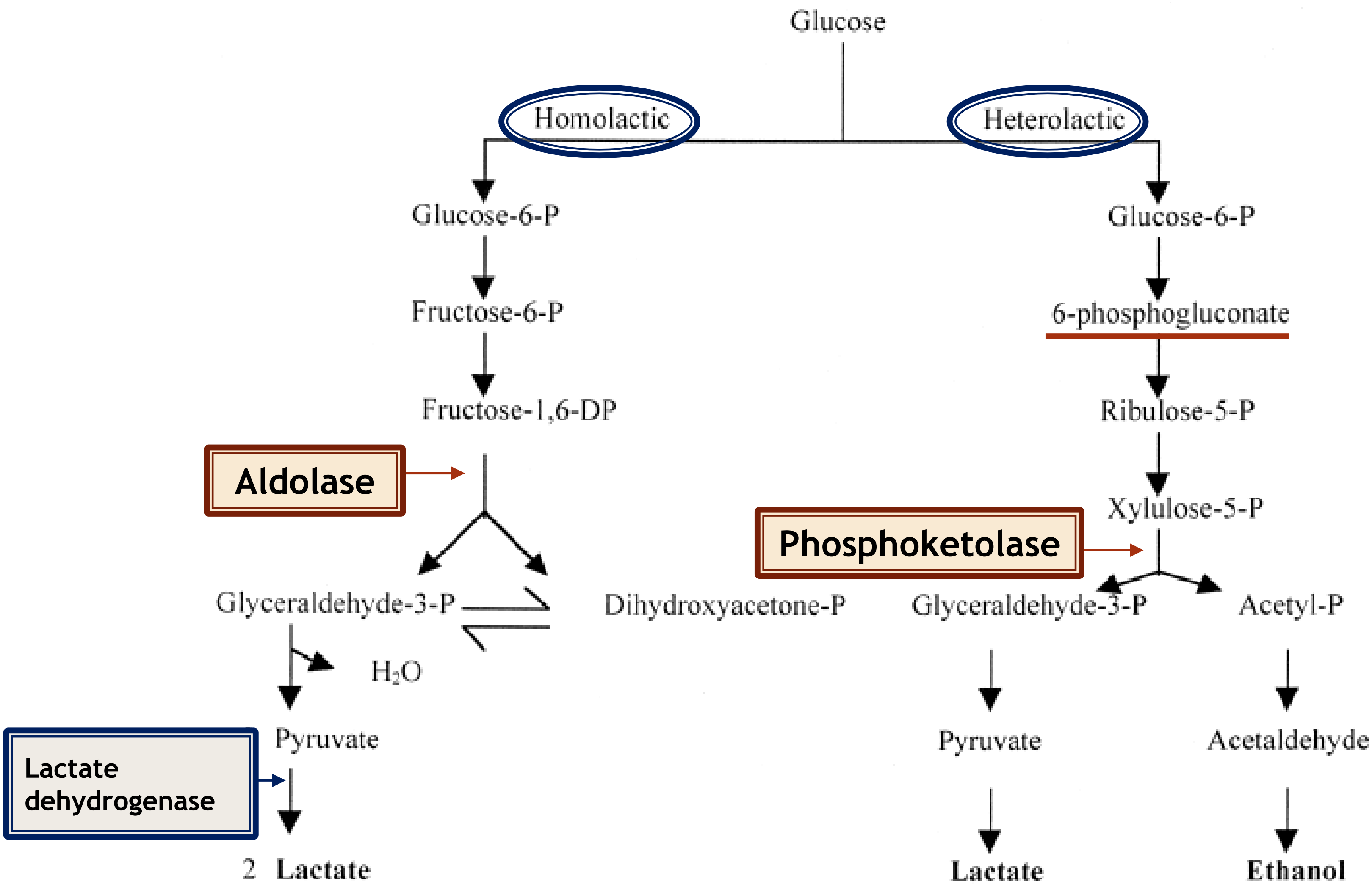
FDE 328
INDUSTRIAL MICROBIOLOGY

Biochemistry of Lactic Acid Fermentation

- Two main sugar fermentation pathways can be distinguished among lactic acid bacteria.

- **Homolactic fermentation:** Glycolysis (Embden-Meyerhof-Parnas pathway) results almost exclusively in lactic acid as the end product under standard conditions, and the metabolism is referred to as homolactic fermentation.

- **Heterolactic fermentation:** The 6 phosphogluconate/phosphoketolase pathway results in significant amounts of other end products such as ethanol, acetate, and CO_2 in addition to lactic acid, and the metabolism is referred to as heterolactic fermentation.



