BIOCHEMISTRY OF LACTIC ACID BACTERIA

Lactic acid bacteria are present in all grape musts and wines. The environmental conditions during the winemaking process determine their ability to multiply. When they develop they metabolize numerous substances and so can play an important role in the transformation of grape must into wine.
The addition of 50 mg.L^{-1} of sodium hydrogen sulfite (sulfur dioxide) inhibits the development of microorganisms. It has a greater activity on bacteria than yeasts.

Metabolism of Sugars

Lactic acid bacteria are chemotrophic, they find the energy required for their entire metabolism from the oxidation of chemical compounds. The oxidation of sugars constitutes the principle energy producing pathway.
Lactic acid bacteria of the genera *Lactobacillus, Leuconostoc* and *Pediococcus*, the important bacteria to winemaking, assimilate sugars by either a homofermentative or heterofermentative pathway.

Homofermentative Metabolism of Hexoses

Homofermentative bacteria transform nearly all of the sugars they use, especially glucose into lactic acid. The homofermentative pathway includes a first phase of all the reactions of glycolysis that lead from hexose to pyruvate. The terminal electron acceptor in this pathway is pyruvate which is reduced to lactic acid. See Figure 1. In fermentation, pyruvate is decarboxylated to ethanal, which is the terminal electron acceptor, being reduced to ethanol.

Heterofermentative Metabolism of Hexoses

Bacteria using the heterofermentative pathway, which includes *Leuconostoc* (the most important bacterium in enology) use the pentose phosphate pathway. In this pathway, NADPH is generated as glucose is oxidized to ribose 5-phosphate. This five-carbon sugar and its derivatives are components of important biomolecules such as ATP, CoA, NAD^+, FAD, RNA and DNA. NADPH is the currency of readily available reducing power in cells (NADH is used in the respiratory chain). This pathway occurs in the cytosol.

After being transported into the cell, a glucokinase phosphorylates the glucose into glucose 6-P (glucose 6-phosphate). Its destination is completely different from the glucose 6-P in the homofermentative pathway. Two oxidation reactions occur: the first leads to gluconate 6-P and the second, accompanied by a decarboxylation, forms ribulose 5-P. See Figure 2. In each of these reactions a molecule of $NADP^+$ is reduced. Ribulose 5-P can then be epimerized either to ribose 5-P or to xylulose 5-P.

Xylulose 5-P is then cleaved into acetyl-phosphate and glyceraldehydes 3-phosphate. See Figure 3. The glyceraldehyde 3-phosphate is metabolized into lactic acid by following the same pathway as in the homofermentative pathway. The acetyl-phosphate has two possible destinations, depending on environmental conditions.

This molecule can be successively reduced into ethanal and ethanol, in which case the molecules of the coenzyme NADPH formed during the two oxidation reactions of glucose at the beginning of the heterofermentative pathway, are reoxidized. This reoxidation is essential for regenerating the coenzymes necessary for this pathway. The final products are then lactate and ethanol.

Or the acetyl-phosphate can produce acetate (acetic acid) through the enzyme acetate kinase. This reaction also yields a molecule of ATP. The final products of this pathway are then lactate and acetate.

Bacteria of the genus *Leuconostoc* preferentially produce lactate and ethanol in a slightly aerated environment and lactate and acetate in an aerated environment.

