

# Food Fermentation Technology

Part 2

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**EPFL Course ENG-436**

**Only for Teaching Purposes  
Personal Copy**

Common Fermentation Organisms in Food									
Lactobacillaceae		Streptococcaceae, Enterococcaceae		Other bacteria		Fungi		Yeasts	
<i>Lactobacillus</i>	L.	<i>Lactococcus</i>	Lc.	<i>Acetobacter</i>	Ac.	<i>Aspergillus</i>	A.	<i>Saccharomyces</i>	S.
<i>Leuconostoc</i>	Lu.	<i>Streptococcus</i>	Sc.	<i>Staphylococcus</i>	St.	<i>Penicillium</i>	P.	<i>Candida</i>	C.
<i>Weissella</i>	W.	<i>Tetragenococcus</i>	T.	<i>Gluconacetobacter</i>	Gl.	<i>Geotrichum</i>	G.	<i>Debaryomyces</i>	D.
<i>Pediococcus</i>	Pc.	<i>Enterococcus</i>	E.	<i>Bacillus; Lentibacillus</i>	Bo.; Lt	<i>Monascus</i>	M.	<i>Kluyveromyces</i>	K.
<i>Oenococcus</i>	O.	Non starter lactic acid bacteria	NSLAB	<i>Brevibacterium</i>	Br.	<i>Rhizopus</i>	R.	<i>Zygosaccharomyces</i>	Z.
				<i>Propionibacterium</i>	Pr.	<i>Botrytis</i>	B.	<i>Blastobotrys</i>	Bl.

# Pure and mixed strain cultivation technologies

- The step from mixed to pure culture is done to control, reproduce and standardize industrial processes. Therefore food industries (such as Dairy, Baking, Brewing, Wine) use increasingly pure –or a limited number of- bacterial, yeast and mold cultures for fermentation processes. These cultures are generally derived from proprietary or public microbial culture collections
- To start a fermentation process, seed cultures with appropriate cell numbers are prepared and then transferred sterile into the chosen fermentation system.

## Liquid/submerged Fermentation



Most fermentations are done in “stirred tank bioreactors”. Allow to heat-sterilize liquid media and perform fermentation under sterile, controlled (T, pH, pO<sub>2</sub>) conditions.

## Solid-state Fermentation



Solid-state fermentation is characterized by low water content. A Koji fermenter, e.g. for “culinary biohydrolysates” using the “Koji mold”, which is inoculated as pure culture. Many traditional fermentations, e.g. Salami, cheese or sauerkraut are solid-state, but they do not always rely on pure cultures.

