Food Biotechnology

Part 1

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EPFL Course ENG-436

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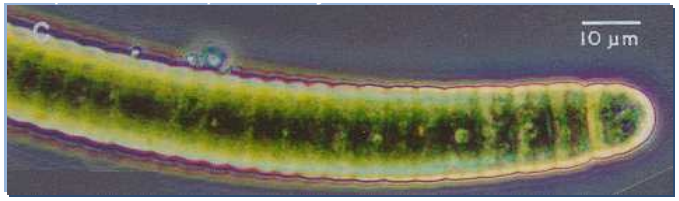
Personal Copy

Fermentation – Origin and History

- Earth — 4.5 billion years
- Fossil Microorganisms (blue green algae) — 3.3 – 3.5 billion years



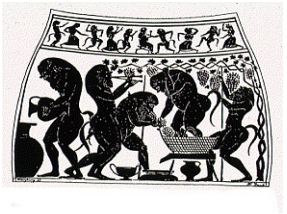
- Plants — 2 billion years
  - Became food supply for MOs
  - MOs recycled plants for energy
- Thus, million of years before humans appeared, all chemical and enzyme reactions needed for food fermentation, were present as recycling reactions used by microorganisms to digest plant material
- Recycling and fermentation are acceptable as long as the products are safe with attractive taste, aroma and texture
- The human race has depended upon fermented foods as major sources of food and energy over millennia

Fermentation is a Natural way to preserve and add Taste/Texture to Food

• 7000 B.C.	Milk in sheep stomach poach/rennet	Euphrat/Tigris
• 6000-3000 B.C.	Coagulated sour milk – Dahi, Laban	India, Egypt
• 6000 B.C.	Winemaking	Near East, Greece
• 3500 B.C.	Breadmaking	Egypt
• 3200-1200 B.C.	Cheese – Soft, pickled	Egypt, Greece
• ~ 2000 B.C.	Yogurt	Turkey, Bulgaria, Iraq
• 1500 B.C.	Meat sausages	Ancient Babylonians
• 1000 B.C.	Soy sauce	China
• 300 B.C.	Preservation of vegetables	China
• 500-1000 A.D.	Cereal-legume based foods	India









Photos from Wikipedia

Man has learned to master the microorganisms that naturally inhabit food raw materials

In all cultures over the globe, man has learned to master the microorganisms that naturally inhabit food raw materials by developing by "trial and error" fermented food products of delicious taste, smell and texture, and with various health benefits.

Photos from Wikipedia

With natural sciences and industrialization, food fermentation and the mastering of food microorganisms has been revolutionized.

Main Benefits by Fermentation (see also slide 5)

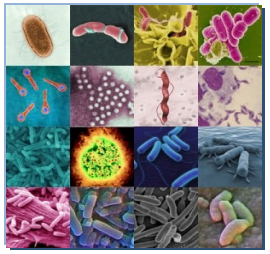
1. **Suppress harmful microorganisms** through formation of organic acids, alcohol, antimicrobial compounds

2. Enhance the diet through development of **nice flavors and textures**

3. **Enrich food** with proteins, essential amino acids, fatty acids, vitamins

4. **Remove anti-nutritional factors** or improve "bioavailability"

5. **Probiotics** - Provide beneficial effects in the gut of humans & animals

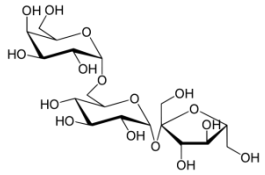


http://www.nbafoodadvocate.com/2009/11/18/top-ten-worst-foodborne-illness-complications-part-i/

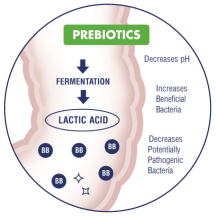
Cocoa fermentation, Ecuador
Photo: C.E. Hansen

http://en.wikipedia.org/wiki/Tempeh

http://news.clas.virginia.edu/biology/x16277.xml



http://en.wikipedia.org/wiki/File:Raffinose.svg



http://www.nestle-nutrition.com

Microorganisms - General

- From Greek: mikrós, "small" and organismós, "organism") or microbe
- Unicellular or lives in a colony of cellular organisms.
- Microbiology began with Anton van Leeuwenhoek's discovery of micro-organisms in 1675, using a microscope of his own design.

Eubacteria

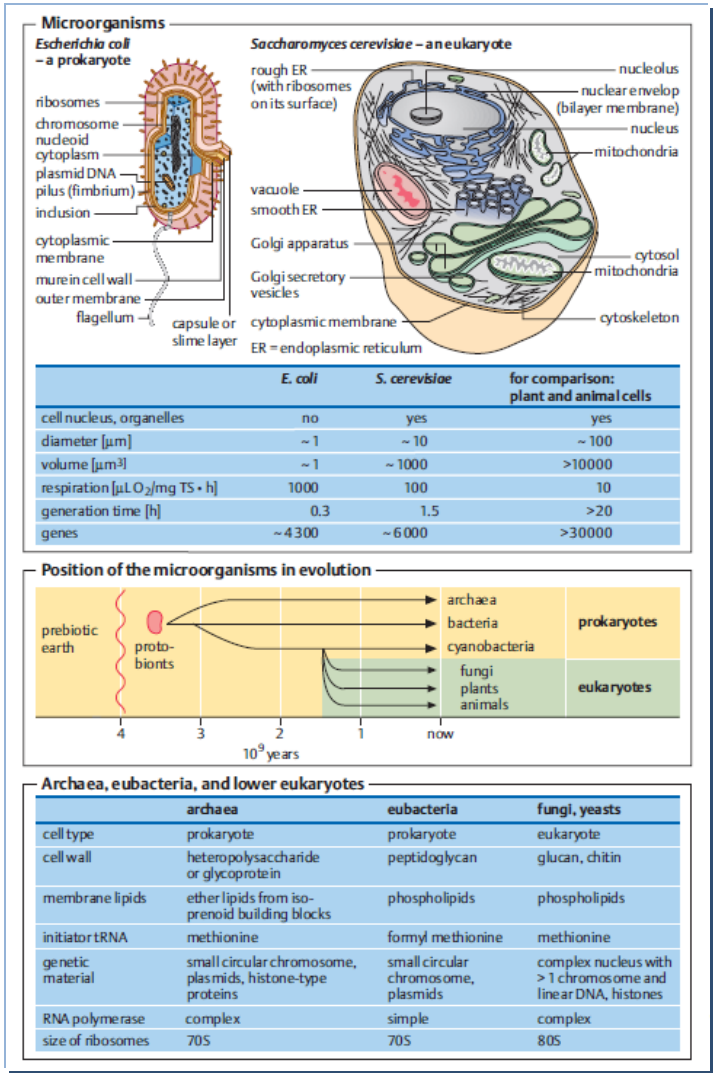
- Unicellular, 1 µm. No cell nucleus
- Small circular chromosome, plasmids

Archaebacteria

- Oldest form of life on earth
- Anaerobes, highly specialized. Enzymes adapted to extreme conditions

Yeast and Fungi

- Eucaryotes. Cell nucleus
- 70'000 different strains classified
- Fungal cell walls contain chitin, unlike the cell walls of plants, which contain cellulose.

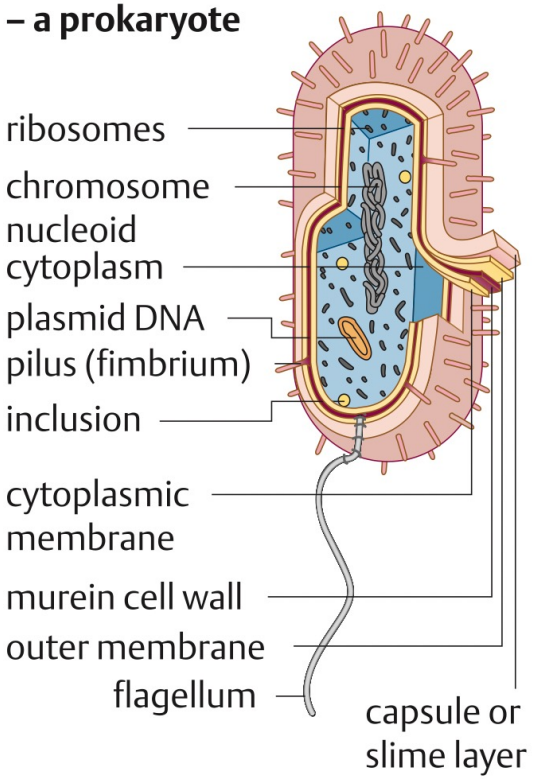


Biotechnology
(ed. R. Schmid, Wiley-VCH, 2016

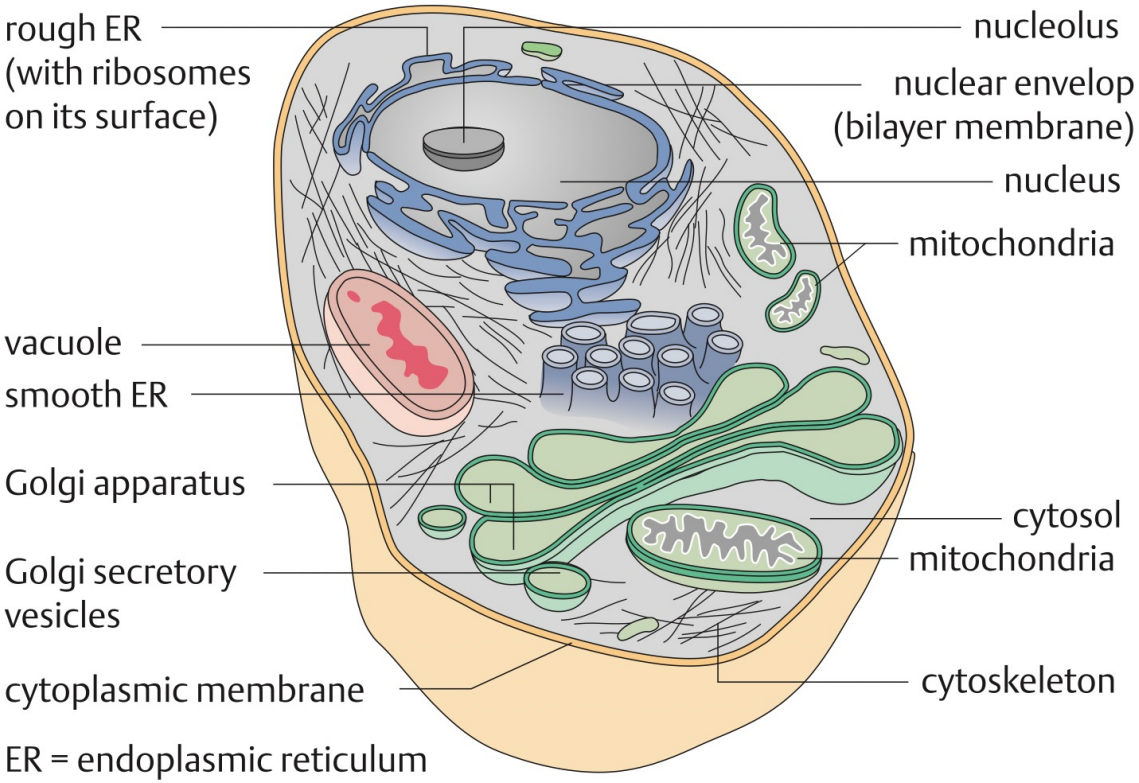
Microorganisms

No need to remember details

Escherichia coli – a prokaryote



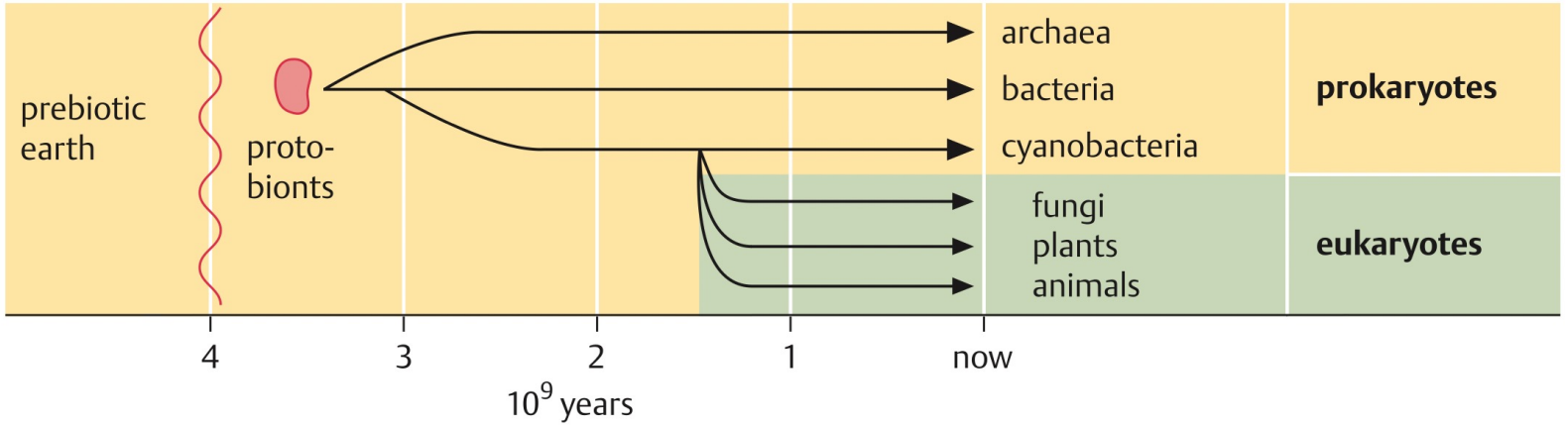
Saccharomyces cerevisiae – a eukaryote



	E. coli	*S. cerevisiae*	for comparison: plant and animal cells
cell nucleus, organelles	no	yes	yes
diameter [µm]	~ 1	~ 10	~ 100
volume [μm^3]	~ 1	~ 1000	>10 000
respiration [µL O_2/mg TS • h]	1000	100	10
generation time [h]	0.3	1.5	> 20
genes	~ 4 300	~ 6 000	> 30 000