Homofermentative Metabolism of Fructose-1,6-biphosphate
Formation of Lactic Acid

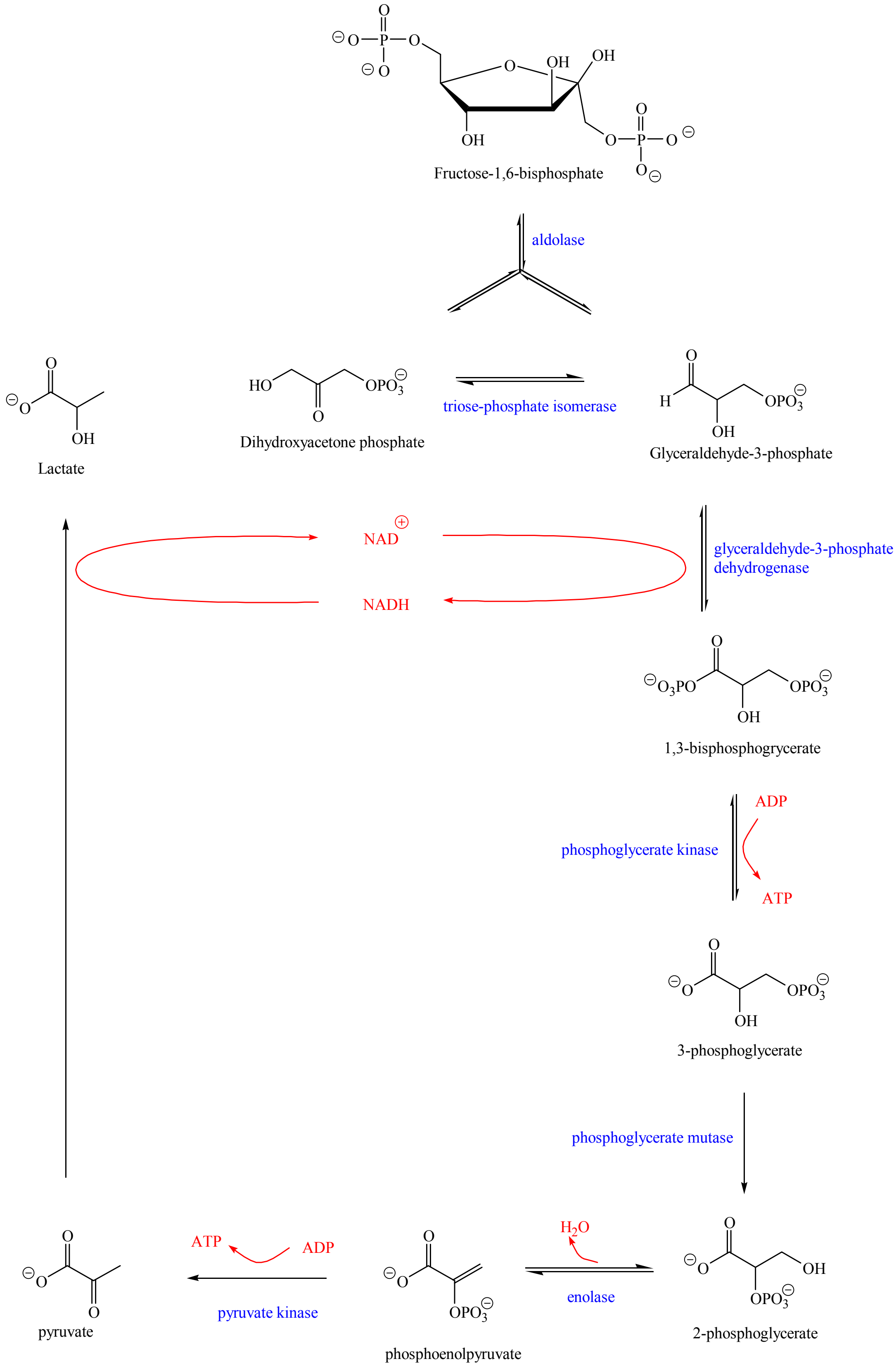


FIGURE 1

Heterofermentative Metabolism of Glucose
Pentose Phosphate Pathway
Glucose to Xylulose