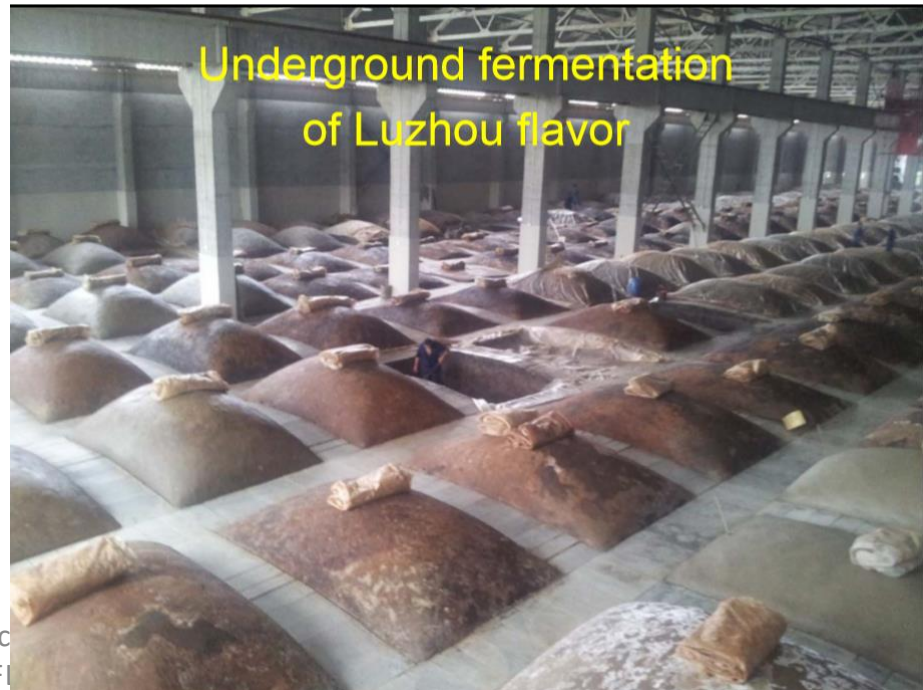
# Pictures for illustration

## Solid state fermentation

Storage of *Daqu* (6 months)



Underground fermentation  
of Luzhou flavor



# Mastering the Microflora in Raw material, Fermentation Process & Product

## In Raw material(s)

- As first step of fermentation processes, measures are taken to eliminate or at least drastically reduce the contaminating microflora from the raw materials. This is feasible in submerged, but is often difficult in solid-state fermentation processes. The most common sterilization or sanitation measure is thermal treatment.

## In Fermentation Process

- Whenever possible, process conditions are chosen that the desired (inoculated) microorganism can multiply and perform its function optimally, and that contaminating microflora cannot multiply to significant levels

## In Product

- Measures are taken to suppress further proliferation of microorganisms in the product. Depending on product characteristics, this is achieved via appropriate physico-chemical parameters ( $T$ ,  $pH$ ,  $a_w$ ,  $pO_2$ ) e.g. via drying, freezing, salting, acidification.



## In Raw Material(s)

- Killing all or the majority of contaminating microorganisms



## In Fermentation Process

- Controlling microbial propagation via
  - choice of physico-chemical parameters
  - "protective" microorganisms



## In Product

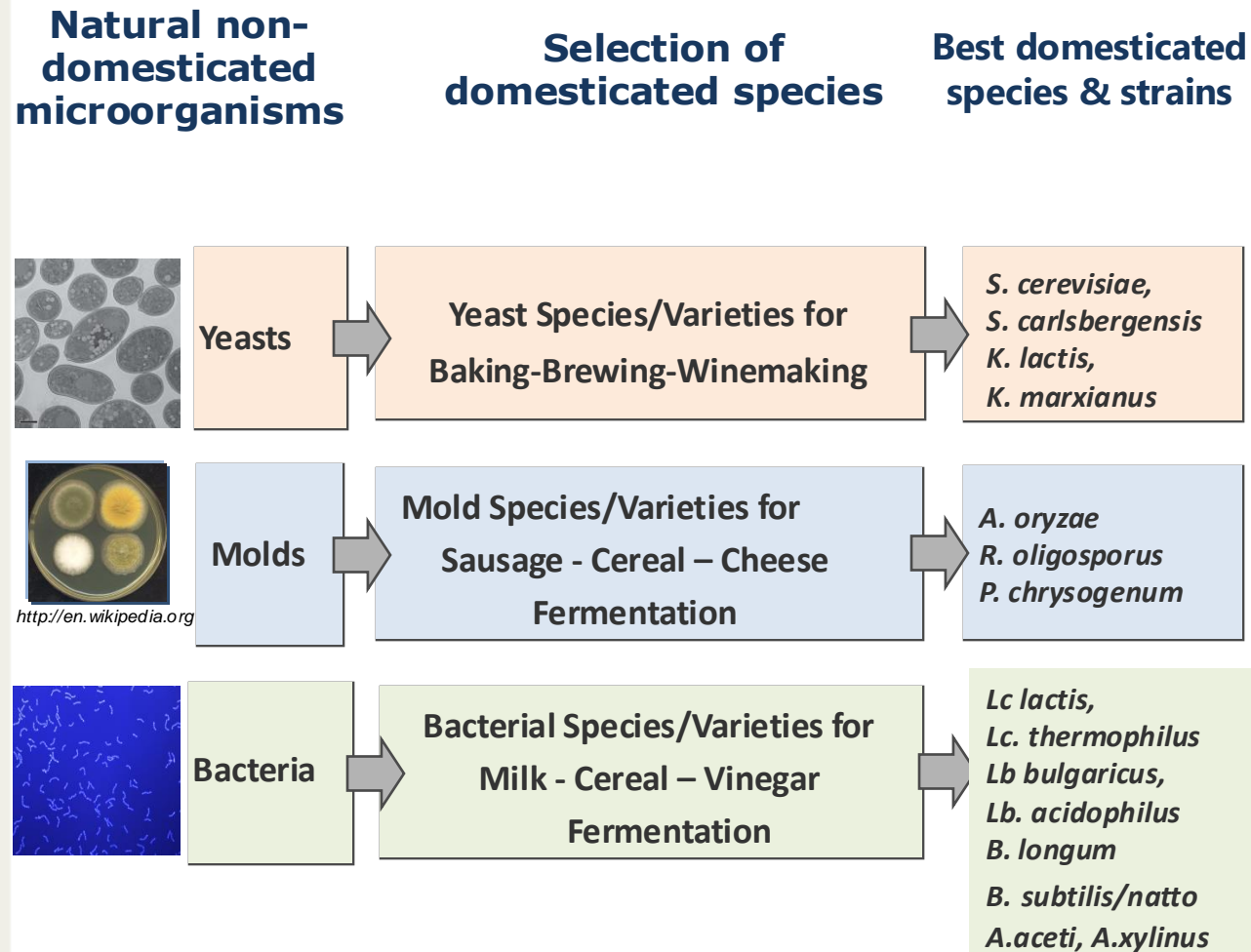
- Suppressing further proliferation of microorganisms
- Is this always the case?***

