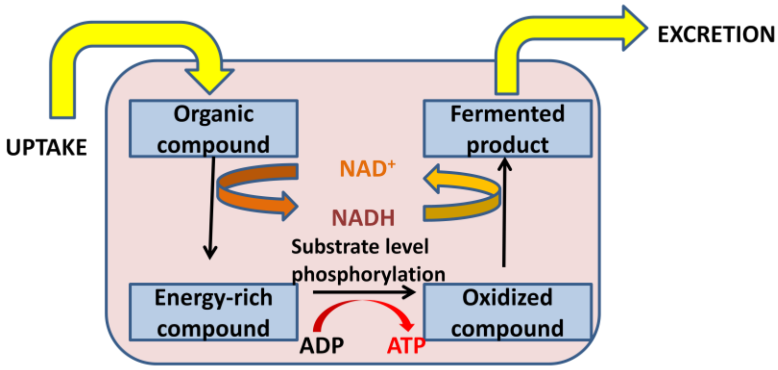
FERMENTATION

- Differs from respiration in redox considerations and mechanism of ATP synthesis
- No exogenous electron acceptors
- In anoxic habitats, if electron acceptors like sulfate, nitrate, ferric iron are in inadequate quantities, then organic compounds are catabolized by fermentation
- ATP is synthesized by substrate level phosphorylation

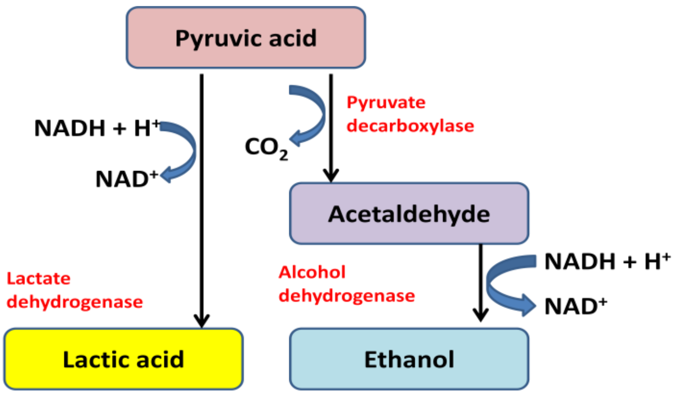


Energy-rich compounds involved in substrate-level phosphorylation

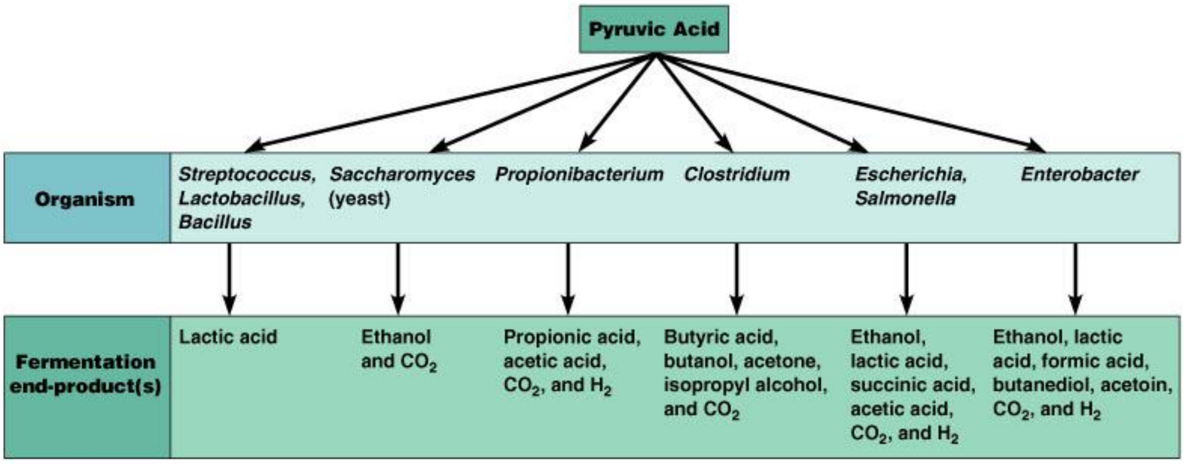
Compound	Free energy of hydrolysis
Acetyl-CoA	-35.7
Propionyl-CoA	-35.6
Butyryl-CoA	-35.6
Succinyl-CoA	-35.1
Acetyl phosphate	-44.8
1,3-bisphosphoglycerate	-51.9
Phosphoenolpyruvate	-51.6
Adenosine phosphosulfate	-88.0