Microorganisms - General

Position of the microorganisms in evolution



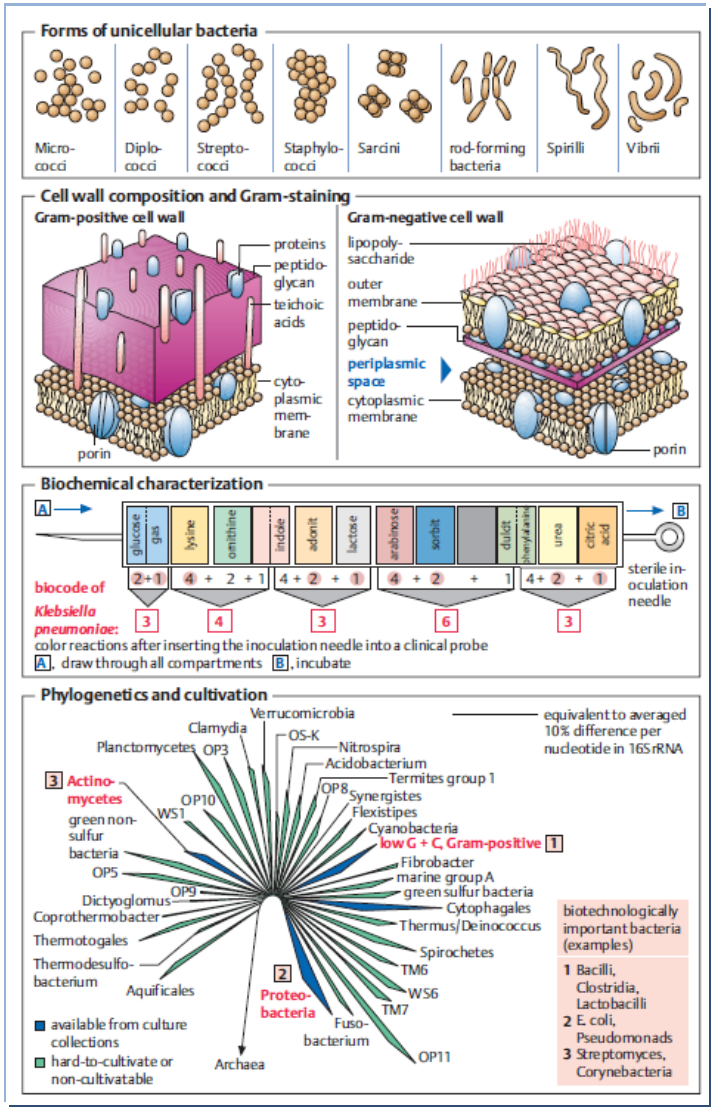
Archaea, eubacteria, and lower eukaryotes

	archaea	eubacteria	fungi, yeasts
cell type	prokaryote	prokaryote	eukaryote
cell wall	heteropolysaccharide or glycoprotein	peptidoglycan	glucan, chitin
membrane lipids	ether lipids from iso-prenoid building blocks	phospholipids	phospholipids
initiator tRNA	methionine	formyl methionine	methionine
genetic material	small circular chromosome, plasmids, histone-type proteins	small circular chromosome, plasmids	complex nucleus with > 1 chromosome and linear DNA, histones
RNA polymerase	complex	simple	complex
size of ribosomes	70S	70S	80S

Biotechnology and Genetic Engineering (ed. R. Schmid, Wiley-VCH, 2016

Bacteria

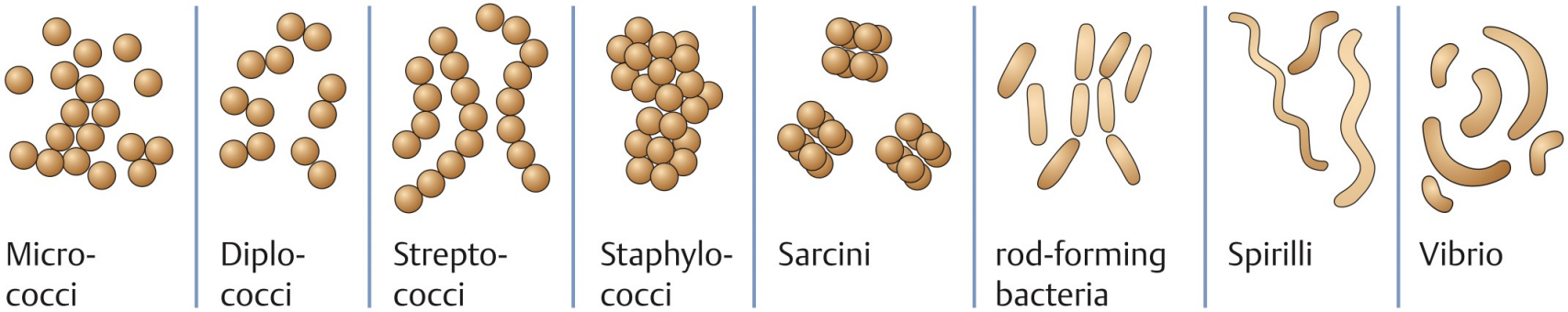
- First observed by Antonie van Leeuwenhoek in 1676, using a single-lens microscope
- >6000 strains isolated
- Oldest method for classification based on morphology under microscopy
- Staining according to H.C. Gram (cell wall structure)
  - Gram Positive: One cell membrane covered by thick murein cell wall
  - Gram Negative: Two cell membranes, enclosing a periplasmic space. Outer membranecovered by thin murein cell wall with lipopolysaccharides
- Other criteria for characterization and taxonomy:
  - Oxygen response, type of energy generation, electron donors, carbon source, phages
  - Analytical profile (API test based on growth on different substrates)
  - Antibiotics response, immuno profiling
  - Genotyping (complete sequencing, 16S and 23S ribosome DNA sequencing)



Pocket Guide to Biotechnology and Genetic Engineering (ed. R. Schmid, Wiley-VCH, 2003

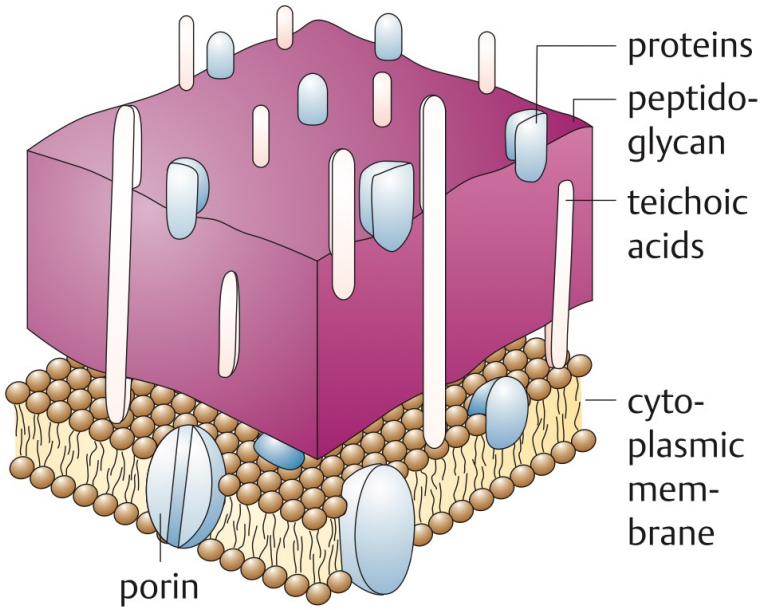
Bacteria

Forms of unicellular bacteria

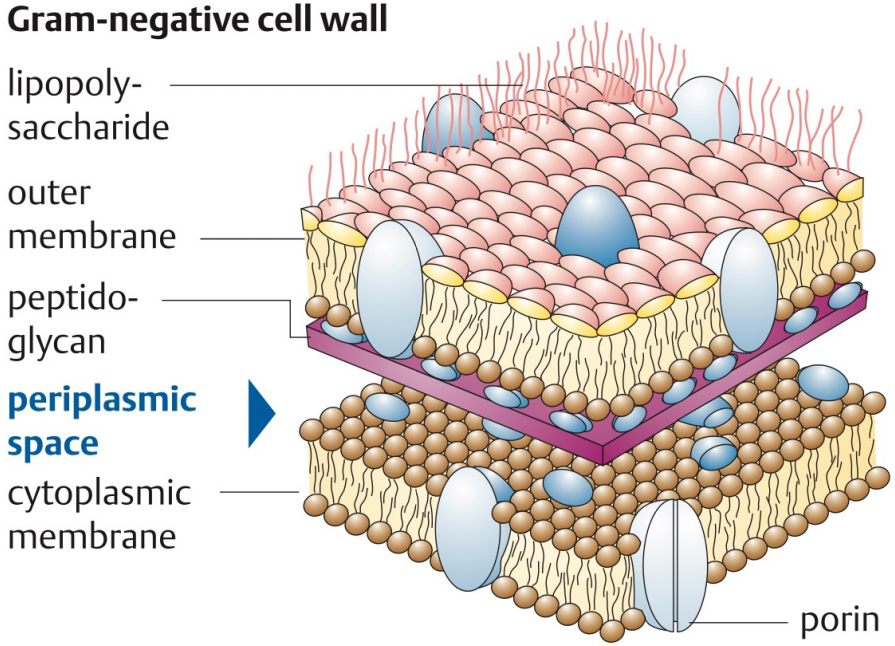


Cell wall composition and Gram-staining

Gram-positive cell wall



Gram-negative cell wall

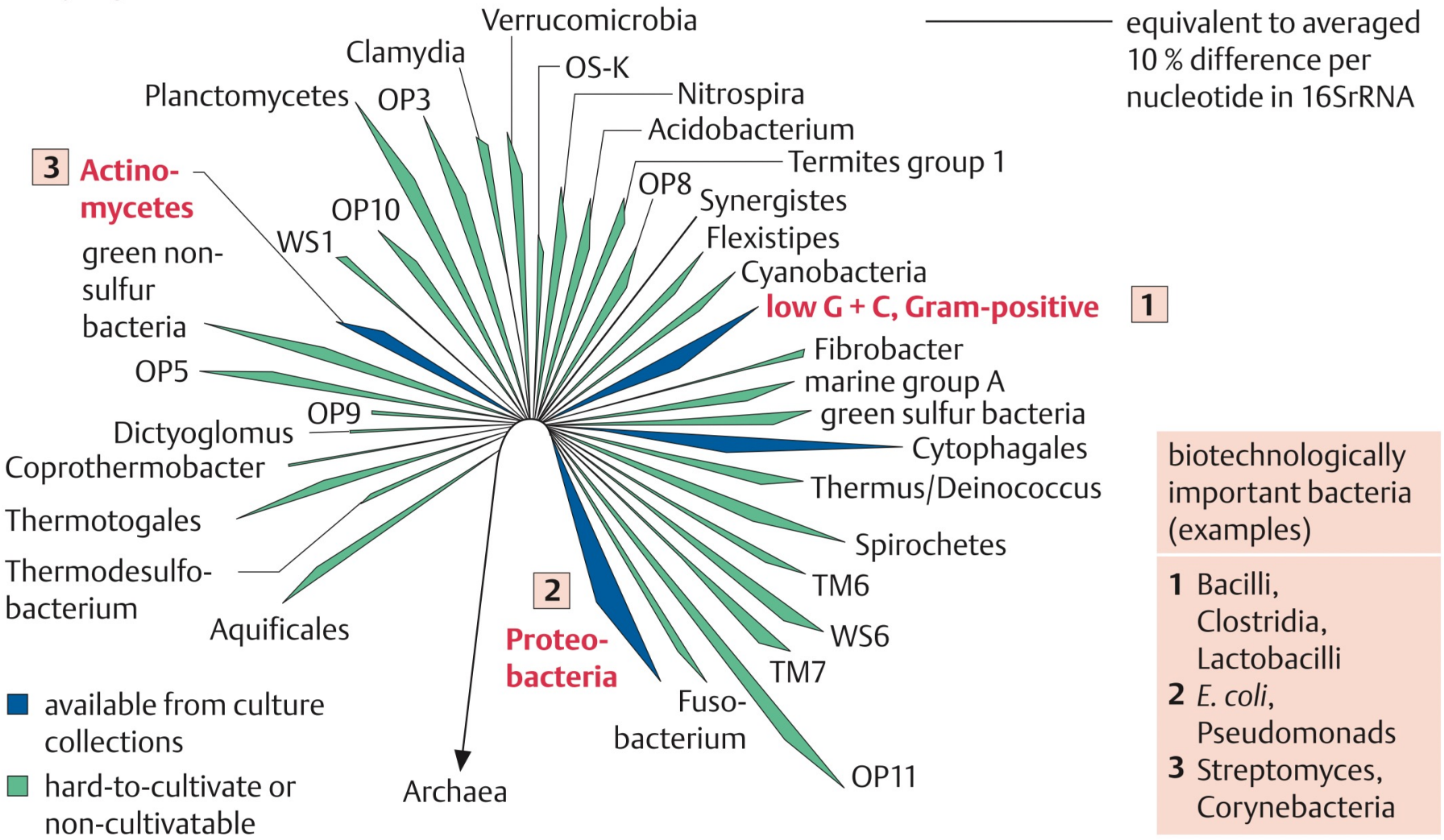


Pocket Guide to Biotechnology and Genetic Engineering (ed. R. Schmid, Wiley-VCH, 2003

Bacteria

No need to remember details

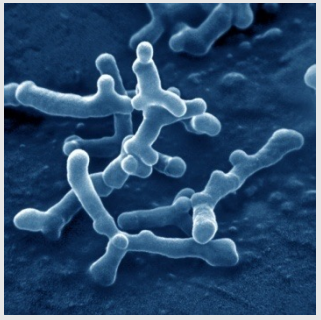
Phylogenetics and cultivation

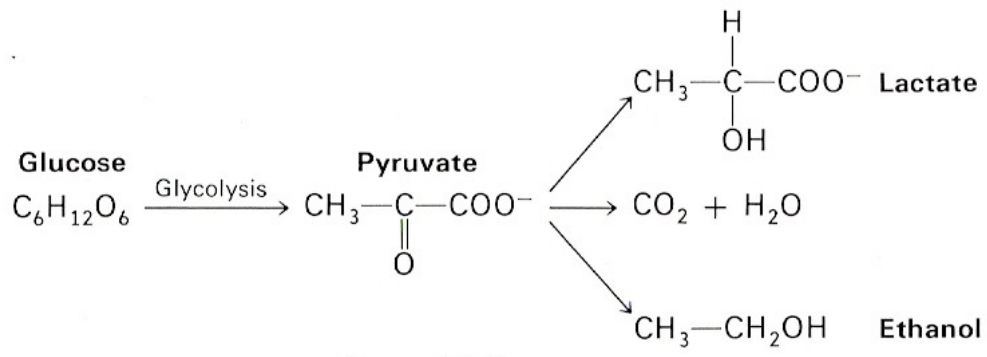


Pocket Guide to Biotechnology and Genetic Engineering (ed. R. Schmid, Wiley-VCH, 2003