# “Domestication” of Food Microorganisms

- Microorganism were always in the raw materials, not to please man, but for their own cause. Seen from the angle of these microorganisms, fermented food was therefore a “side effect”
- Man learned to appreciate the value of this “side effect” and started to “domesticate” food-borne microorganisms, i.e. to adapt the natural microorganisms to his special needs. Domestication probably went in two stages.
  - First stage: Fit needs of traditional food fermentation
  - Second stage: Fit needs of industrial food fermentation

# Examples of domestication of yeasts, molds and bacteria for fermented food applications

- The best domesticated species and strain variants come out of a pool of natural microbial species
- After used successfully for the production of fermented food for years, they are listed as “Generally Regarded As Safe” (GRAS), according to the US regulations or having “Qualified Presumed Safety” (QPS) status, according to EU regulations

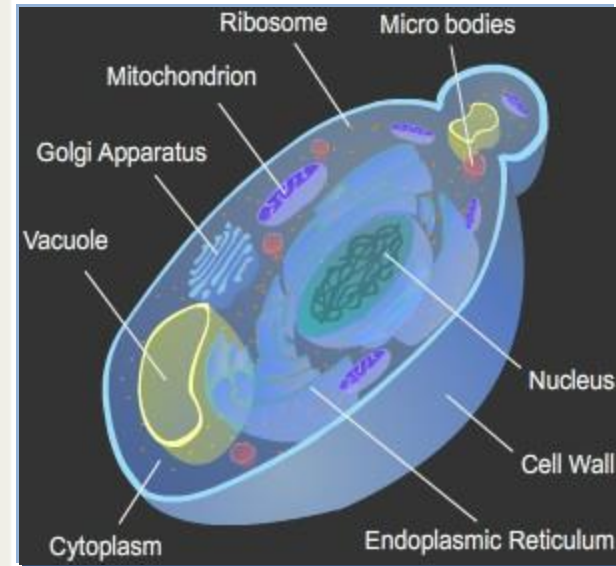


# Yeast - History

- "Yeast" comes from Old English gist, gyst, and from the Indo-European root yes-, meaning boil, foam, or bubble.
- One of the earliest domesticated organisms. Archaeologists in Egyptian ruins found 4,000-year-old grinding stones and baking chambers for yeasted bread
- In 1680, the Dutch, Anton van Leeuwenhoek, first microscopically observed yeast, but did not consider them to be living organisms, but rather globular structures.
- In 1857, Louis Pasteur proved "Mémoire sur la fermentation alcoolique" that fermentation was conducted by living yeasts and not by a chemical catalyst.
- Late 18th century, 2 strains identified: *Saccharomyces cerevisiae* (top fermenting), *S. carlsbergensis* (bottom fermenting). *S. cerevisiae* was sold by Dutch for bread since 1780; while around 1800, Germans started producing *S. cerevisiae* as cream. In 1825 a method was developed to remove liquid to prepare yeast as solid blocks.