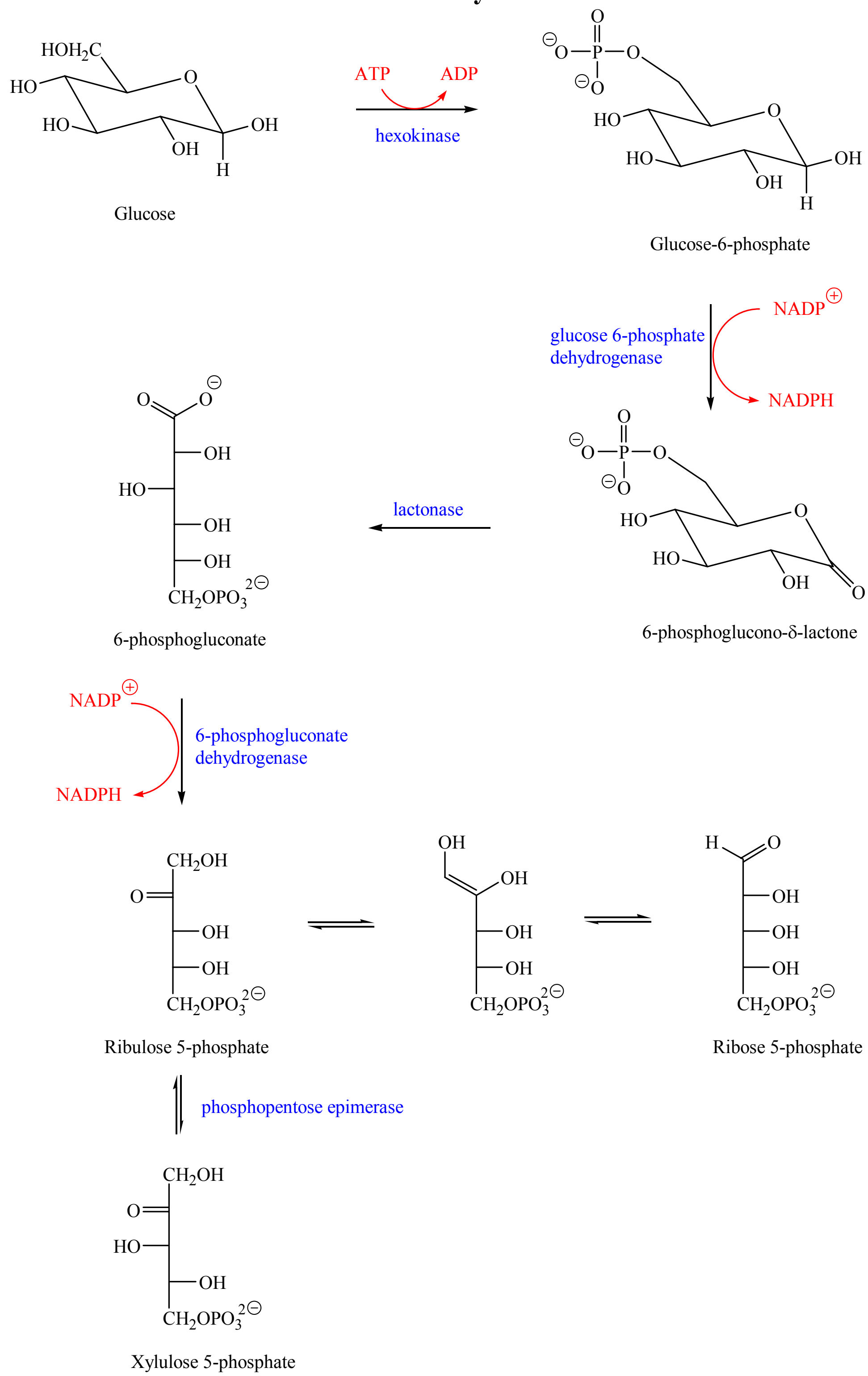


FIGURE 2

Heterofermentative Metabolism of Glucose
Pentose Phosphate Pathway
Xylulose to Lactic acid and Ethanol