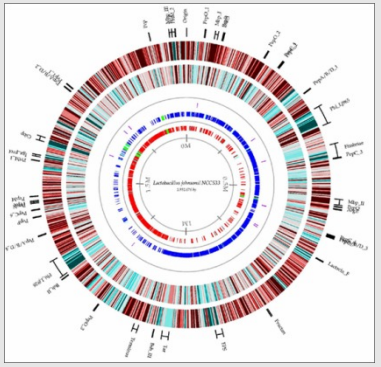
Fermentation - Definition

- *"Fermentation is the process of deriving energy from oxidation of organic compounds, such as carbohydrates, and using an endogenous electron acceptor, which is usually an organic compound".*
- *"An anaerobic (without oxygen) cellular process in which organic foods are converted into simpler compounds, and chemical energy (ATP) is produced."*





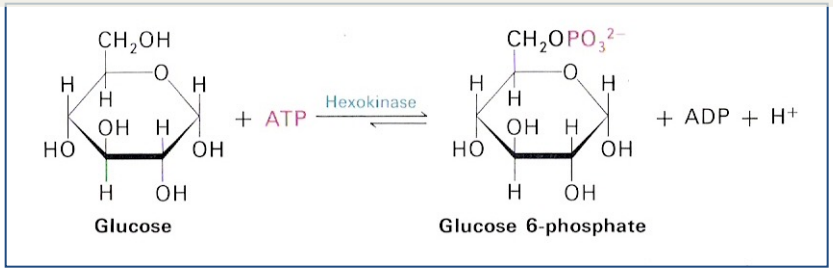
Biochemistry. L. Stryer (W.H. Freemann and Co, San Fransisco, 1981

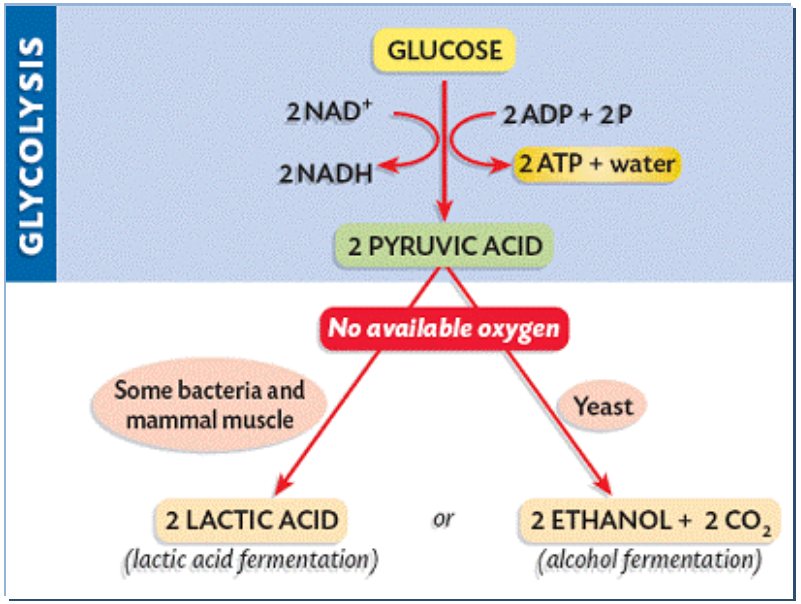


- *"The process by which complex organic compounds, such as glucose, are broken down by the action of enzymes into simpler compounds without the use of oxygen. Fermentation results in production of energy in the form of two ATP molecules, and produces less energy than the aerobic process of cellular respiration. The other end products of fermentation differ depending on the organism. In many bacteria, fungi, and animals cells (muscle cells), fermentation produces lactate, CO_2 and water. In yeast and most plant cells, fermentation produces ethyl alcohol, CO_2 and water".*

Glycolysis

- Glyolysis is the sequence of reactions that converts glucose into pyruvate with the production of 2 ATP
- Required for lactic acid & ethanol fermentation





http://leavingbio.net/RESPIRATION-(higher%20level).htm

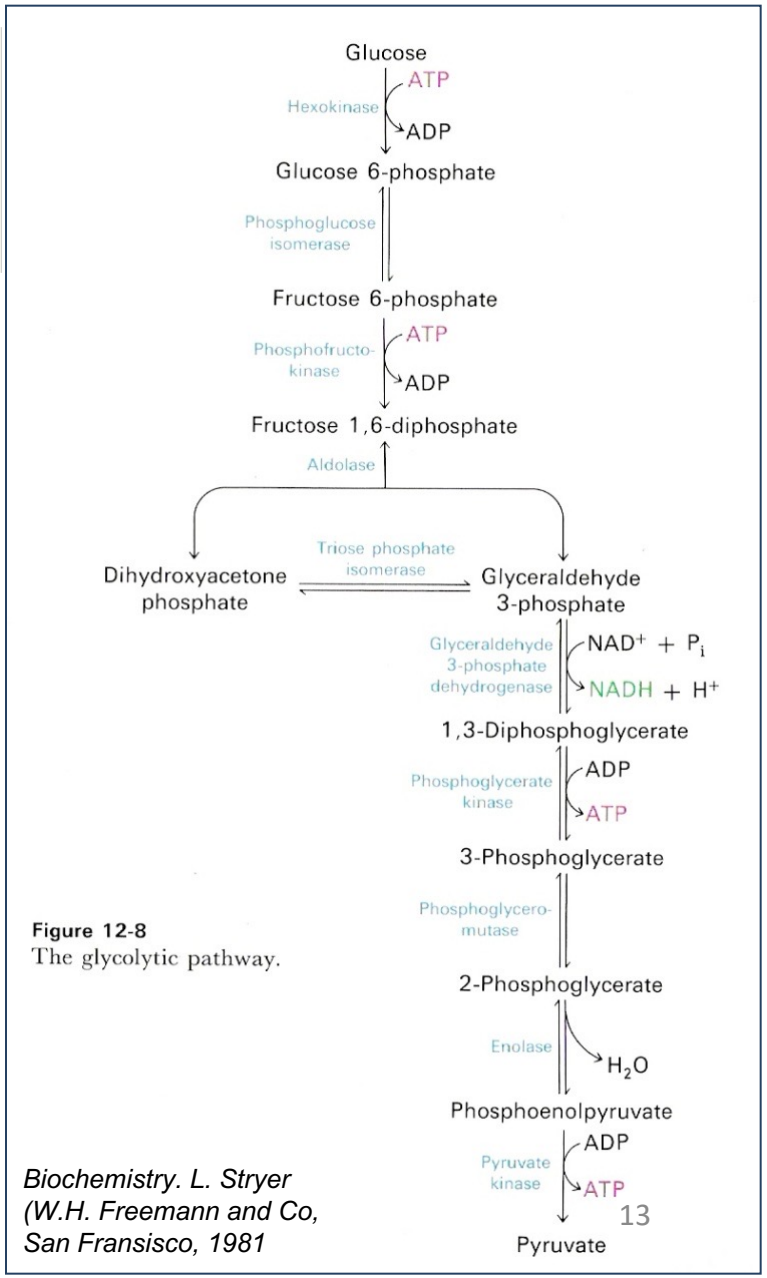


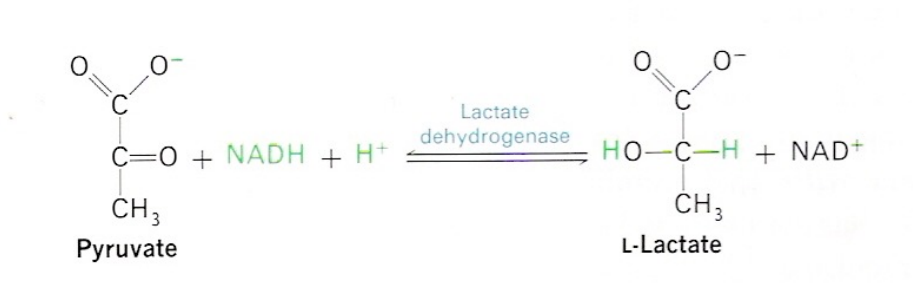
Figure 12-8
The glycolytic pathway.

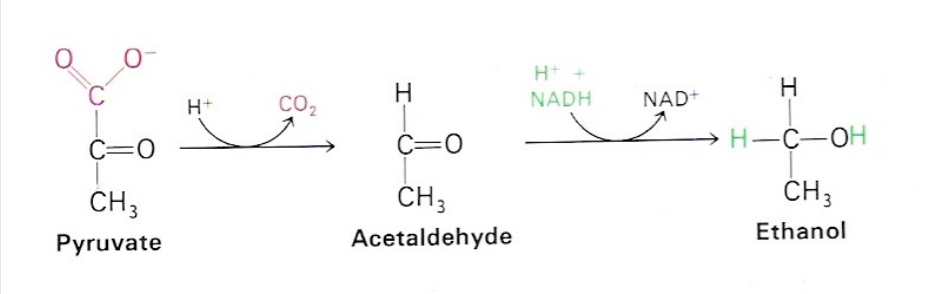
Biochemistry. L. Stryer (W.H. Freemann and Co, San Fransisco, 1981

Fermentation:
Pyruvate metabolised to e.g. Lactate and Ethanol

- Fermentation is important in anaerobic conditions when there is no oxidative phosphorylation to maintain the production of ATP (Adenosine triphosphate) by glycolysis

- During fermentation, pyruvate is metabolised to various different compounds.
  - Homolactic fermentation is the production of lactic acid from pyruvate
  - Alcoholic fermentation is the conversion of pyruvate into ethanol and carbon dioxide
  - Heterolactic fermentation is the production of lactic acid as well as other acids and alcohols





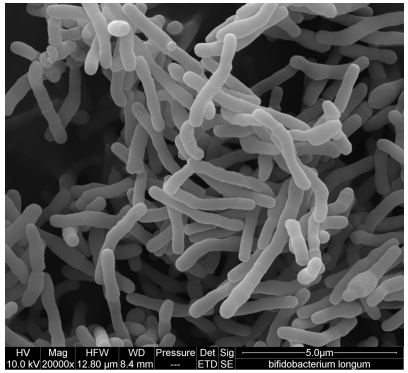
- Fermentation does not necessarily have to be carried out in an anaerobic environment. For example, even in the presence of abundant oxygen, yeast cells greatly prefer fermentation to oxidative phosphorylation, as long as sugars are readily available for consumption

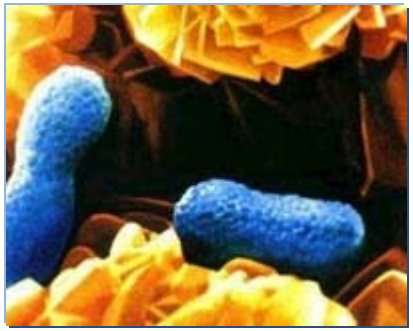
- Sugars are the most common substrate of fermentation, and typical examples of fermentation products are ethanol, lactic acid, and hydrogen. However, more exotic compounds can be produced by fermentation, such as butyric acid and acetone.

Anaerobe and Aerobe Microorganisms

- **An anaerobic organism** or anaerobe does not require oxygen for growth. It could possibly react negatively and may even die if oxygen is present. There are three types:
  1. Obligate anaerobes: Can not use oxygen for growth and are even harmed by it. Use fermentation or anaerobic respiration
  2. Aerotolerant organisms: Can not use oxygen for growth, but tolerate the presence. Strictly fermentative
  3. Facultative anaerobes: Can grow without oxygen but can utilize oxygen if present. Use aerobic respiration in presence of oxygen. Without oxygen some of them ferment, some use anaerobic respiration.