# Yeast

- Eukaryotic micro-organisms classified in Fungi kingdom. 1.500 species described, estimated to be only 1% of all fungal species.
- Most reproduce asexually by budding. A few by mitosis
- Yeast size vary by species, typically 3–8  $\mu\text{m}$  in diameter. Some yeasts can reach over 40  $\mu\text{m}$ .
- Prefer acidic pH 3.5–5.0. Usually do not form mycelia
- Important in biotechnology
  - *Saccharomyces cerevisia* (brewing, wine-making, baking)
  - *Candida utilis*
  - *Schizosaccharomyces pombe*
  - *Hansenula polymorpha*
  - *Pichia pastoris*
- Pathogenic yeasts found within *Candida* genus (e.g. skin)
- Cell wall: glucans, mannans. High in proteins (enzymes)
- In 1996, *S. cerevisiae* became first eukaryote to have entire genome sequenced. > 12 million base pairs and around 6000 genes. On the forefront of genetic research



<http://www.biocourseware.com/iphone/cell/>

# Domestication of Yeast

- Brewer's yeast, Baker's yeast and "Academic" (laboratory) yeasts are genetic variants of the same or closely related species *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*
- The latter is a genetic hybrid of *S. cerevisiae* and *S. monacensis*
- The three yeast variants show increasing degree of domestication and accessibility to "modern" genetic strain development.

Brewer's Yeast  
Low  
domestication



**Lager-Beer**  
*S. carlsbergensis*

Difficult access to  
genetic strain  
development

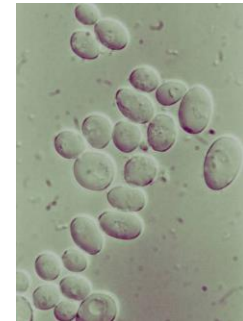
Baker's Yeast  
Medium  
domestication



**Dough**  
*S. cerevisiae*

Traditional  
genetic strain  
development

"Academic" Yeast  
High  
domestication



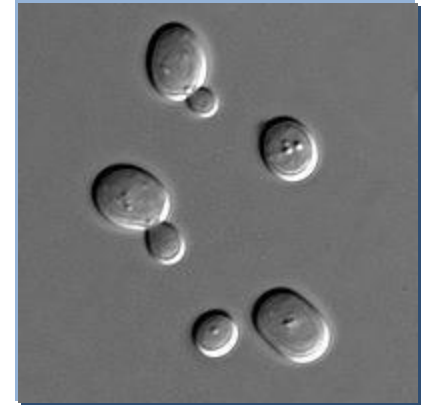
Various  
laboratory  
cell lines,  
S288C,  
X2180-1A,  
CEN.PK,  
ENZ WA-1B

**Laboratory**  
*S. cerevisiae*

Traditional & recombinant  
genetic strain  
development

# Yeast (Bread)

- Dough Rising:
  - Air is incorporated into the dough by mixing and the yeast is able to establish respiratory-type metabolism.
  - Few minutes after mixing, all oxygen is consumed by the yeast.
  - Anaerobic conditions: yeast metabolism is geared towards fermentation.  $\text{CO}_2$  produced
- Acidification: by organic acids
- Flavour production by secondary fermentation
- Substrates:
  - Monosaccharides (glucose, fructose, galactose)
  - Sucrose (after invertase in the yeast)
  - Maltose (from starch). 2 glucose by maltase. In Europe most industrial yeasts have constituent maltosepermease (transport) and maltase.