Energy of hydrolysis of ATP (ATP $\longrightarrow$ ADP + Pi = -31.8 kJ/mol)

Anaerobic metabolism of glucose



- Pyruvate decarboxylase is a key enzyme of ethanol fermentation.in *Zymomonas* (bacteria) and yeast
- Ethanol is produced through different reactions in saccharolytic clostridia, heterofermentative lactic acid bacteria and enteric bacteria.
- These bacteria oxidize pyruvate to acetyl-CoA before reducing it to ethanol. They do not possess pyruvate decarboxylase



Fermentative diversity of bacteria

Lactic acid fermentation by Lactic acid bacteria (LAB)

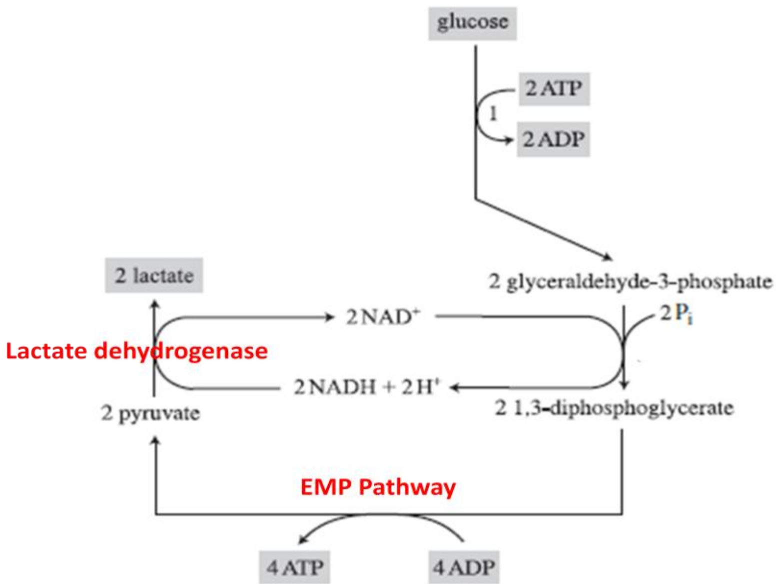
- Gram positive rods and cocci
- Grow anaerobically, but are aerotolerant
- Produce lactate as a major fermentation product
- Some LAB produce only lactate from sugars while others produce acetate and ethanol in addition to lactate. The former are referred to as homofermentative and the latter heterofermentative LAB.

Homofermentative LAB ferment sugars through the EMP pathway
Heterofermentative LAB ferment sugars through the phosphoketolase pathway