- **An aerobic organism** or aerobe can survive and grow in an oxygenated environment.
  - In aerobic respiration, the pyruvate produced by glycolysis is further oxidized completely, generating additional ATP and NADH in the citric acid cycle and by oxidative phosphorylation

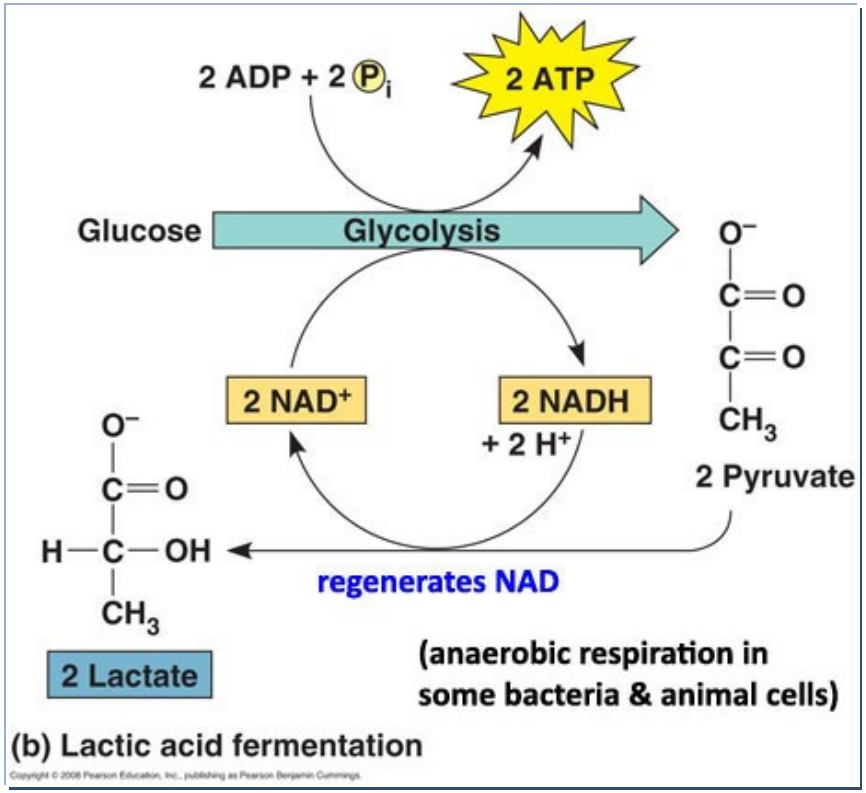




http://www.scienceknowledge.org/2010/05/23/the-genome-of-bacillus-subtilis-natto-sequencing/

Lactic Acid Fermentation

- Simplest type of fermentation. Redox reaction
- In anaerobic conditions, the cell's primary mechanism of ATP production is glycolysis. Glycolysis reduces – transfers electrons to – NAD+, forming NADH. However, there is only a limited supply of NAD+ available.
- For glycolysis to continue, NADH must be oxidized – have electrons taken away – to regenerate NAD+. This is usually done through an electron transport chain in a process called oxidative phosphorylation; however, this mechanism is not available without oxygen
- Instead, NADH donates its extra electrons to pyruvate formed during glycolysis. Since the NADH has lost electrons, NAD+ regenerates and is again available for glycolysis. Lactic acid is formed by the reduction of pyruvate

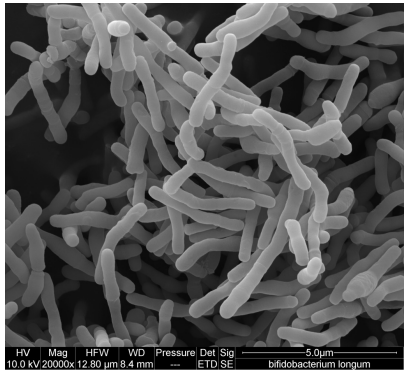


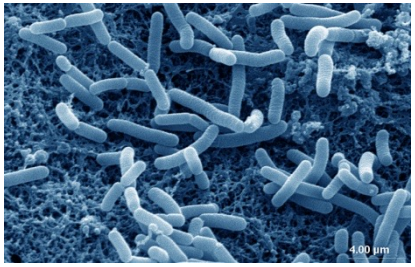
http://www.foodnetworksolution.com

- In homolactic fermentation, one molecule of glucose is converted to two molecules of lactic acid: $C_6H_{12}O_6 \rightarrow 2\ CH_3CHOHCOOH$.

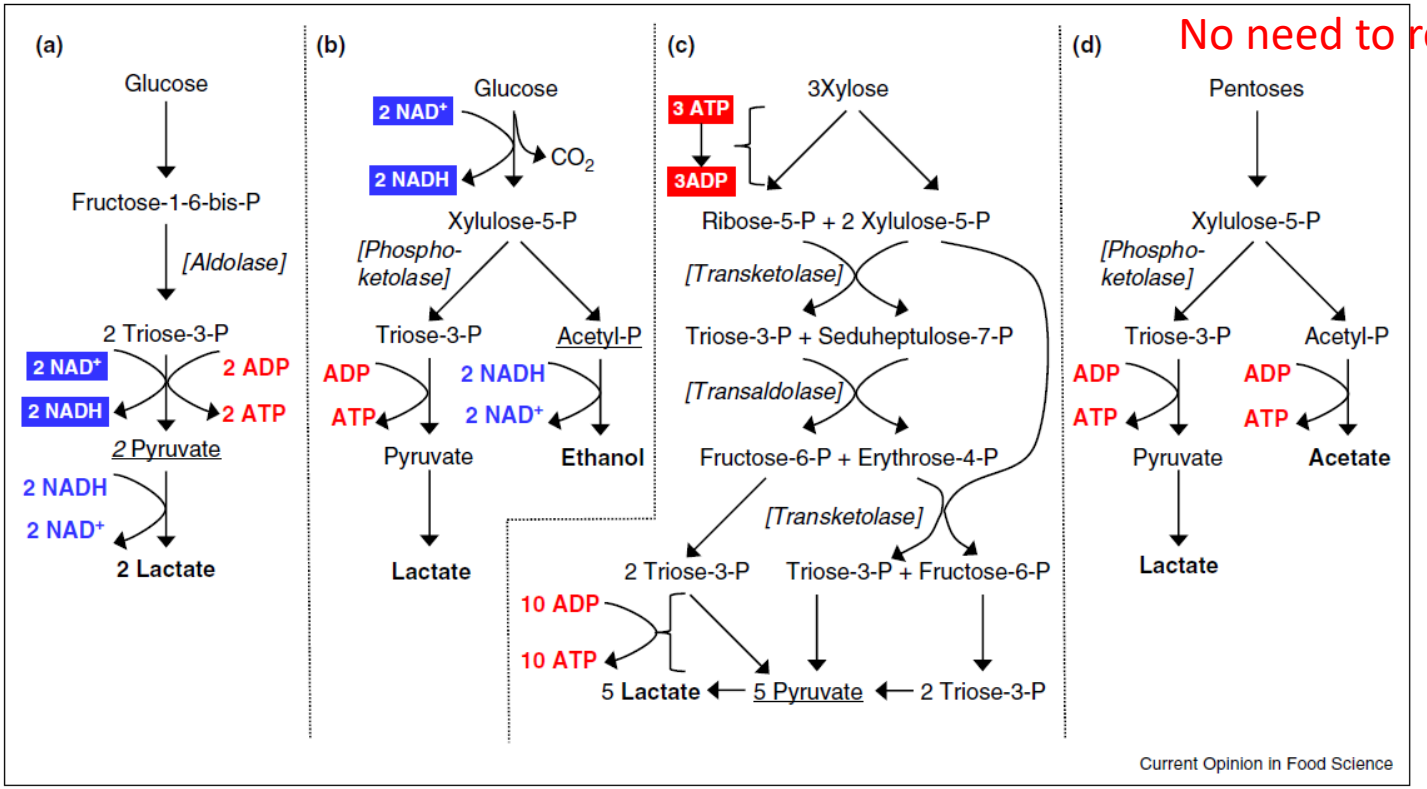
Lactic Acid Bacteria (LAB)

- Gram-positive, acid-tolerant, generally non-sporulating, non-respiring rod or cocci.
- Usually found in decomposing plants and lactic products.
- Produce lactic acid as end-product of carbohydrate fermentation.
- Throughout history, linked to acidification that inhibits growth of spoilage/pathogene bacteria. Bacteriocins, produced by several LAB strains, provide additional hurdle for spoilage and pathogenes.
- Lactic acid & metabolic products contribute to flavour & texture
- Industrial importance: Generally recognized as safe (GRAS) status, due to long history in food and healthy microflora of human mucosa
- *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus, Streptococcus* as well as the more peripheral *Aerococcus, Carnobacterium, Enterococcus, Oenococcus, Sporolactobacillus, Tetragenococcus, Vagococcus, Weisella*





Several types of Glycolysis in LABs. The most common is the Emden–Meyerhof–Parnas (EMP pathway)



No need to remember details

Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. M.G. Gänzle. Current Opinion in Food Science 2015, 2: 106-117

a) Homofermentative metabolism of hexoses via the Emden–Meyerhoff pathway
b) Heterofermentative metabolism of hexoses via the phosphoketolase pathway
c) Homofermentative metabolism of pentoses via the pentose phosphate pathway
d) Heterofermentative metabolism of pentoses via the phosphoketolase pathway

Comparison of metabolic properties of homolactic and heterolactic metabolism

Comparison of metabolic properties of homolactic and heterolactic metabolism.

	Homolactic metabolism	Heterolactic metabolism[a]
Metabolism of glucose	Emden–Meyerhoff pathway	Phosphoketolase pathway[b]
Metabolism of galactose	Tagatose pathway and/or Leloir pathway	Leloir pathway
Metabolism of fructose	Emden–Meyerhoff pathway	Mannitol-dehydrogenase[c], phosphoketolase pathway
Metabolism of pentoses	Phosphoketolase pathway or pentose phosphate pathway; sequential metabolism of hexoses and pentoses	Phosphoketolase pathway; simultaneous metabolism of hexoses and pentoses
Preferred substrate	Glucose	Fructose[c,d], sucrose and/or maltose
Alternative products from pyruvate	Formate, ethanol, and acetate; lactate, or acetoin	Lactate, acetate (acetoin)[e]
Alternative end products from acetyl-phosphate	Acetate	Ethanol or acetate[e]
Products of lactate metabolism	Acetate, formed by stationary cultures at aerobic conditions, or acetoin	1,2-Propanediol and acetate[f]

[a] All species in the *Lactobacillus vaccinostercus*, and *L. collinoides* groups, most species in the *L. reuteri*, *L. brevis*, *L. buchneri* and *L. fructivorans* groups, *L. rossiae*, *L. siliginis*, *L. floricola*; and all Leuconostococaceae (Genera Fructobacillus, *Leuconostoc*, *Oenococcus*, and *Weissella*).
[b] Not all heterofermentative LAB grow with glucose as sole carbon source.
[c] Mannitol dehydrogenase is absent in most strains of *Weissella*.
[d] *Fructobacillus* spp. preferentially ferment fructose. Several *Fructobacillus* species do not produce ethanol from fructose as they apparently lack an alcohol dehydrogenase.
[e] Acetoin formation during co-metabolism of hexoses or pentoses and citrate is observed in *Lu. mesenteroides* and *Oenococcus* spp. but not in heterofermentative lactobacilli. Diacetyl results from chemical oxidation of α-acetolactate, an intermediate of acetoin formation.
[f] This pathway is found only in *L. buchneri* and few other species.

Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. M.G. Gänzle. Current Opinion in Food Science 2015, 2: 106-117

Utilization of Lactose

- Most lactic acid bacteria (*Lactobacilli, Bifidobacteria, Streptococci*) use glucose as primary energy source. Exception is *Streptococcus thermophillus (*uses lactose)

- Two properties required to metabolise lactose

  1. Intracellular transport system allowing lactose uptake. E.g. *Lactococcus lactis* transports lactose into the cell using the phospho-enolpyruvate phosphotransferase system which phosphorylates lactose to lactose-6-phosphate.

  2. Ability to produce the enzyme β-galactosidase (Lactase). This enzyme breaks down lactose into β-galactose & glucose, which can be metabolised to produce energy. Most LABs convert galactose to glucose-6-phosphate by the Leloir pathway and thus into the glycolytic pathway.

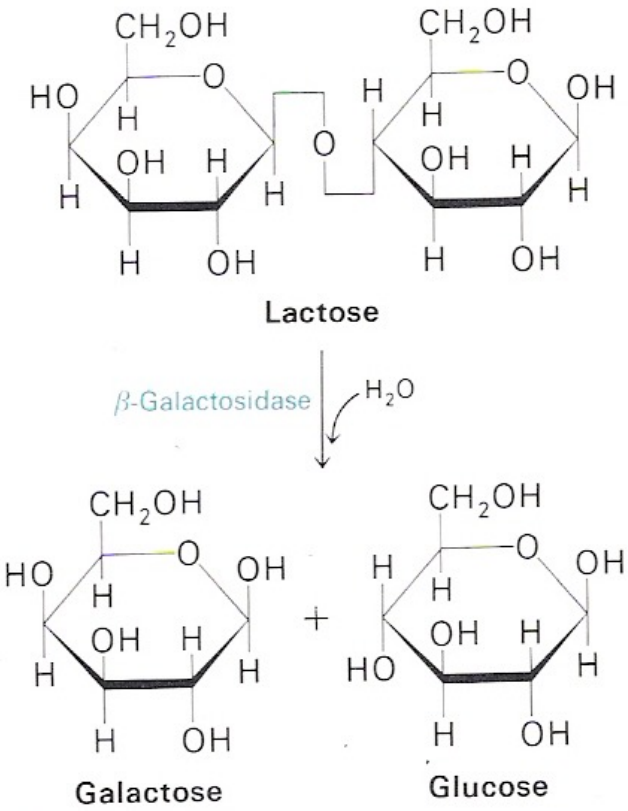


Figure 28-1
Lactose is hydrolyzed by β-galactosidase.

Biochemistry. L. Stryer (W.H. Freemann and Co, San Fransisco, 1981

Ethanol Fermentation (alcoholic fermentation)

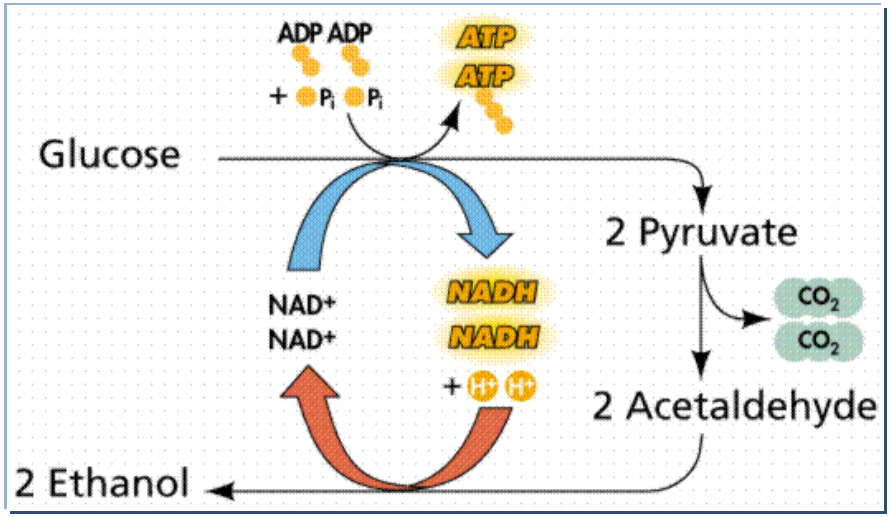
- Ethanol fermentation (by yeast and some types of bacteria) breaks pyruvate down into ethanol and carbon dioxide

  $C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

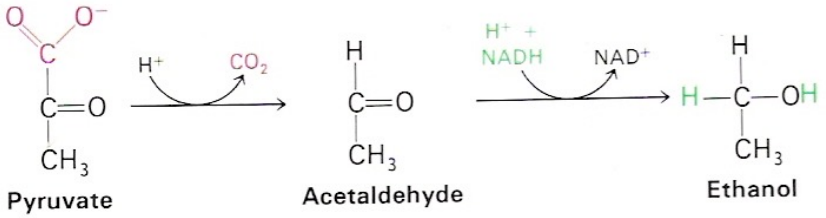
- One glucose molecule is converted into two ethanol molecules and two CO_2 molecules

- $C_6H_{12}O_6 + 2\ ADP + 2\ Pi + 2\ NAD+ \rightarrow 2\ CH_3COCOO- + 2\ ATP + 2\ NADH + 2\ H_2O + 2H$

- Usually only one of the products is desired
  - Bread-making: alcohol is baked out
  - Alcohol production, CO_2 is released into the atmosphere or used for carbonating the beverage.