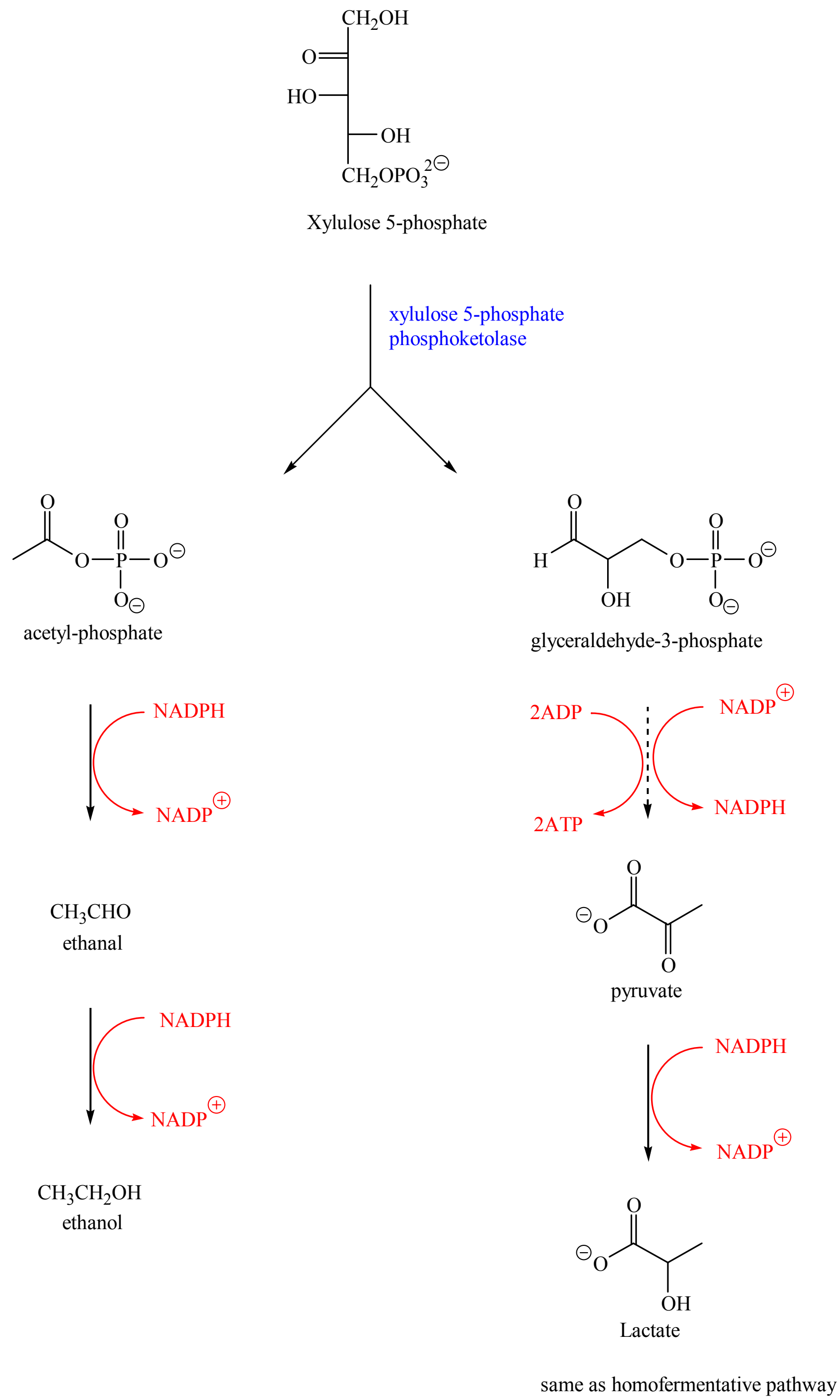


FIGURE 3

Heterofermentative Metabolism of Glucose
Pentose Phosphate Pathway
Xylulose to Lactic acid and Acetate

