METHODS OF PRESERVING MICROORGANISMS

Several methods have been devised for preserving microbial cultures. None of them can be said to apply exclusively to industrial microorganisms. Furthermore, no one method is suitable for preserving all organisms. The method most suited to any particular organism must therefore be determined by experimentation unless the information is already available. Methods employed in the preservation of microorganisms all involve some limitation on the rate of metabolism of the organism. A low rate of spontaneous mutation exists during the growth of microorganisms, about once in every 109 division.
Lowering the metabolic rate of the organism will further reduce the chances of occurrence of mutations. Preservation methods will be discussed under the following three headings, although it should be understood that in practice the methods combine one or more of the following three principles. The principles involved in preserving microorganisms are:
(a) reduction in the temperature of growth of the organism;
(b) dehydration or desiccation of the medium of growth;
(c) limitation of nutrients available to the organism.
All three principles lead to a reduction in the organisms metabolism.

(a)Microbial Preservation Methods Based on the Reduction of the Temperature of Growth
1. Preservation on agar with ordinary refrigeration (4 – 10°C)
2.Preservation in Deep Freezers at about -20°C, or between -60°C and -80°C
3.Storage in low temperature liquid or vapor phase nitrogen (-156°C to -196°C)

(b)Microbial Preservation Methods Based on Dehydration
1.Drying on sterile silica gel
Many organisms including actinomycetes and fungi are dried by this method. Screw-cap tubes half-filled silica gel are sterilized in an oven. On cooling a skim-milk suspension of spores and the cells of the fungus or actinomycetes is placed over the silica gel and cooled. They are dried at 25°C, cooled and stored in closed containers containing desiccants.
2.Preservation in sterile dry soil
3.Preservation on sterile filter paper
4. Freeze-drying (drying with freezing), lyophilization

(c)Microbial Preservation Methods Based on the Reduction of Nutrients

Storage in distilled water

Many organisms die in distilled water because of water absorption by osmosis. However some have been known to survive for long periods in sterile distilled water. Usually such storage is accompanied by refrigeration; some organisms are however, harmed by refrigeration. Among organisms which have been stored for long periods with this method are *Pseudomonas solanaceanum, Saccharomyces cerevisiae*, and *Sarcina lutea.*

The attractiveness of this method is its simplicity and inexpensiveness; since so few organisms seem to be storable in this manner, it should not, for fear of losing the organism, be adopted as the sole method for storing a newly acquired or isolated organism until it has been shown to be suitable.

The **Embden–Meyerhof–Parnas (EMP) pathway**

which is the most common route and is found in all major groups of organisms, including filamentous fungi, yeasts and many bacteria (Fig. 3.1). This pathway
can operate under anaerobic or aerobic conditions and consists of a sequence of 10 enzyme-catalysed reactions located within the cytoplasmic matrix.

The three key regulatory enzymes of the pathway (hexokinase, hosphofructokinase
and pyruvate kinase) act irreversibly All other steps are freely reversible, which
is important for the biosynthetic role of the pathway during glucose synthesis .

The irreversible steps have bypasses to enable the pathway to operate in this anabolic mode. for each glucose molecule oxidized to two pyruvate molecules, the net gain is only two ATP, due to its consumption in the earlier reactions.

Glucose (C6) + 2ADP + 2Pi + 2NAD+→ 2 pyruvate (C3) + 2ATP + 2NADH +2H+

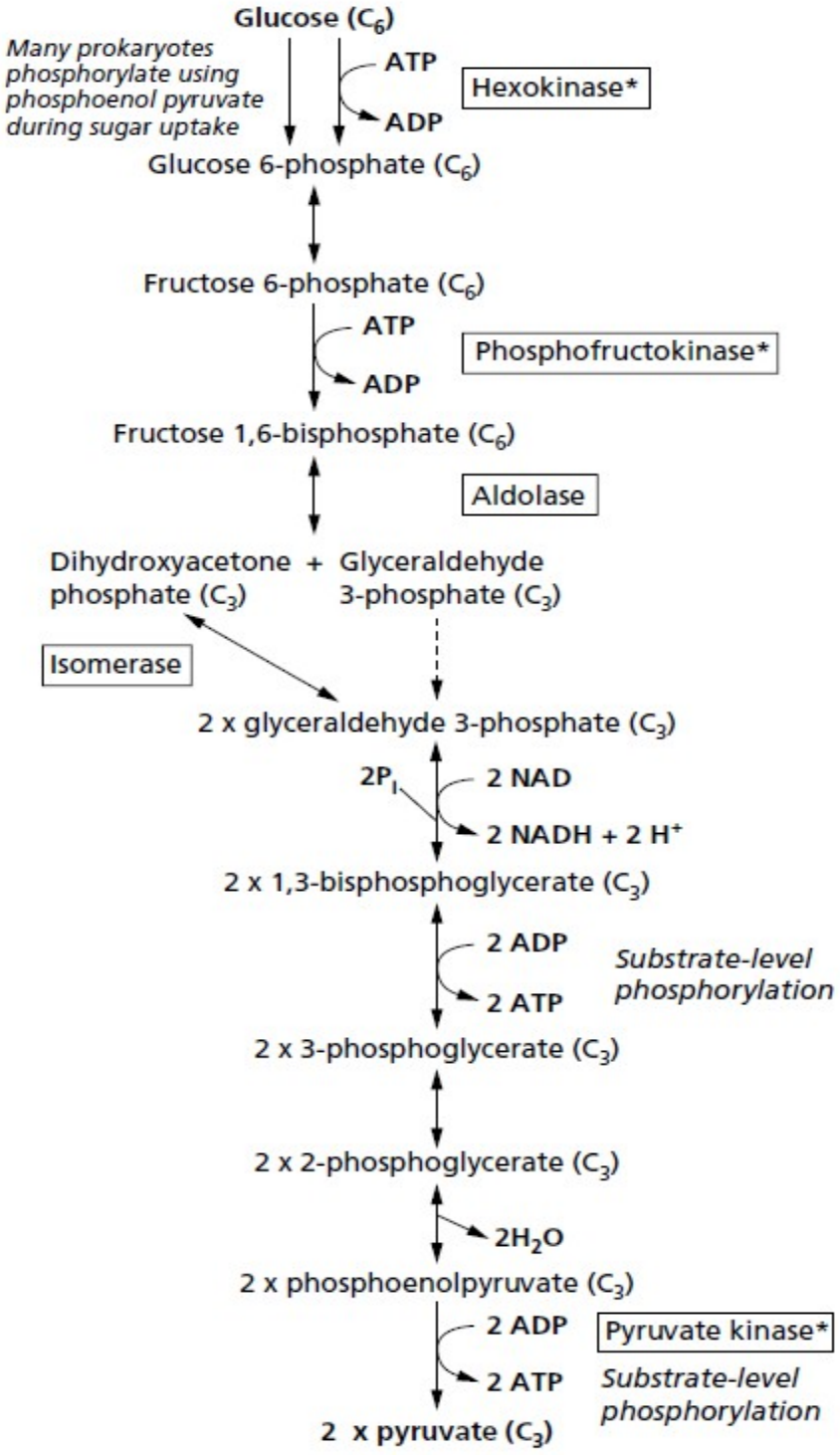

Fig. 3.1 Embden–Meyerhof–Parnas pathway (*irreversible steps).