Homolactate fermentation

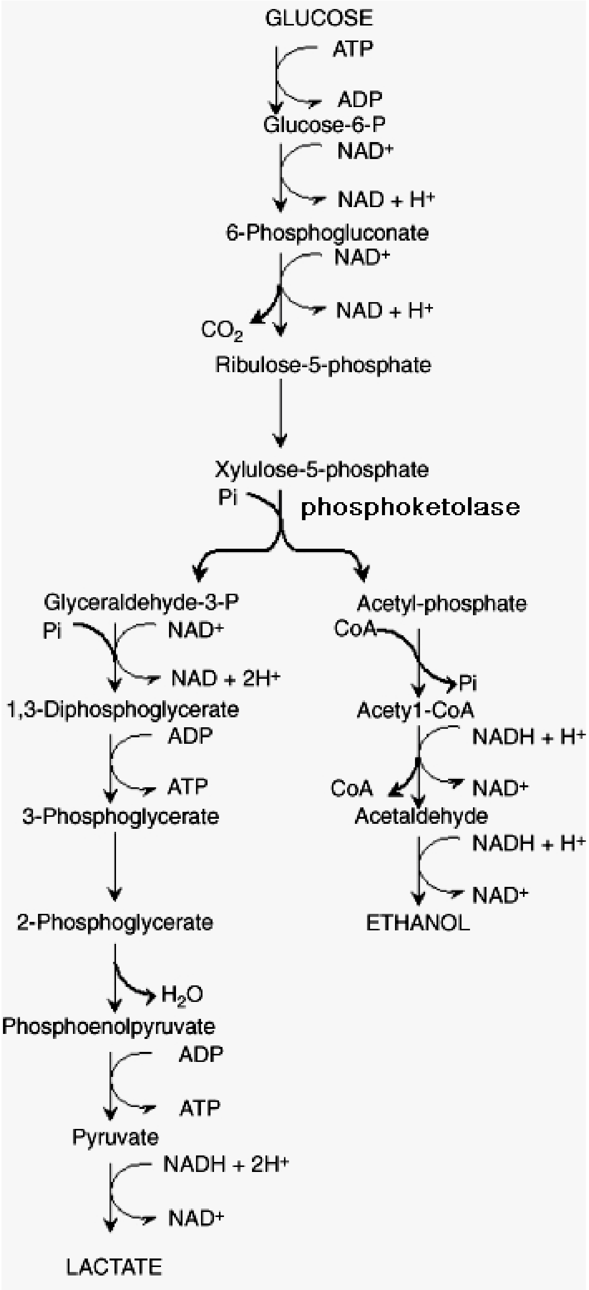
- Homofermentative LAB include most species of *Lactobacillus*, *Sporolactobacillus*, *Pediococcus*, *Enterococcus* and *Lactococcus*.
- They use hexoses through the EMP pathway to generate ATP.
- Lactate dehydrogenase reoxidizes the NADH reduced during the EMP pathway using pyruvate as the electron acceptor
- As fermentation proceeds, lactate accumulates lowering the intracellular pH.
- Lactate dehydrogenase is active in acidic conditions producing lactate as the major product.

HOMOFERMENTATIVE PATHWAY OR HOMOLACTIC FERMENTATION



Heterolactate fermentation (Phosphoketolase pathway or Heterofermentative pathway)

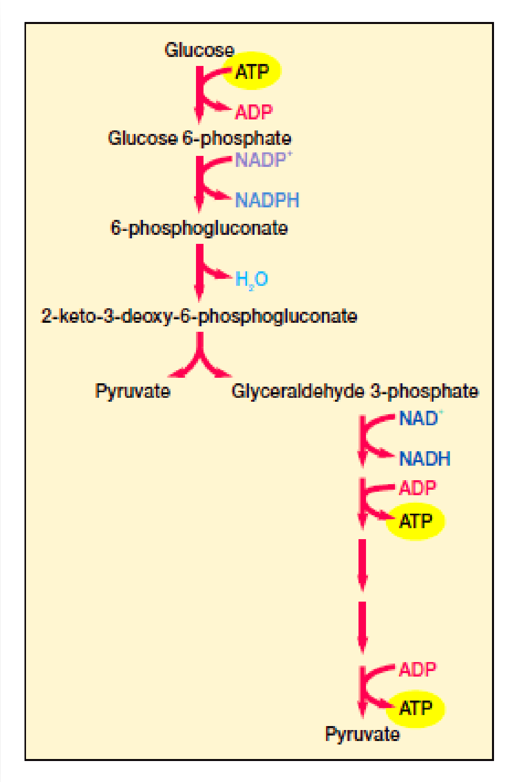
- Species of *Leuconostoc*, *lactobacillus* (few species) and *Bifidobacterium* produce ethanol and acetate in addition to lactate.
- Heterofermentative LAB oxidize glucose-6-phosphate to ribulose-5-phosphate.
- Epimerase converts ribulose-5-phosphate to xylulose-5-phosphate, before cleavage to glyceraldehyde-3-phosphate and acetyl-phosphate by the action of phosphoketolase.
- Glyceraldehyde-3-phosphate is metabolized to lactate as in the homolactate fermentation generating ATP.
- Acetyl-phosphate is reduced to ethanol acting as the electron acceptor to oxidize the NADH reduced in the glucose-6-phosphate oxidation process.
- One ATP per hexose is available from this fermentation.