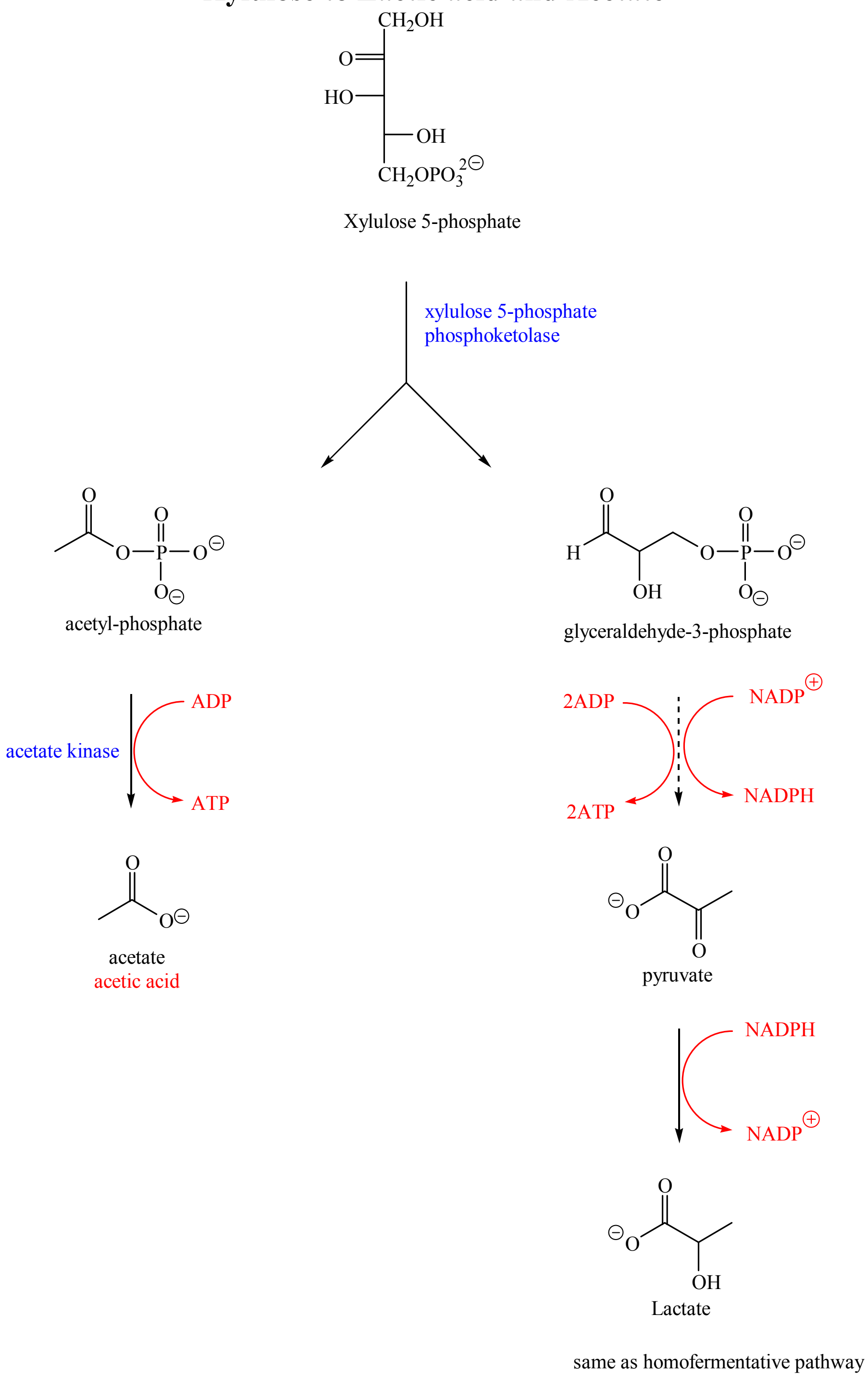
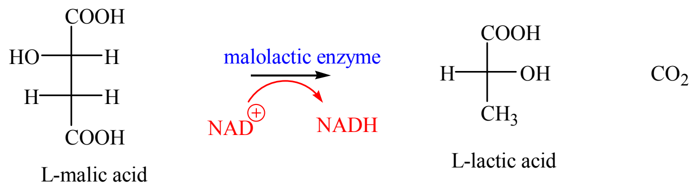


FIGURE 4

Metabolism of Malic Acid

The majority of bacterial species preponderant in wine after alcoholic fermentation break down malic acid.
The most important bacterium in enology is the heterofermentative bacteria, *Leuconostoc oenos*. This bacterium forms D-lactic acid from glucose and L-lactic acid from L-malic acid.



The major distinction between wine and vinegar is the amount of acetic acid. The amount of acetic acid is estimated from the volatile acidity or VA (the wine is steam distilled and the distillate titrated with sodium hydroxide using phenolphalein). A small amount of acetic acid is produced by yeasts, in particular *Saccharomyces cerevisiae* to the extent of 100-300 mg.L^{-1} (see Figure 5, Yeast Biochemistry, Sugars).

Bacteria degrade must and wine sugars with a different affinity depending on the species. In general, bacterial development occurs after yeast development. Since the yeast has consumed the sugars, the lactic acid formed from them is small compared to the amount produced from malic acid.

L. oenos can and does produce acetic acid from glucose (see figures 3 & 4), but since the lactic acid formed from them is low, so is the acetic acid.

An increase in the VA (acetic acid) of a wine coupled with an abnormal amount of lactic acid (>300 mg.L^{-1}) indicates lactic disease and suggests the *L. oenos* fermented a significant quantity of sugars.

The next topic deals with acetic acid bacteria, the winemaker’s nightmare.