Fermentation of glucose in homofermentatives and heterofermentatives

Biochemistry of Lactic Acid Fermentation

- There are two major pathways for hexose (e.g., glucose) fermentation within LAB.

- Major fermentation pathways of glucose:

1. Homolactic fermentation (Glycolysis, Embden-Meyerhof-Parnas pathway)
2. Heterolactic fermentation (6-phosphogluconate/phosphoketolase pathway)

1. Homolactic fermentation (Glycolysis, Embden-Meyerhof-Parnas pathway):

- The main difference between the metabolism of hexoses by homofermentatives and heterofermentatives is the presence of the **aldolase**, which is a key enzyme of glycolysis.

- Since the homofermentative LAB contain **aldolase**, they produce two molecules of lactic acid from glucose by the glycolytic pathway.

- Homofermentative LAB ferment 90% of glucose and produce lactic acid as the major end product of glucose fermentation.

- Homofermentative LAB include *Lactococcus*, *Streptococcus*, *Pediococcus*, *Enterococcus*, and some species of *Lactobacillus* such as *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. delbrueckii* subsp. *lactis*, *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus*, *Lb. helveticus*, and *Lb. salivarius*.

- They ferment glucose via the glycolysis to pyruvate, which is then converted into lactic acid.

- Homofermentative LAB generate two molecules of lactic acid per molecule of glucose and produce approximately twice as much energy per molecule of glucose as heterofermentatives.

1. Homolactic fermentation (Glycolysis, Embden-Meyerhof-Parnas pathway):

- **Glycolysis (Embden-Meyerhof-Parnas pathway)** is characterized by the formation of fructose-1,6-diphosphate (FDP), which is split by a **FDP aldolase** into dihydroxyacetonephosphate (DHAP) and glyceraldehyde-3-phosphate (GAP).
- GAP (and DHAP via GAP) is then converted to pyruvate.
- Under normal conditions, i.e., excess sugar and limited access to oxygen, pyruvate is reduced to lactic acid by a NAD^+-dependent lactate dehydrogenase (nLDH), thereby reoxidizing the NADH formed during the earlier glycolytic steps.
- A redox balance is thus obtained, lactic acid is virtually the only end product, and the metabolism is referred to as a homolactic fermentation.
- Homofermenters use the Embden–Meyerhof–Parnas pathway to generate two moles of lactate per mole of glucose and derive approximately twice as much energy per mole of glucose as heterofermenters.

2. Heterolactic fermentation (6-phosphogluconate/phosphoketolase pathway):

- The other main fermentation pathway has had several designations, such as the pentose phosphate pathway, the pentose phosphoketolase pathway, the hexose monophosphate pathway and the 6-phosphogluconate pathway.
- (We will refer to it as the 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway, thereby recognizing a key step in the metabolic sequence (the phosphoketolase split) and at the same time distinguishing it from the bifidum pathway, which also involves phosphoketolase but does not have 6-phosphogluconate as an intermediate.)

- It is characterized by initial dehydrogenation steps with the formation of **6-phosphogluconate**, followed by decarboxylation.

- The remaining pentose-5-phosphate is split by **phosphoketolase** into GAP and acetyl phosphate.

- GAP is metabolized in the same way as for the glycolytic pathway, resulting in lactic acid formation.

- When no additional electron acceptor is available, acetyl phosphate is reduced to ethanol via acetyl CoA and acetaldehyde. Since this metabolism leads to significant amounts of other end products (CO_2, ethanol) in addition to lactic acid, it is referred to as a heterolactic fermentation.