End products are ethanol, lactic acid and CO_2

- Do not contain aldolase enzyme, hence cannot break down fructose-bisphosphate to triose phosphate
- Phosphoketolase converts pentose phosphate to acetyl phosphate and G3P
- Triose phosphate produces lactic acid
- Acetyl phosphate is reduced to acetaldehyde and further to ethanol.

Entner–Doudoroff (ED) pathway



- First identified in *Pseudomonas saccharophila* by Entner and Doudoroff
- Main glycolytic pathway in prokaryotes that do not possess enzymes of the EMP pathway.
- ED pathway functions as the main glycolytic pathway in other Gram negative bacteria such as *Zymomonas* and *Azotobacter*, *Escherichia coli*, and Gram positive bacteria such as *Arthrobacter* and *Cellulomonas* species
- Glucose-6-phosphate is converted to 6-phosphogluconate via phosphogluconolactone, as in the HMP pathway.
- 6-phosphogluconate is dehydrated to 2-keto-3- deoxy-6-phosphogluconate (KDPG) by 6-phosphogluconate dehydratase.
- KDPG aldolase splits its substrate into pyruvate and glyceraldehyde-3-phosphate (G3P).
- G3P is oxidized to pyruvate as in the EMP pathway.
- **The key enzymes of this pathway are 6-phosphogluconate dehydratase and KDPG aldolase.**
- Net gain of one ATP per glucose oxidized in this pathway.

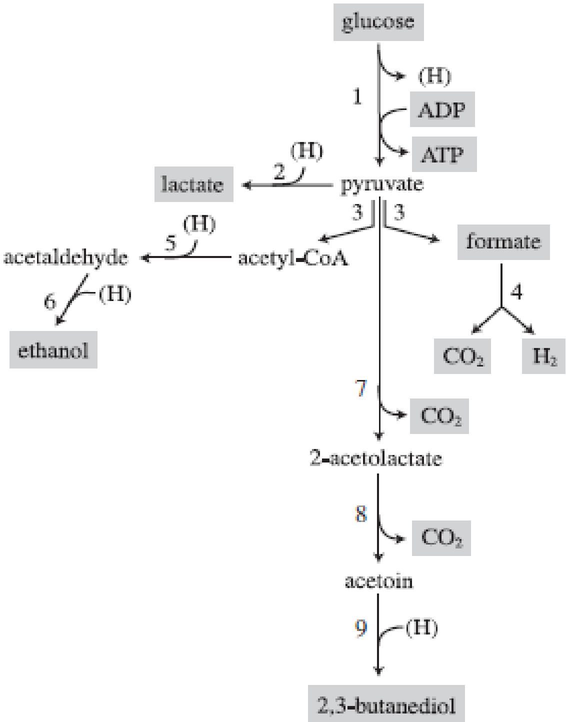
Butanediol and mixed acid fermentations

Enteric bacteria

- Gram negative straight rods, peritrichous flagella or nonmotile
- Nonsporulating
- Facultative aerobes
- Catalase positive, oxidase negative
- Ferment glucose
- Reduce nitrate to nitrite