Wikipedia



# Yeast Extract

## Yeast Extract

- Made by extracting the cell contents (removing the cell walls); they are used as food additives or flavourings, or as nutrients for bacterial culture media. They are often used to create savory flavors and umami taste sensations. Monosodium glutamate (MSG) is used for umami. Yeast extracts in liquid form can be dried to a light paste or a dry powder.

## Autolyzed Yeast

- Containing the cell walls or autolyzed yeast extract consists of concentrations of yeast cells that are allowed to die and break up, so that the yeasts' endogenous digestive enzymes break their proteins down into simpler compounds (amino acids and peptides).

## Hydrolyzed yeast

- Hydrolyzed yeast or hydrolyzed yeast extract is another version used as flavouring. Exogenous enzymes or acids are used to hydrolyze the proteins



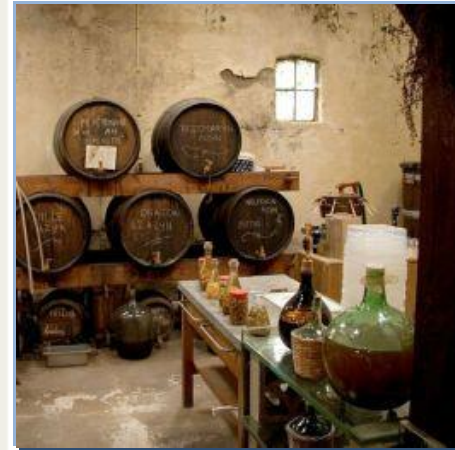
<https://commons.wikimedia.org/wiki/Category:Marmite?uselang=de>



<https://vegemite.com.au/>

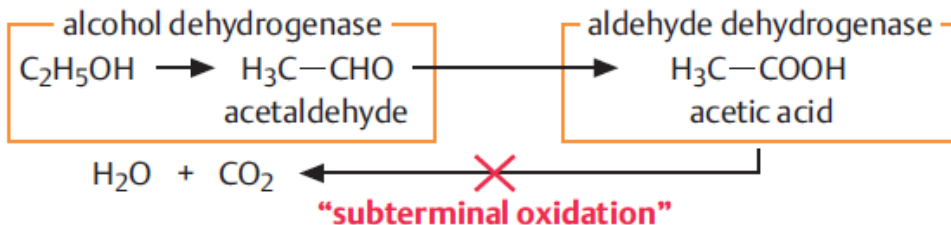
# Vinegar Fermentation

- Vinegar is an acidic liquid produced from fermentation of ethanol in a process that yields its key ingredient, acetic acid.
- pH of table vinegar ranges from 2.4 to 3.4 (higher if diluted).
- Acetic acid: 4-8% for table vinegar and up to 18% for pickling vinegar.
- Ethanol is first produced as result of yeast fermentation of sugars from different sources (e.g. wine, cider, beer, fermented fruit juice)
- Ethanol is then oxidized to acetic acid by acetic acid bacteria (AAB).
- Acetic acid bacteria derive energy from oxidation of ethanol to acetic acid during fermentation. Gram-negative, aerobic, rod-shaped.
- Aeration is crucial in the fermentation process.
  - Excess air can ruin the product by complete oxidation of carbohydrates to  $\text{CO}_2$  by yeast and other aerobic bacteria
  - Too little air will lead to high concentrations of alcohol resulting in the death of the acetic acid bacteria.

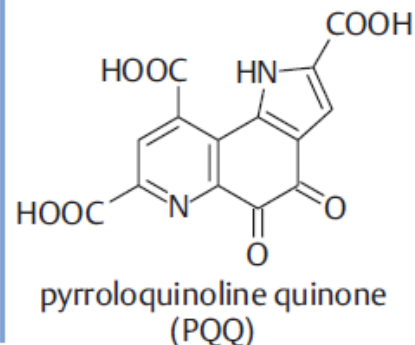


<http://hubpages.com/hub/Vinegar-Love-to-the-Mother>

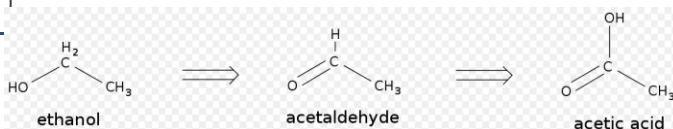
## Biosynthesis by *Acetobacter* sp.