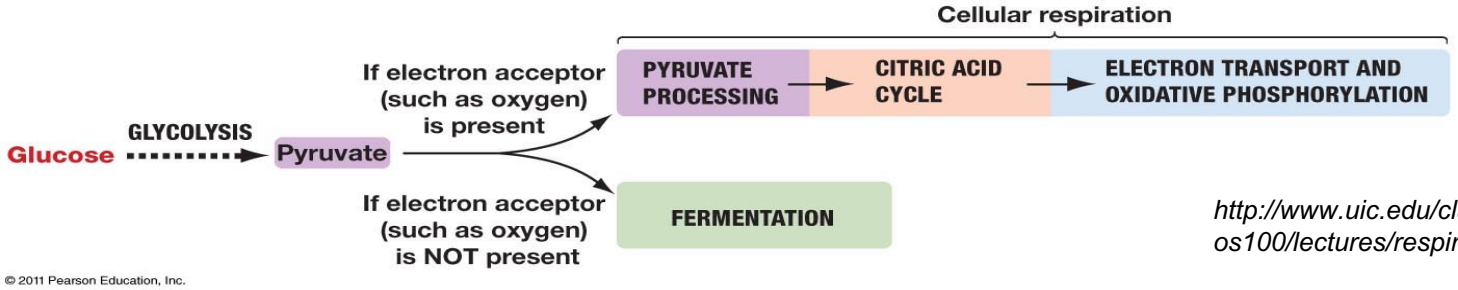
http://www.lookfordiagnosis.com/images.php?term=Fermentation&lang=4



- *Pyruvate decarboxylase*
- *Alcohol dehydrogenase*

Respiration

- Respiration is a controlled combustion; the energy in the C-H bonds are released slowly in small catalyst driven reactions. Theoretical yield: 38 ATP in procaryotes.
- Both burning a glucose molecule and slow oxidation via cellular respiration would yield same amount of energy (686 kcal/mol)
- Eukaryotes: 36 ATP (2 ATP used to transport NADH produced in glycolysis into the mitochondria - hence the lower yield)



http://www.uic.edu/classes/bios/bios100/lectures/respiration.htm

TABLE 5.3 **ATP Yield During Prokaryotic Aerobic Respiration of One Glucose Molecule**

Source	ATP Yield (Method)
Glycolysis	
1. Oxidation of glucose to pyruvic acid	2 ATP (substrate-level phosphorylation)
2. Production of 2 NADH	6 ATP (oxidative phosphorylation in electron transport chain)
Preparatory Step	
1. Formation of acetyl CoA produces 2 NADH	6 ATP (oxidative phosphorylation in electron transport chain)
Krebs Cycle	
1. Oxidation of succinyl CoA to succinic acid	2 GTP (equivalent of ATP; substrate-level phosphorylation)
2. Production of 6 NADH	18 ATP (oxidative phosphorylation in electron transport chain)
3. Production of 2 FADH	4 ATP (oxidative phosphorylation in electron transport chain)
	Total: 38 ATP

Glycolysis, Krebs cycle, ATP, Electron transport chain and chemiosmosis

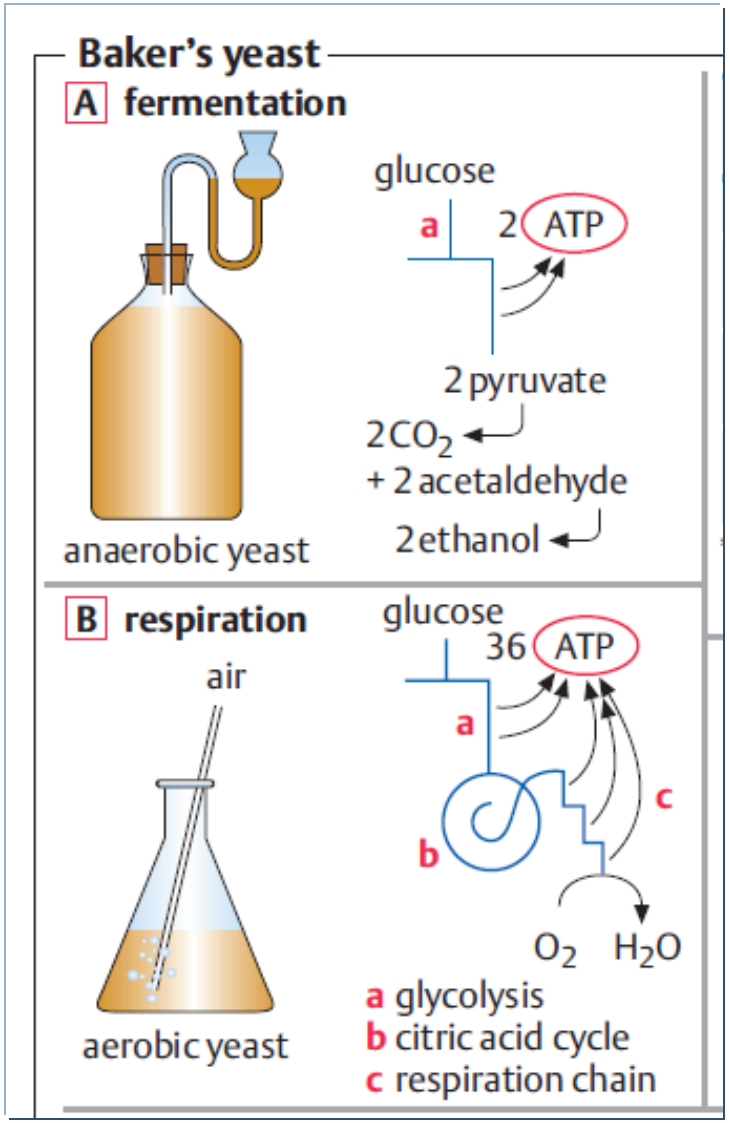
Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings.

http://classes.midlandstech.com/carterp/Courses/bio225/chap05/ss4.htm

Yeast - Respiration

- Yeast can use oxygen if it is present
- In absence of oxygen, yeast switch to anaerobic
- The anaerobic end products are CO_2 and ethanol. Also known as alcoholic fermentation.
- The "Pasteur effect": Occurs when a facultative anaerobic cell is provided with oxygen, so that its high rate of glucose metabolism is slowed down
- A shift from slow aerobic to rapid anaerobic glucose consumption happens if you are unable to provide oxygen to mitochondria. Glucose is consumed faster in attempt to produce ATP via the less efficient fermentation to lactate, and lactic acid accumulates in your muscles.

(https://courses.cit.cornell.edu/biomi290/z.OldWebSite/MOVIES/PASTEUR.HTML)



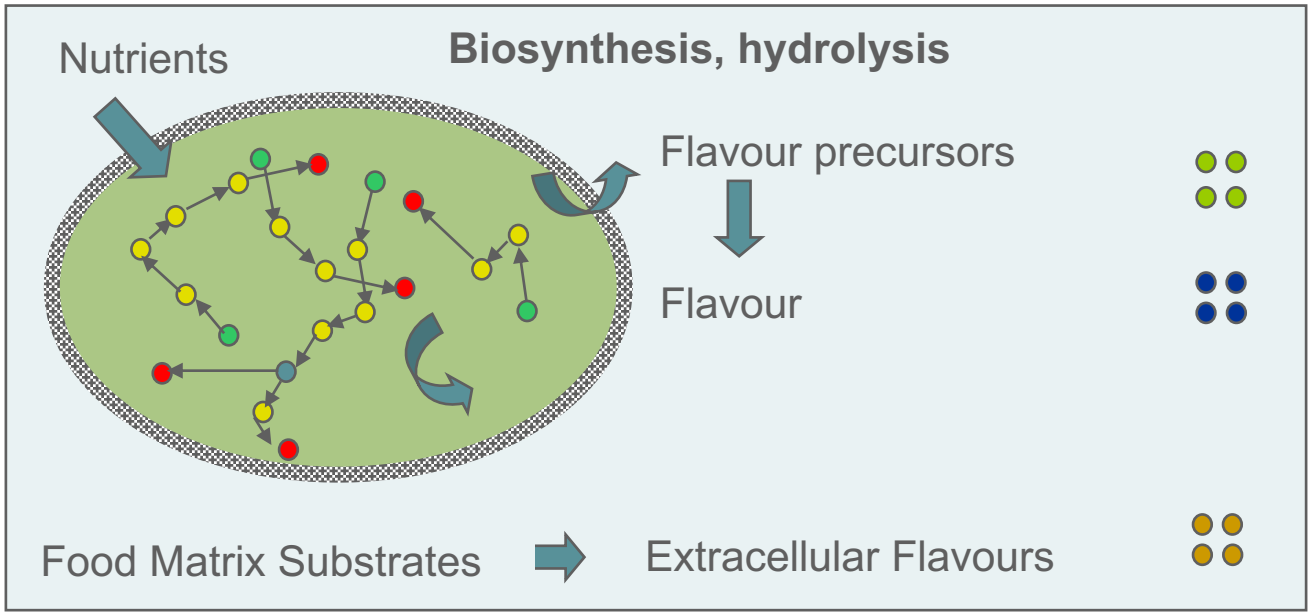
Pocket Guide to Biotechnology and Genetic Engineering (ed. R. Schmid, Wiley-VCH, 2003

Question

- You are Heineken; What Yeast fermentation condition would you prefer, and why?

- You are Lallemand (Yeast producing company); What fermentation conditions would you prefer, and why?

Flavour Generation by Fermentation



In Food, the flavour/flavour precursor generation is often followed by thermal flavour generation

Flavor formation is complex and can be separated into two sub-processes

1. The generation of precursor molecules
2. The conversion of precursor molecules into aroma compounds

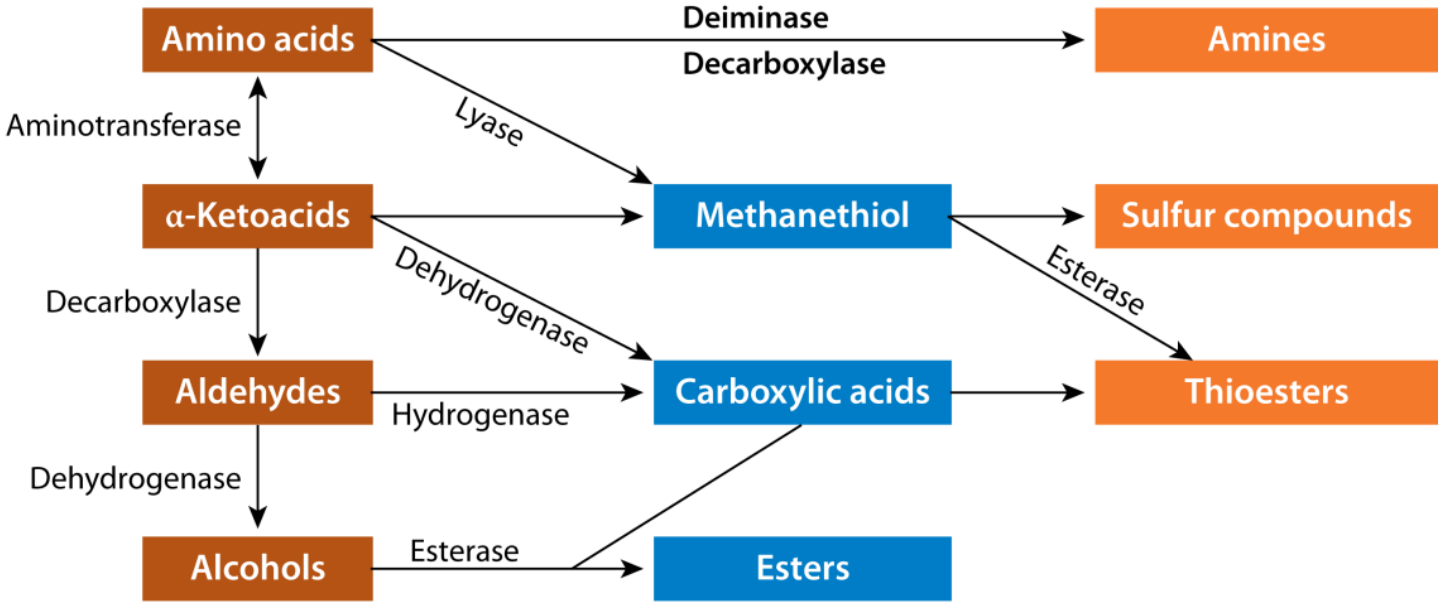
Origin of the precursors and enzymes:

1. Synthesized directly by the LAB
2. Present in the food matrix (proteins, carbohydrates, lipids)
3. Secreted Enzymes or released by LAB cell lysis

Schmid EJ and Kleerebezem (2014) Production of aroma compounds in lactic fermentation. Annu. Rev. Food Sci. Technol. 5:313-326

Flavor Generation by Cytosolic metabolic pathways: Amino acid degradation (1)

- Conversion of Amino acids into alcohols, aldehydes, acids, esters and sulfur compounds to produce specific flavor profiles.



Schematic representation of the most important amino acid conversion pathways for flavor formation in cheese manufacturing. The chemical compounds and corresponding interconversion enzyme classes are indicated; the most dominant flavor components formed via these pathways are listed in Table 1.