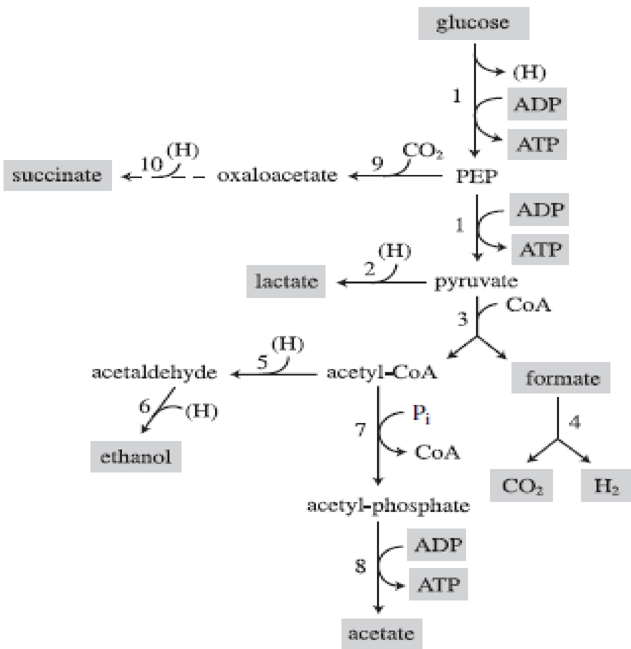
Include mixed acid fermenters: *Escherichia*, *Salmonella*, *Shigella*, *Citrobacter*, *Proteus*, *Yersinia*
Include Butanediol producers: *Enterobacter*, *Klebsiella*, *Serratia*

Butanediol fermentation



Facultative anaerobes such as *Erwinia*, *Klebsiella* and *Serratia* produce 2,3-butanediol in addition to lactate and ethanol.
1-EMP pathway, 2-lactatedehydrogenase, 3- pyruvate:formate lyase, 4-formate:hydrogen lyase, 5-acetaldehyde dehydrogenase, 6- alcohol dehydrogenase, 7-2-acetolactate synthase, 8- 2-acetolactate decarboxylase, 9-2,3-butanediol dehydrogenase.

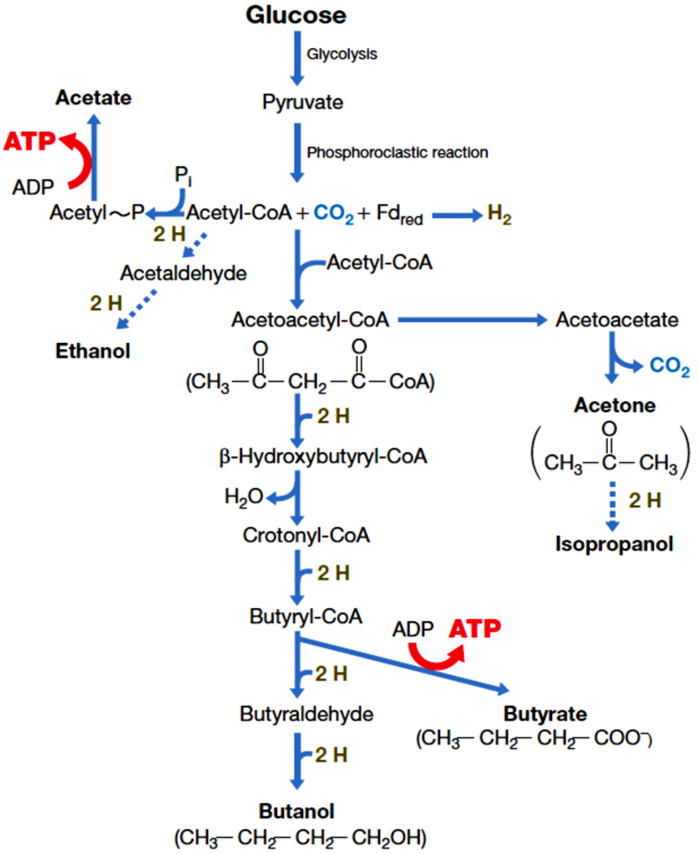
Mixed acid fermentation
Facultative anaerobes belonging to the genera *Escherichia, Salmonella, Shigella, Enterobacter* and others ferment sugars to lactate, acetate, formate, succinate and ethanol in the absence of electron acceptors.