the membrane-bound, PQQ-dependent dehydrogenases transfer the electrons generated by the oxidation of ethanol *via* ubiquinone to a membrane-bound terminal oxidase



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



# Chilled Pizza Dough and Baker's Yeast

## The Problem and the Solution



The Problem:  
Too high activity even under refrigeration



The solution:  
Very little activity under refrigeration  
but normal activity at higher  
temperatures

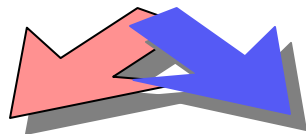
# A need for Low Temperature Inactive (LTI) Baker's Yeast

## Requirements:

**“Wild” Baker's yeast”**  
(*Saccharomyces cerevisiae*)

- **Activity** – to guarantee an open dough texture a residual yeast activity during storage or immediately prior to baking must remain.
- **Consumer acceptance**– the new baker's yeast variety had to be obtained purely by classical strain development methods.

**Yeast  
producers**



**Nestlé  
Research**

Selection für  
strong CO<sub>2</sub> development

Selection for strongly reduced  
CO<sub>2</sub> development under refrigeration

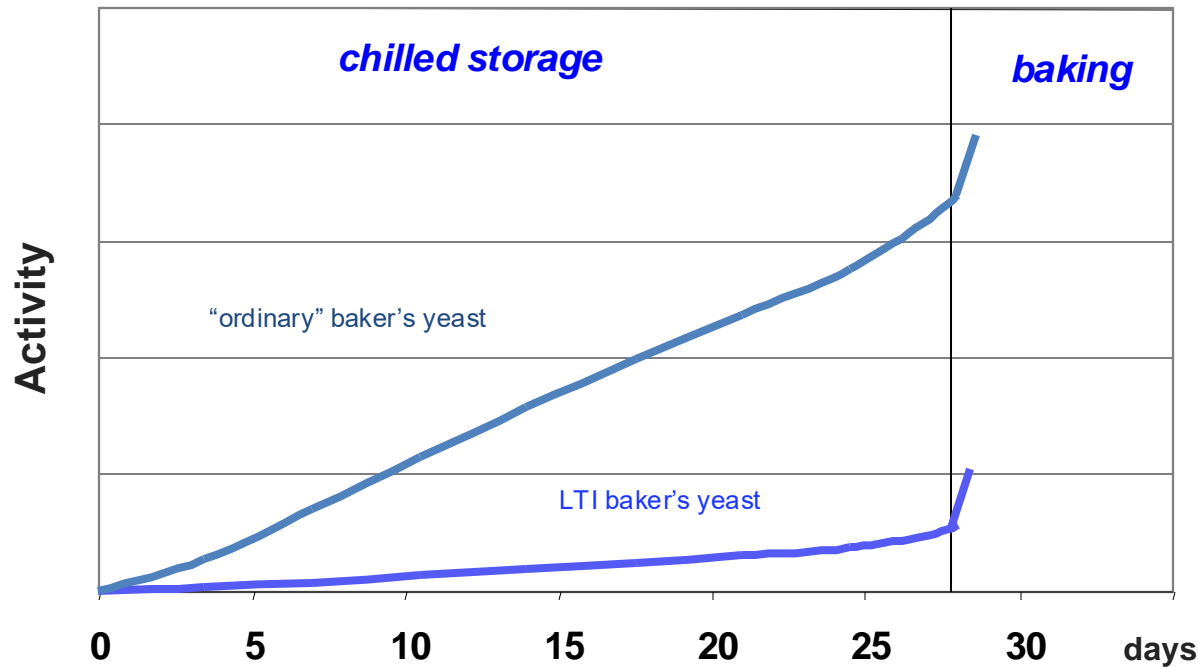
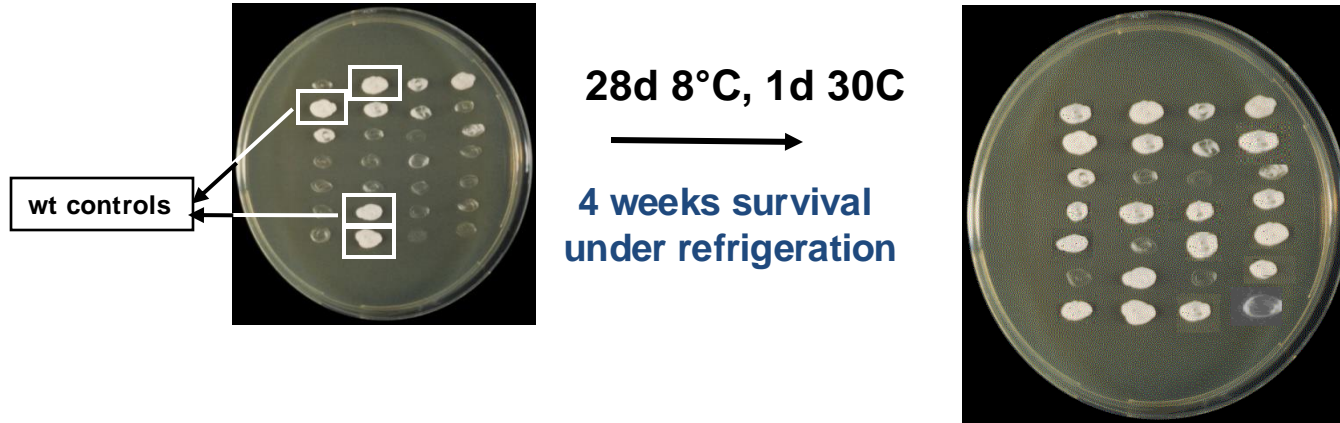
**“Ordinary”  
Baker's yeast varieties**