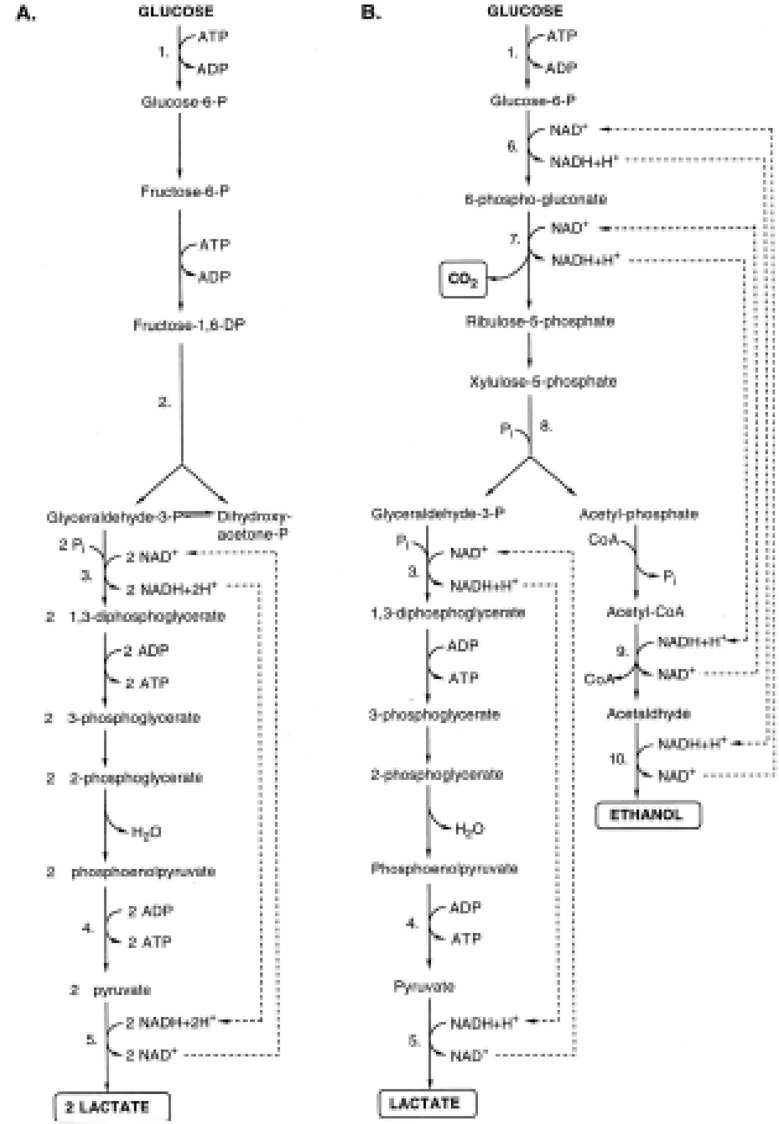


Figure 2 Major fermentation pathways of glucose: (A) homolactic fermentation (glycolysis, Embden-Meyerhof-Parnas pathway); (B) heterolactic fermentation (6-phosphogluconate/phosphoketolase pathway). Selected enzymes are numbered: 1. Glucokinase; 2. fructose-1,6-diphosphate aldolase; 3. glyceradehyde-3-phosphate dehydrogenase; 4. pyruvate kinase; 5. lactate dehydrogenase; 6. glucose-6-phosphate dehydrogenase; 7. 6-phosphogluconate dehydrogenase; 8. phosphoketolase; 9. acetaldehyde dehydrogenase; 10. alcohol dehydrogenase.

2. Heterolactic fermentation (6-phosphogluconate/phosphoketolase pathway):

- Heterofermentative LAB such as *Leuconostoc*, *Oenococcus*, and some *Lactobacillus* such as *Lb. brevis*, *Lb. buchneri*, *Lb. fermentum*, and *Lb. reuteri* use pentose phosphate pathway and produce especially CO_2 and ethanol along with lactic acid.

- Since they lack aldolase, they cannot break down fructose 1,6-disphosphate into triose phosphate.

- But they oxidize glucose 6-phosphate to 6-phosphogluconate.

- Then, 6-phosphogluconate is decarboxylated to pentose phosphate, and CO_2 is released.

- The produced pentose phosphate is converted to triose phosphate and acetyl phosphate by **the key enzyme phosphoketolase**.

- Acetyl phosphate is used as an electron acceptor and is reduced by NADH to **ethanol**.

- Heterofermentative LAB produce more flavor and aroma compounds such as acetaldehyde and diacetyl when they are compared to homofermentative LAB.

- In general, the term “homofermentative LAB” refers to those in the group that use the glycolytic pathway for glucose fermentation, whereas “heterofermentative LAB” are those that use the 6-PG/PK pathway.

- However, it should be noted that some LAB regarded as homofermentative use the 6-PG/PK pathway when metabolizing certain substrates.

- Some *Lactobacilli* such as *Lb. casei*, *Lb. curvatus*, *Lb. plantarum*, and *Lb. sakei* behave as **facultatively heterofermentative** depending on the presence of pentose sugars and gluconate due to having both aldolase and phosphoketolase enzymes.

- For example; *Lactobacillus plantarum* uses hexoses homofermentatively but pentoses heterofermentatively.

- In theory, homolactic fermentation of glucose results in 2 mol of lactic acid and a net gain of 2 ATP per mol glucose consumed.

- Heterolactic fermentation of glucose through the 6-PG/PK pathway gives 1 mol each of lactic acid, ethanol, and CO_2 and 1 mol ATP/mol glucose.