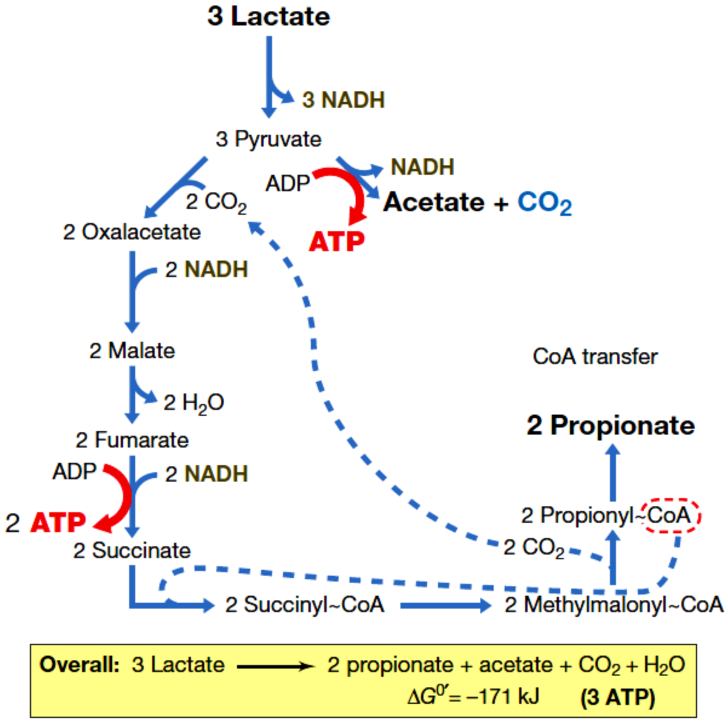
1-EMP pathway, 2-lactate dehydrogenase, 3-pyruvate:formate lyase, 4-formate:hydrogen lyase, 5-acetaldehyde dehydrogenase, 6-alcohol dehydrogenase, 7-phosphotransacetylase. 8-acetate kinase, 9-phosphoenolpyruvate (PEP) carboxylase, 10-enzymes of the TCA cycle.

Butyric acid and butanol/acetone fermentation by *clostridium acetobutylicum*

- Different Clostridia ferment sugars, amino acids, purines and pyrimidines, etc.
- Production of acetate and butyrate lead to ATP synthesis by substrate level phosphorylation
- Production of butyrate (neutral pH)
- Production of acetone and butanol (acidic pH)



Propionic acid fermentation by *Propionibacterium*



- The reaction in which fumarate is reduced to succinate is linked to ETS and formation of PMF that yields ATP by oxidative phosphorylation
- Thus both substrate-level and oxidative phosphorylation occur to form ATP

Propionic acid bacteria (*Propionibacterium* and *Propionigenium*)

- Gram positive, anaerobes
- *Propionibacterium* ferment lactic acid, carbohydrates, polyhydroxyalcohols, producing primarily propionic acid, acetic acid and CO_2
- ***Propionigenium modestum*** (phylogenetically unrelated to Propionibacterium) ferments succinate to propionic acid (Unusual fermentation)
    - inhabits marine and fresh water anoxic sediments
    - No substrate-level phosphorylation
    - Sodium-translocating ATPase produces ATP

Fermentation by *Clostridium* spp.

Characteristics of some Clostridia

- Obligate anaerobes
- Endospore formers
- Produce energy by substrate-level phosphorylation
- Ferment diversity of compounds

Cellulose ➔ acetate, lactate, succinate, ethanol, CO_2, H_2 (*C. cellobioparum*)
Sugars, starch ➔ Acetone, butanol, ethanol, isopropanol, butyric acid, acetate, propionate, CO_2, H_2 (*C. acetobutylicum, C. perfringens, C. butyricum*)

- Some *Clostridium* sp. are able to ferment amino acids like glutamate, glycine, alanine, cysteine, etc. to yield fatty acid-CoA derivatives such as acetyl-, butyryl- and caproyl-CoA.
- From these ATP is produced by substrate-level phosphorylation.
  Amino acids ➔ Acetate, other fatty acids, NH_3, CO_2 and sometimes H_2
  May produce exotoxins (*C. tetani, C. botulinum, C. sporogenes, C. putrifaciens*)

Suggested Readings:

Prescott, Harley and Klein’s Microbiology, Willey, Sherwood, Woolverton, McGraw-Hill
Bacterial Physiology and Metabolism, Kim and Gadd, Cambridge University Press
Brock Biology of Microorganisms, Madigan, Martinko, Dunlap, Clark, Pearson Education