Schmid EJ and Kleerebezem (2014) Production of aroma compounds in lactic fermentation. Annu. Rev. Food Sci. Technol. 5:313-326

Flavor Generation by Cytosolic metabolic pathways: Amino acid degradation (1)

Table 1 Flavor compounds derived from amino acids via their corresponding α-ketoacids and subsequent catabolic pathways

Amino acid	α-Ketoacid	Aldehyde	Carboxylic acid	Alcohol	Esters
Ile	α-keto-3-methyl-pentanoic acid	2-methylbutanal	2-methyl butyric acid	2-methylbutanediol	
Leu	α-ketoisocaproic acid	3-methylbutanal **2-methylpropanal**[a]	3-methyl butyric acid 2-methyl propanoic acid	3-methylbutanediol 2-methylpropanol	ethyl-3-methylbutanoate
Val	α-ketoisovaleric acid	2-methylpropanal	2-methyl propanoic acid	2-methylpropanol	ethyl isobutanoate
Phe	phenyl pyruvate	**benzaldehyde**[a] phenylacetaldehyde	benzoic acid phenylacetic acid	phenylmethanol phenylethanol	ethyl benzoate phenylethyl acetate
Trp	indole-3-pyruvate	indole-3-acetaldehyde	indole-3-acetic acid		
Met	α-keto methylthio butyric acid	methional **methylthio-acetaldehyde**[a]	methylthiobutyric acid	methionol methanethiol	ethyl-3-methyl-thiopropionate methylthioacetate

[a]Metabolite names in bold can be formed by nonenzymatic chemical reactions.

No need to remember details

Schmid EJ and Kleerebezem (2014) Production of aroma compounds in lactic fermentation. Annu. Rev. Food Sci. Technol. 5:313-326

Flavor Generation by Cytosolic metabolic pathways: Threonine Degradation

- Yogurt starter cultures convert threonine into acetaldehyde and glycine with the enzyme; threonine aldolase
- Acetaldehyde is considered to be the main aroma compound in yogurt
- Threonin metabolism is also correlated with production of butter, vanilla-like aroma compounds like 2,3-pentadione (Figure)

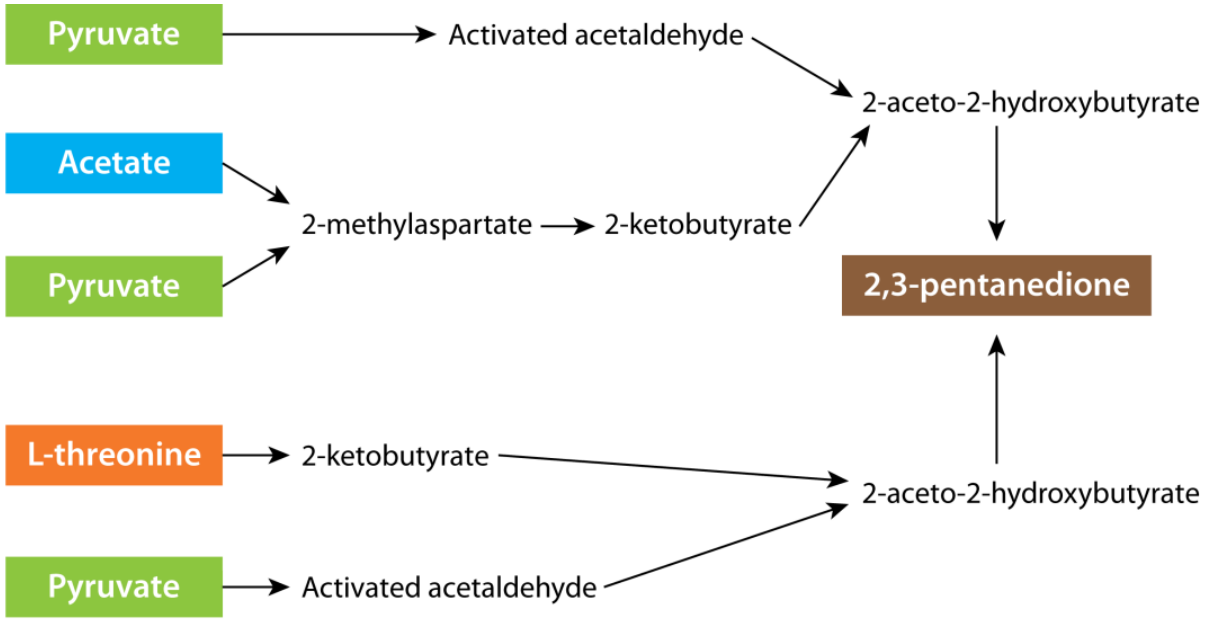


Figure 3 Metabolic pathways for 2,3-pentanedione biosynthesis from pyruvate, acetate, and L-threonine. Figure adapted from Ott et al. (2000).

Schmid EJ and Kleerebezem (2014) Production of aroma compounds in lactic fermentation. Annu. Rev. Food Sci. Technol. 5:313-326

Flavor Generation by Cytosolic metabolic pathways: Citrate Metabolism

- Citrate found in many fermentable foods (fruit, vegetables, milk).
- Conversion of Citrate into diacetyl, acetoin, butanediol and acetaldehyde
- Only few LAB are capable to degrade citrate
- Need to have a plasmid-encoded citrate transporter gene (e.g. *L. lactis, Leuconostoc mesenteroides*).

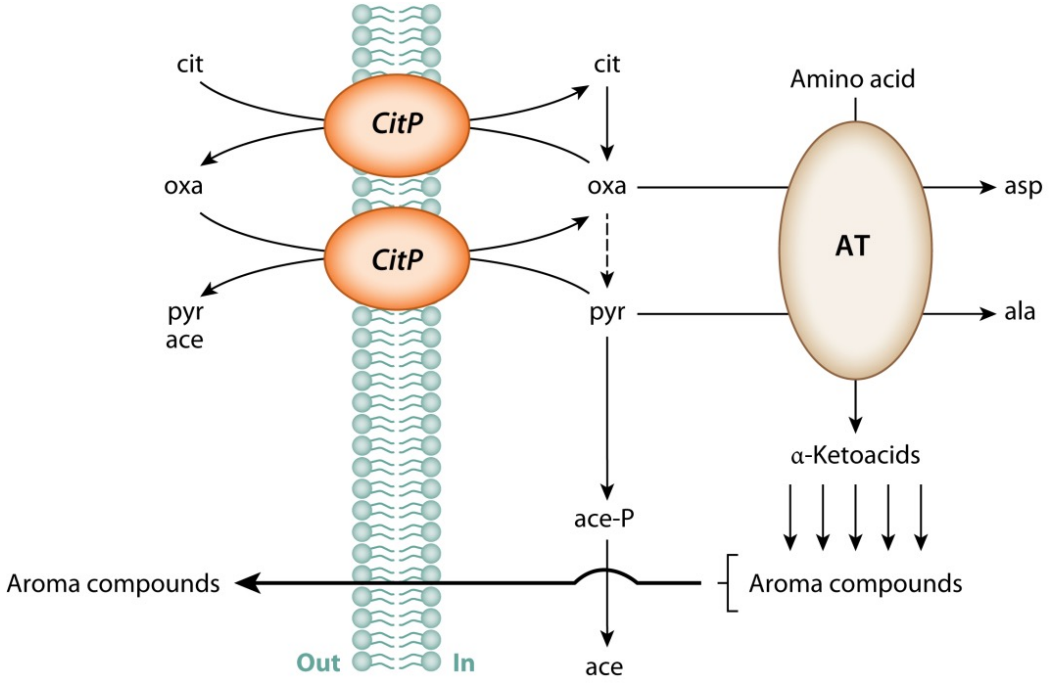


Figure 2 Citrate-driven transamination of amino acids and subsequent aroma formation. Abbreviations: cit, citrate; oxa, oxalacetate; pyr, pyruvate; asp, aspartate; ala, alanine; ace, acetate; ace-P, acetyl phosphate; CitP, citrate permease; AT, aminotransferase. Figure adapted from Pudlik & Lolkema (2012a).

Schmid EJ and Kleerebezem (2014) Production of aroma compounds in lactic fermentation. Annu. Rev. Food Sci. Technol. 5:313-326

From traditional to industrial food fermentation
Definition

"Foods invaded by bacteria producing toxins or by fungi producing mycotoxins are dangerous to man. If the products of invasion are ill-smelling, off-flavored or toxic, human consumers try to avoid them and the foods are described as spoiled"

"If the products are pleasantly flavored, have attractive aromas and textures and are nontoxic, the human consumer accepts them and they are described as fermented foods"

For further reading :K. H. Steinkraus (2002) Fermentations in World Food Processing. Comprehensive Reviews in Food Science and Food Safety: Vol 1. 23-32

From traditional to industrial food fermentation

The essential 5 steps from traditional to industrial Food Fermentation

Step 1: Identifying Microorganisms as cause of Fermentation

Step 2: Creating "Microbial Mothers" – starter cultures

Step 3: Taking the "Microbial Zoo" apart

Step 4: Pure cultivation Technology and Pure culture collections

Step 5: Mastering the microflora in Raw materials, Fermentation Process and Product

Creating "Microbial Mothers"

- Creation of "microbial mothers" is a man-made copy of what nature did all the time
- After time and growth cycles in a given raw material (e.g. milk, cereal flour, fruit juice) under given conditions (temperature, oxygen pressure, water activity, pH), a mix of micro-organisms will establish that is stable in its composition and is then called a "microbial mother".
- It contain species that have synergies in their metabolism (e.g. substrate preferences, pH/T optima).
- "Sourdough mothers" for bread or Panettone and "Kefir mothers" (Kefir grains), are the most known industrially-applied examples, while "Vinegar mothers" are mostly used in artisanal processes.

Kefir Mother

http://mondesteph.wordpress.com/page/16/

Kefir

Sourdough Mother

http://www.diggingthedirt.ca/2009/03/01/sourdough-starter-success/

Panettone

Sourdough Bread

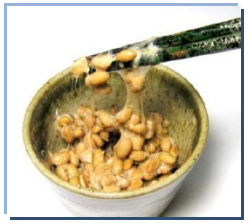
http://www.instructables.com/id/Sourdough-Bread/

Taking the "Microbial Zoo" apart

- Fermented raw materials and products are inhabited by mixtures of bacteria, yeasts or molds.
- From these, food microbiologists can isolate pure strains. These can be stably maintained in cultures collections.

Some examples of fermented raw materials and products

Cereals (e.g. Koji)

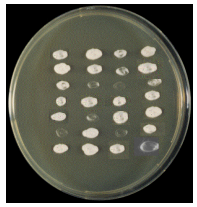


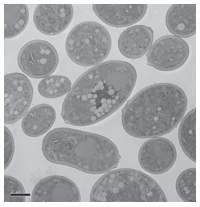
Beans (e.g. Natto)

Meat (e.g. Salami)

Milk (e.g. Yoghurt)

Various isolation and enrichment techniques



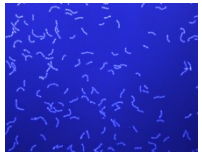


Pure Yeast Strains

http://en.wikipedia.org

http://www.mnstate.edu/ehs/Health&Safety/moldspores.cfm

Pure Mold Strains



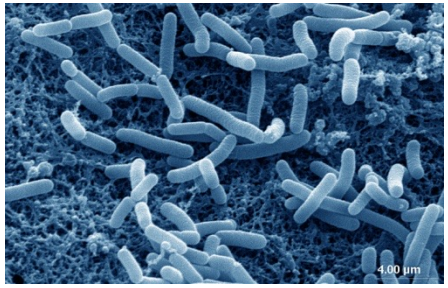
Pure Bacterial Strains

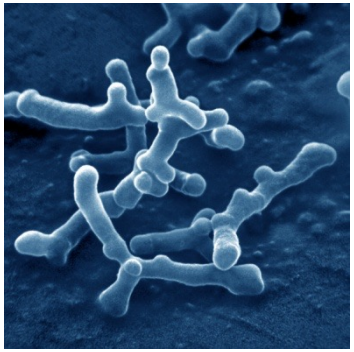
http://www.scienceknowledge.org/2010/05/23/the-genome-of-bacillus-subtilis-natto-sequencing/

http://www.bbcgoodfood.com/content/knowhow/glossary/salami/

http://www.123rf.com/photo_5512926_bowl-of-greek-yogurt--food-and-drink.html

Culture Collections and Strain Identification



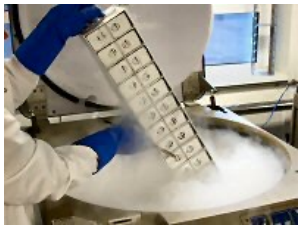


Pure culture collections

- E.g. Nestlé has extensive proprietary culture collections. These serve as sources for proprietary fermentation processes and as reference for strain identification in products.
- Major advantages are their rapid access and their confidential nature.
- Large number of public (mostly universities) and commercial culture collections, which contain many strains, which are patented.
- Depositing patented strains is a standard procedure.
- These can generally only be ordered by signing a declaration that they are used exclusively for research purposes. In addition, the patent holder of the strain is informed about its delivery to an applicant, which may compromise confidentially.

