, 2-3 Butanediol)	Yeasts
H	(Acetone, Isoprpanol, Acetone)	Clostridia (butyric acid)
I	(Propioninate)	Propionic acid bacteria

Fig. 5.3 Products of the Fermentation of Pyruvate by Different Microorganisms

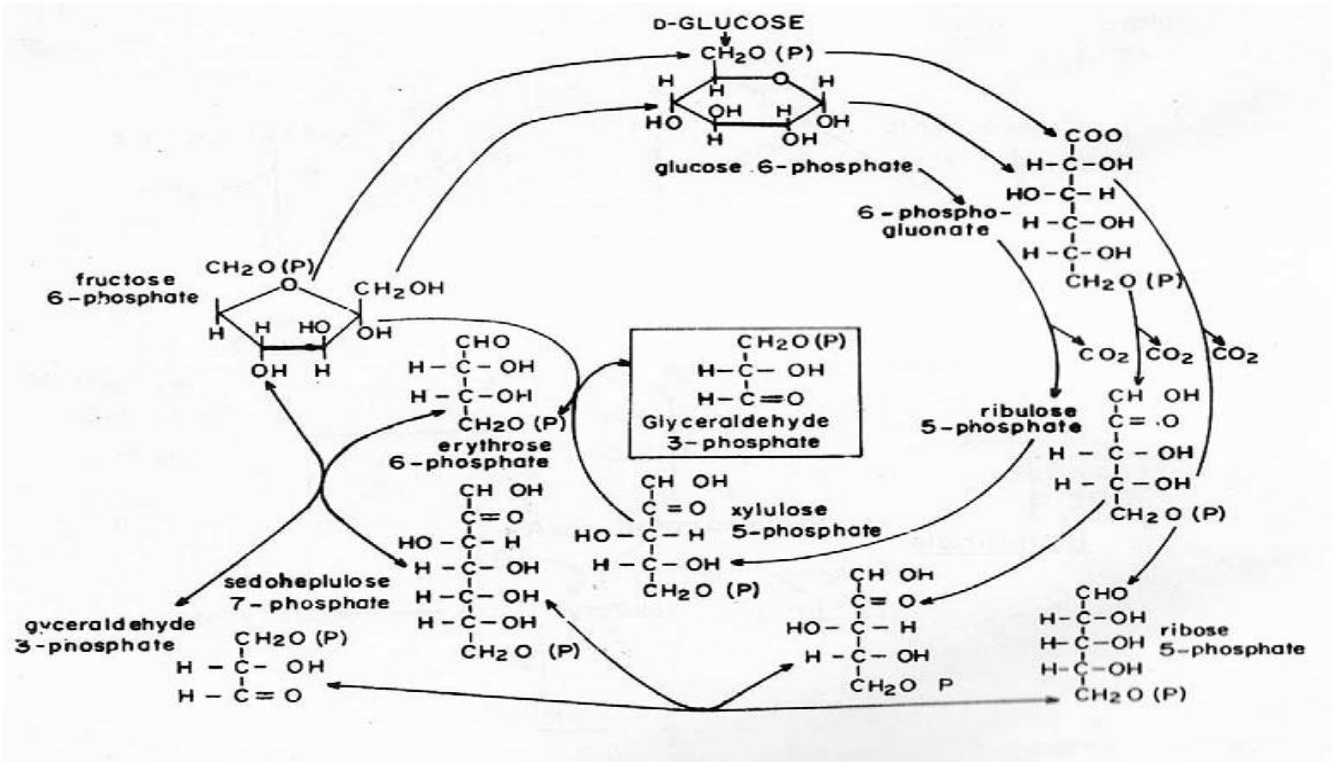


Fig. 5.4 The Pentose Phosphate Pathway

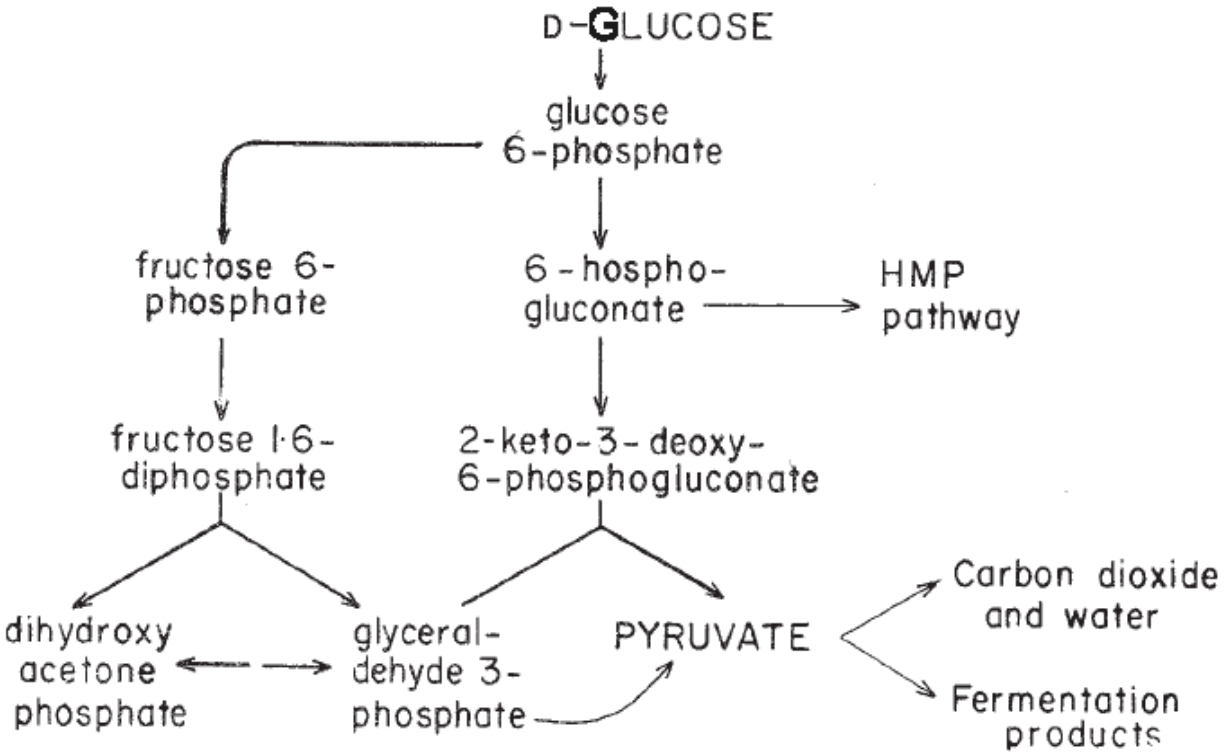


Fig. 5.5 The Enter-Doudoroff Pathway

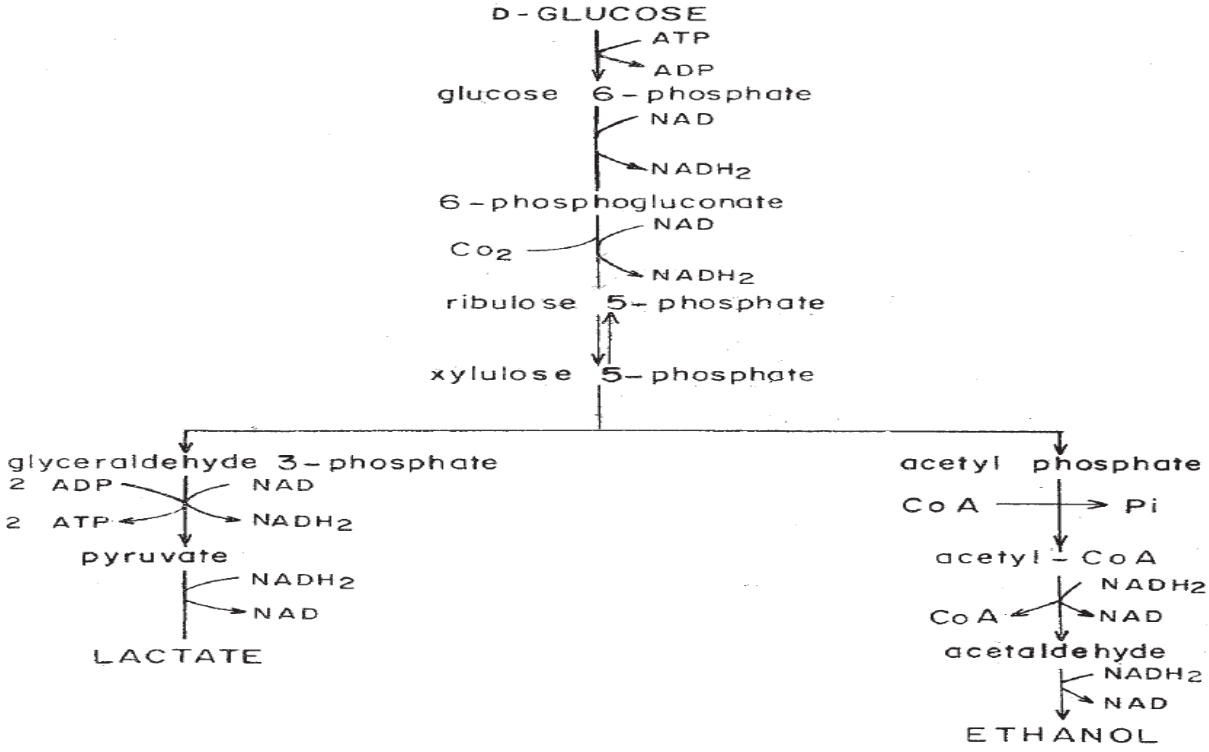


Fig. 5.6 The Phosphoketolase Pathway