E.g. Nestlé has proprietary culture collections

Nestlé Culture Collection (NCC) > 3000 strains



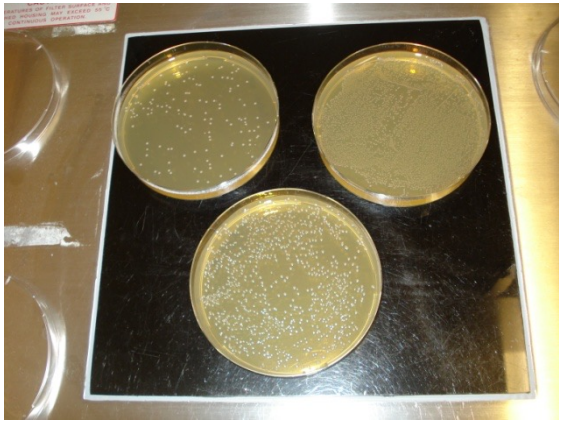
http://www.wfcc.info/
http://www.wdcm.org/

WFCC
World Federation for Culture Collections

Strain Isolation

Product with live bacteria

Dilution

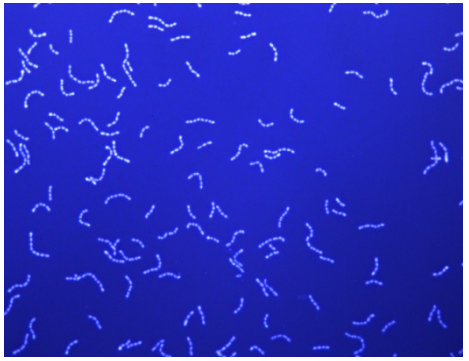


Culture at appropriate medium.
Isolation of individual colonies

MRS Medium

- Bacterial growth medium named in 1960 by: de Man, Rogosa & Sharpe.
- Designed to favour growth of *Lactobacilli*.
- Sodium acetate suppresses many competing bacteria
- Yeast & meat extracts & peptone provide sources of carbon, nitrogen and vitamins.
- Yeast extract contains vitamins and amino acids required by *Lactobacilli*.
- Polysorbate 80 is a surfactant to assists in nutrient uptake by *Lactobacilli*.
- Magnesium sulfate and manganese sulfate provide cations used in metabolism.
- 2% Glucose. pH 6.2

Cell counting



Strain characterization

Bacterial Taxonomy

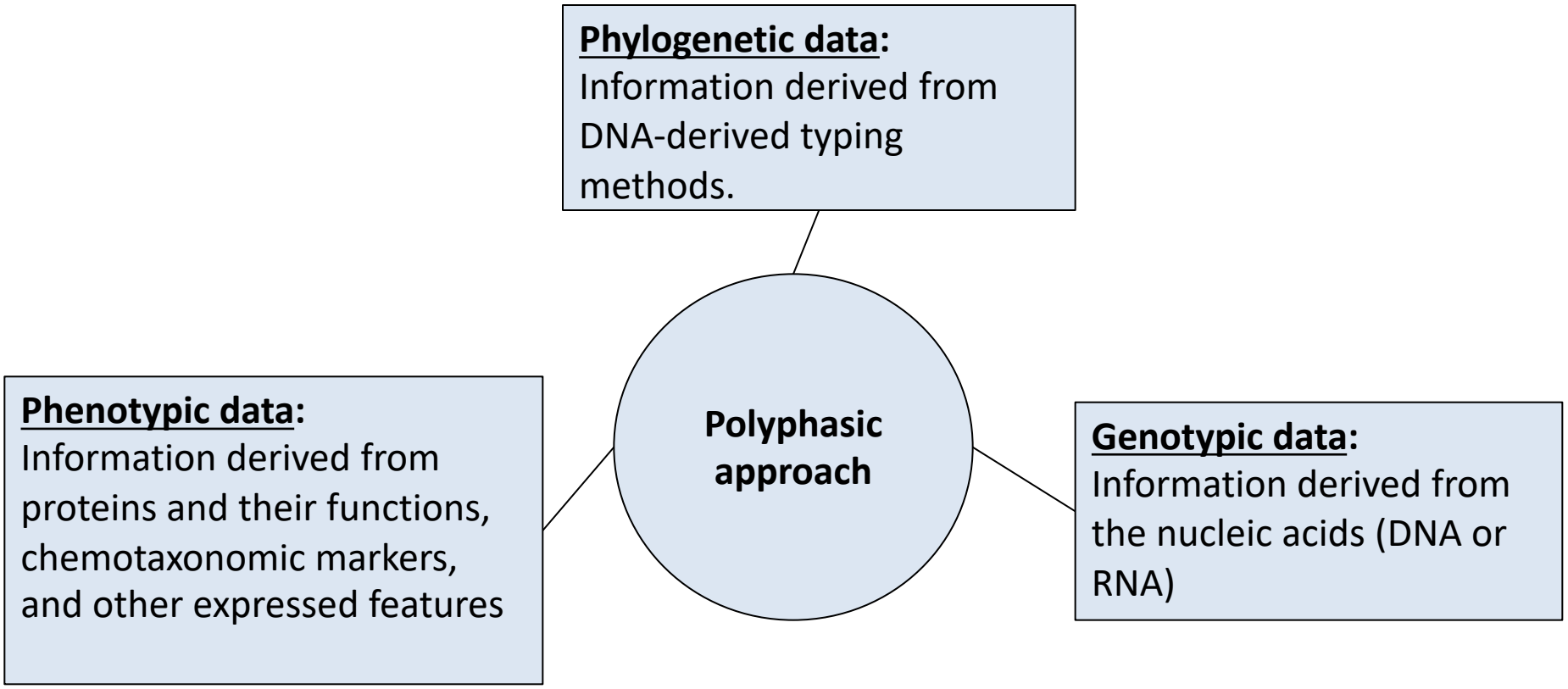
		Humans	Bacteria
I.	Kingdom	Animalia	Bacteria
II.	Phylum	Chordata	Actinobacteria
III.	Class	Mammalia	Actinobacteria
IV.	Order	Primata	Actinomycetales
V.	Family	Hominidae	Bifidobacteriaceae
VI.	Genus	Homo	Bifidobacterium
VII.	Species	Sapiens	lactis
VIII.	Subspecies	Caucasoid	lactis
IX.	Strain	Wilbert Sybesma	Bb12®

Relevant for industrial characterization

Polyphasic approach to bacterial taxonomy

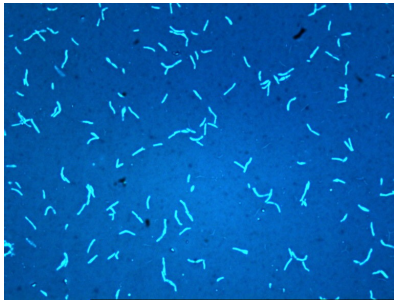
A bacterial species may be regarded as a collection of strains that share many features in common and differ considerably from other strains

Staley and Krieg, Bergey's Manual, 1984

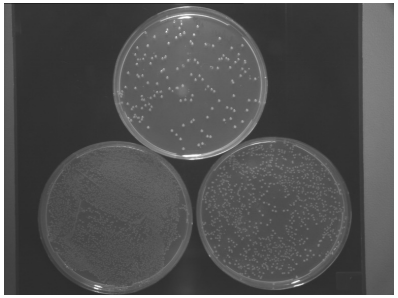


Phenotypic data / methods

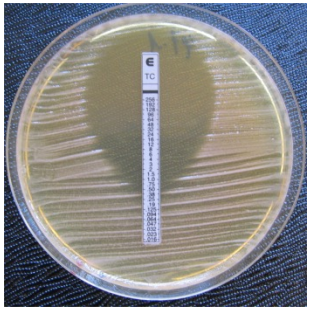
Information derived from proteins and their functions, chemotaxonomic markers, and other expressed features



Morphological description:
- **Shape**
- **Gram coloration**
- **Etc…**



Colony morphology



Antibiotic resistance profiling

Biochemical description:
- **Sugar fermentation profile**
- **Enzyme activity profiling**
- **Lactic acid production**
- **Etc…**

Evaluation of Matrix-Assisted Laser Desorption Ionization–Time-of-Flight Mass Spectrometry in Comparison to 16S rRNA Gene Sequencing for Species Identification of Nonfermenting Bacteria[▽]

A. Mellmann,[1]* J. Cloud,[2] T. Maier,[3] U. Keckevoet,[1] I. Ramminger,[1] P. Iwen,[4] J. Dunn,[5] G. Hall,[6] D. Wilson,[6] P. LaSala,[7] M. Kostrzewa,[3] and D. Harmsen[8]

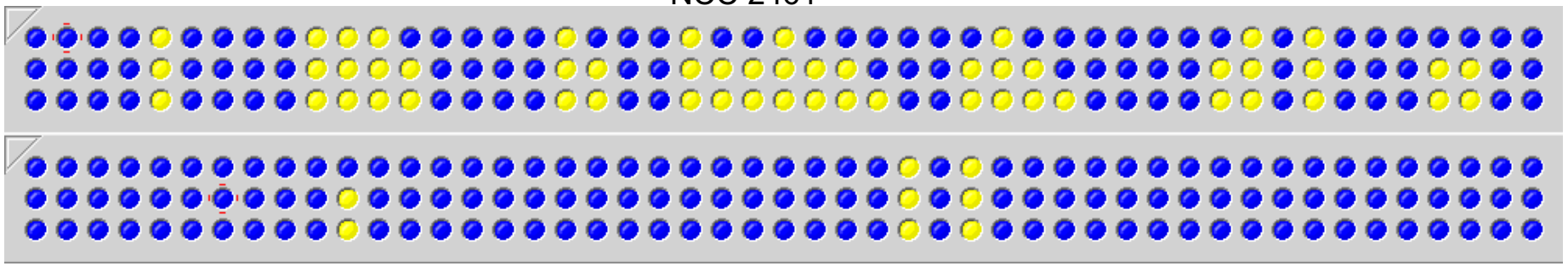
All cell protein profiling using mass spectrometry (MALDI-Tof)

API-50 CH(L) (Biomérieux)

- API 50 CH(L), indented for identification of *Lactobacillus* and related organisms.
- Ready to use system which enable the fermentation of 49 carbohydrates.
- Used to determine the sugar fermentation profile of the bacteria, not for ID purposes.
- Part of the basic characterization of bacteria in a culture collection.

http://www.biomerieux.fr

NCC 2461

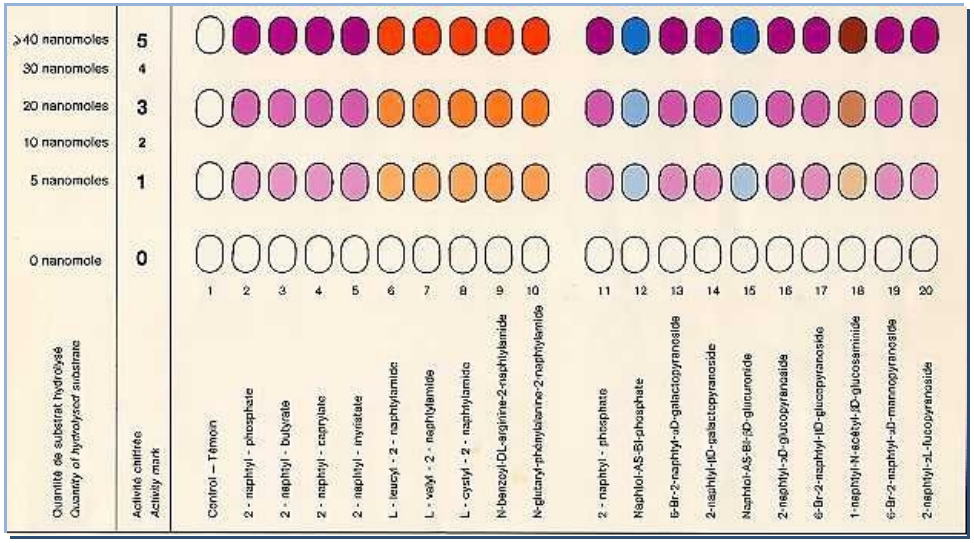


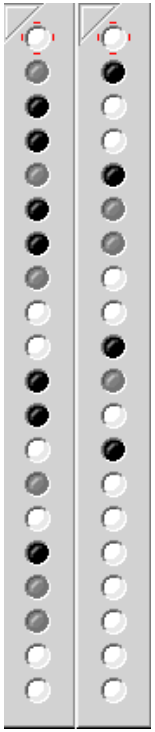
NCC 2496

API-ZYM (Biomérieux)

- Semi-quantitative micromethod designed for the research of enzymatic activities.
- Allows the systematic and rapid study of 19 enzymatic reactions using very small sample quantities.
- Part of the basic characterization of bacteria in culture collections.

http://www.biomerieux-usa.com/clinical/api

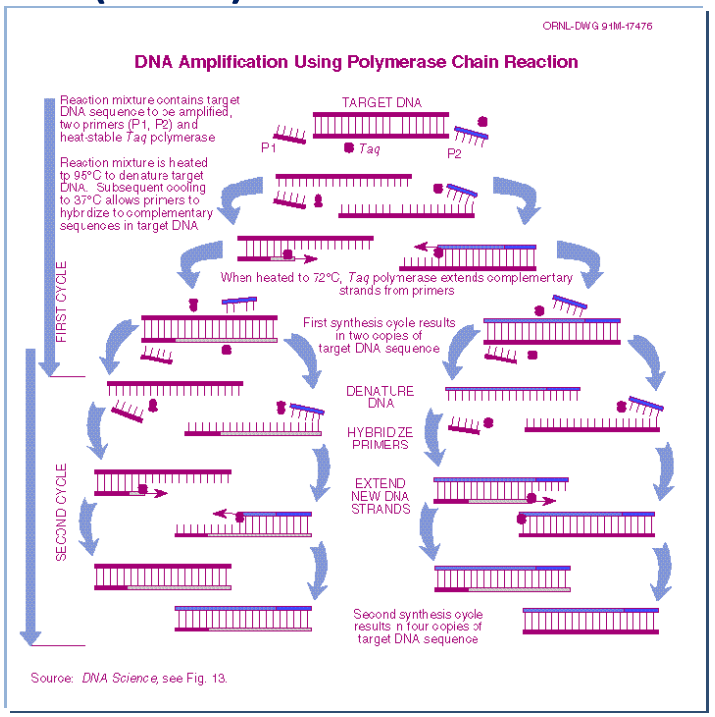




NCC 2461 NCC 2496

Identification - Many approaches based on: Polymerase Chain Reaction (PCR)

- Amplify single/few copies of a piece of DNA. Generate millions of copies of a particular DNA sequence.
- Developed by Kary Mullis (1983). Nobel Prize in Chemistry with Michael Smith (1993)
- Indispensable technique in medicine & biology
  - DNA cloning for sequencing
  - DNA-based phylogeny
  - Functional analysis of genes
  - Diagnosis of hereditary diseases
  - Identification of genetic fingerprints (used in forensic sciences and paternity testing)
  - Detection and diagnosis of infectious diseases
- Thermal cycles of heating & cooling for DNA melting and enzymatic DNA replication. Primers (short DNA fragments) containing sequences complementary to target region along with DNA polymerase are key to enable selective and repeated amplification.



http://www.ucl.ac.uk/~ucbhjow/b200/pcr.htm

Cycles of:

1. **Denaturation**
2. **Annealing**
3. **Extension**

E.g. DNA profiling uses repetitive sequences that are highly variable; variable number tandem repeats (VNTRs), particularly short tandem repeats (STRs). VNTR loci are very similar between closely related humans, but so variable that unrelated individuals are extremely unlikely to have the same VNTRs.

Genotypic data / methods

Earlier

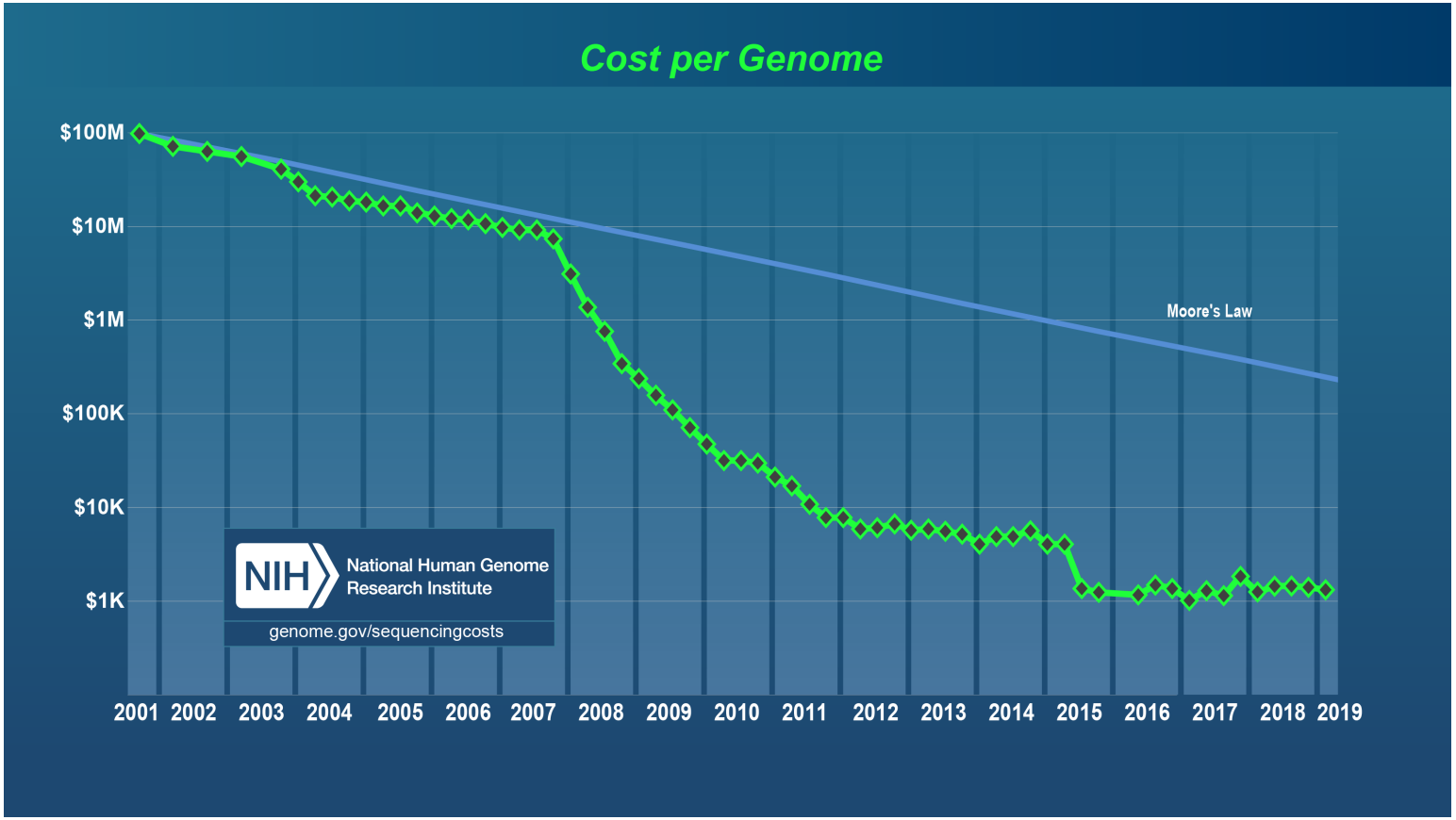
- DNA/DNA or RNA/RNA hybridization

Today

- 16S or 23S rDNA sequencing
- Multi-Locus Sequence Analysis (MLSA), sequencing of several well conserved genes
- Whole genome sequencing

Cost evolution of sequencing

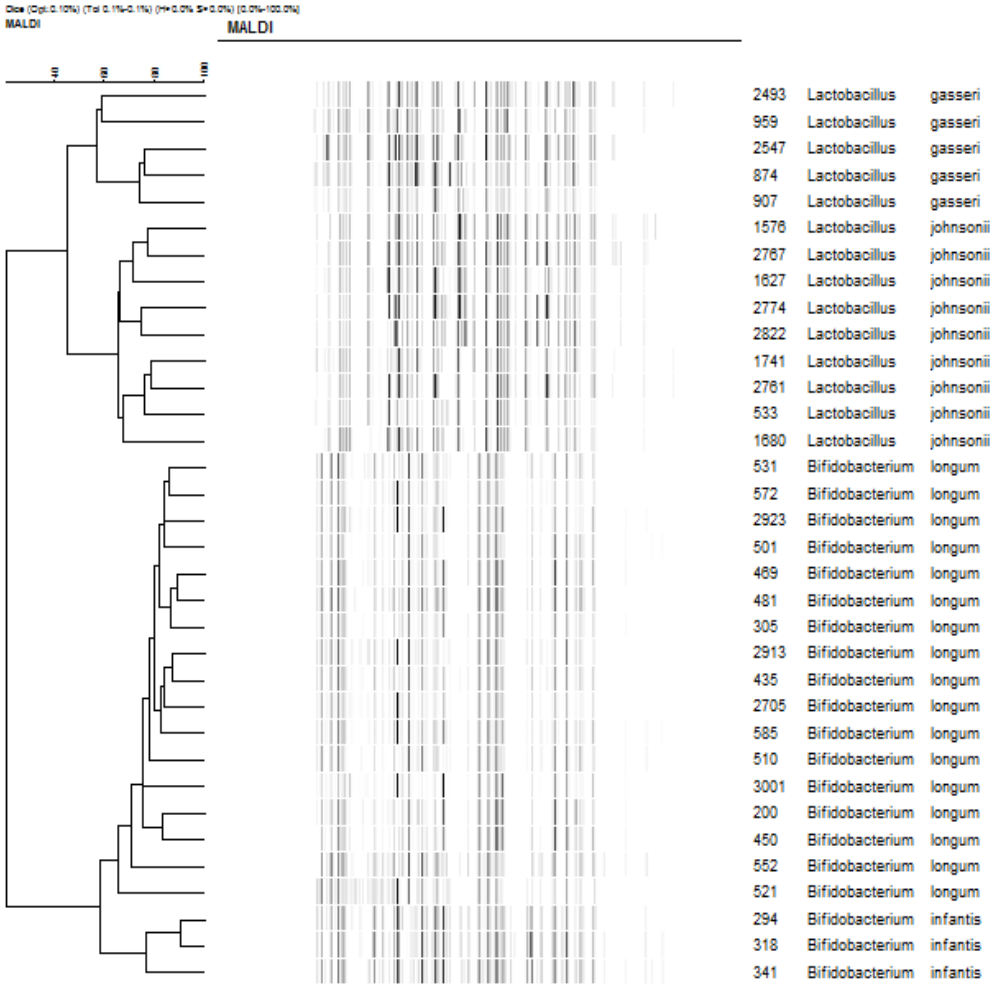
- Question: Will it go below zero? Why, why not?



MinION: A complete DNA sequencer on a USB stick

https://nanoporetech.com/products/minion

Identification by MALDI-Tof MS



English EN Search

European Commission > Food, farming, fisheries > Food Safety > Food > Labelling and nutrition >

Food

Union rules on nutrition and health claims have been established by **Regulation (EC) No 1924/2006**. The Regulation started to apply on 1 July 2007.

This regulation is the legal framework used by food business operators when they want to highlight the particular beneficial effects of their products, in relation to health and nutrition, on the product label or in its advertising.

The rules of the Regulation apply to **nutrition claims** (such as "low fat", "high fibre") and to **health claims** (such as "Vitamin D is needed for the normal growth and development of bone in children").

The objective of those rules is to ensure that any claim made on a food's labelling, presentation or advertising in the European Union is **clear, accurate and based on scientific evidence.**

Food bearing claims that could mislead consumers are prohibited on the EU market.

This not only protects consumers, but also **promotes innovation and ensures fair competition**. The rules ensure the free circulation of foods bearing claims, as any food company may use the same claims on its products anywhere in the European Union.

There are different procedures managed by the Commission for the various types of claims, with regard to their authorisation.

A **public EU Register of Nutrition and Health Claims** lists all permitted nutrition claims and all authorised and non-authorised health claims, as a source of reference and so that full transparency for consumers and food business operators is ensured.

Roadmap to review the Nutrition and Health Claims legislation

https://ec.europa.eu/food/safety/labelling_nutrition/claims_en

Additional slides on strain identification

From traditional to industrial food fermentation
What does it mean?

- **The microbes were "always there" - thus all food raw materials (cereals, vegetables, fruit, milk, meat, etc.) are inhabited by microbes (bacteria, yeasts, molds).**
- **Some are spoiling food and can be dangerous for health or may even be live threatening at high doses.**
- **Some can provide benefits when multiplying and deploying their functionality during the fermentation process, such as**
  - **Enhancing the diet through development of nice flavors, aromas, and textures**
  - **Suppressing the harmful microorganisms through formation of organic acids, alcohol, and antimicrobial compounds,**
  - **Enriching food with proteins, essential amino acids, fatty acids, vitamins**
  - **Removing anti-nutritrional factors or improving "bio-availability"**
  - **Probiotics - Providing beneficial effects in the gut of humans & animals**

From traditional to industrial food fermentation
Identifying Microorganisms as cause of Fermentation

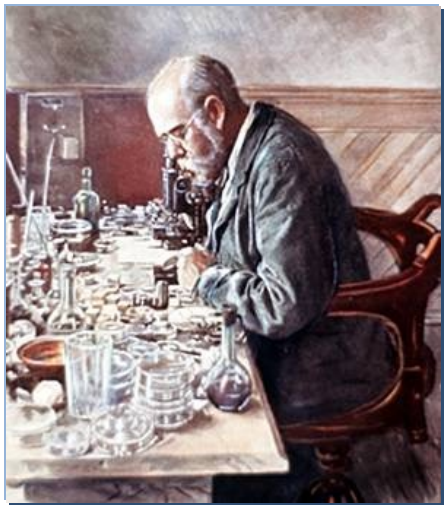
Breakthrough Discoveries

- Only three of many other scientists are shown here. They stand out as those who made the big breakthrough discoveries
- These were achieved always after decade-long fierce fights among scientists of different disciplines, mainly chemists, physicians and biologists

Louis Pasteur
1822-1895

http://fr.wikipedia.org/wiki/Fichier:Albert_Edelfelt_-_Louis_Pasteur_-_1885.jpg

Robert Koch
1843-1910



http://www.questmachine.org/article/Robert_Koch

Eduard Büchner
1860-1917

http://de.wikipedia.org/wiki/Eduard_Buchner

From traditional to industrial food fermentation
Discoveries leading to modern Fermentation

- Louis Pasteur was certainly one key person advancing the scientific principles of food fermentation.
- He was originally a chemist but with extended knowledge in medicine and biology and a strong interest in "applied sciences".
- He demonstrated (among many other things) that alcoholic fermentation is caused by the growth of microorganisms (process called "biogenesis"), and not by "spontaneous generation" as leading chemists claimed.

- For further information see e.g. http://en.wikipedia.org/wiki/Louis_Pasteur

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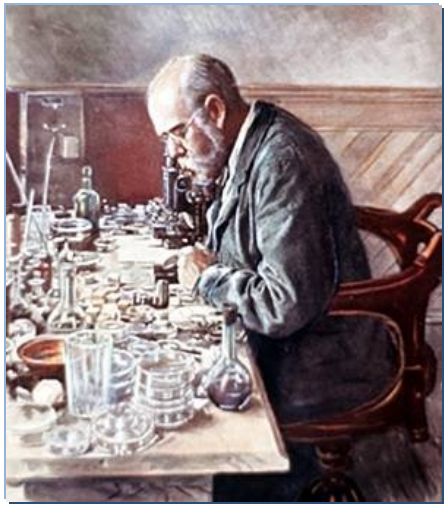
- Robert Koch was a physician and founder of (medical) microbiology
- Father of the "germ theory" (together with Pasteur)
- Koch's postulates are 4 criteria to establish a causal relationship between a microbe and a disease.
  1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms
  2. The microorganism must be isolated from a diseased organism and grown in pure culture
  3. The cultured microorganism should cause disease when introduced in a healthy organism
  4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent
- Co-inventor of the "Kochsche's Plattengussverfahren", thus using the "Petri-dish" (Julius Richard Petri was one of his students), as a technique to isolate "pure" microbial strains.

- **For further information see e.g. http://en.wikipedia.org/wiki/Robert_Koch)**

Robert Koch
1843-1910



http://www.questmachine.org/article/Robert_Koch

From traditional to industrial food fermentation
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- Eduard Büchner was a physician who won the Nobel Prize for unequivocally demonstrating that a "cell-free extract" of yeast cells was the cause of the biotransformation (fermentation) of sugar to alcohol. Thus he gave the final blow to "vitalism" (see http://en.wikipedia.org/wiki/Vitalism)
- Postulated that the functions of a living organism are due to a "vital principle distinct from biochemical reactions."
- Postulated that the fermentation process by yeast was due to proteins (enzymes) they contain. Thus he made the link between microbial physiology and biochemistry.

- For further information see e.g. http://en.wikipedia.org/wiki/Eduard_Buchner

Eduard Büchner
1860-1917

http://de.wikipedia.org/wiki/Eduard_Buchner

Green Light Bioscience

https://www.greenlightbiosciences.com

Inside these tanks, yeasts are busily fermenting grape juice into wine. Why do winemaking tanks like these need pressure-release valves?

A. The yeasts produce O2 gas by cellular respiration.
B. The yeasts produce CO2 gas by lactic acid fermentation.
C . The yeasts produce CO2 gas by alcohol fermentation.
D. The yeasts produce CO2 gas by cellular respiration.

https://www.khanacademy.org/science/biology/cellular-respiration-and-fermentation/variations-on-cellular-respiration/a/fermentation-and-anaerobic-respiration