**LTI  
Baker's yeast variety**



# Strain construction and selection

## 1) EMS mutagenesis and selection for desired phenotype:



# Domestication of Dairy Bacterial strains

- “Natural” Dairy starters were isolated as pure strains from artisanal fermented milk
- This yielded domesticated strain variants of the species *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for “standard” yoghurt and mainly *Lactobacillus lactis*, *Lb. helveticus* and *Lb. casei* for cheese. For Kefir production the “milk yeast” *Kluyveromyces lactis* was isolated
- For “functional” milk drinks, the same strains are used, but probiotic strains are added. Pure probiotic strains from *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lb. johnsonii* and *Lb. rhamnosus* were isolated from intestinal tracts of animals and human infants. A probiotic yeast *Saccharomyces boulardii* was isolated (paramedical products)

## Artisanal Fermented Milk e.g. Kefir, Kumis



<http://mondesteph.worpress.com/page/16/>

*Lactobacillus* sp.  
*Streptococcus* sp.  
“Milk yeasts”

## “Standard” Yoghurt (& Cheese)



*Sc. thermophilus*  
*Lb. bulgaricus*  
*Lb. helveticus*  
*Lb. lactis*  
*Lb. helveticus*  
*Lb. casei*  
*K. lactis*

## “Functional” milk drinks



*Sc. thermophilus*  
*Lb. bulgaricus*  
*Lb. helveticus*  
*Lb. lactis*  
+ Probiotic strains  
*Lactobacillus* sp.  
*Bifidobacteria* sp.  
(*S. boulardii*)

# Yogurt (1)

- Yoghurt, yogurt or yogourt (Turkish: yoğurt) is produced by bacterial fermentation of milk. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yoghurt its texture and its characteristic tang.
- Cow's milk is most commonly used to make yoghurt, but milk from water buffalo, goats, sheep, camels and yaks is also used in various parts of the world. Soya yoghurt, a non-dairy yoghurt alternative, is made from soy milk
- The milk is first heated to about 80 °C to kill undesirable bacteria and to denature the milk proteins so that they set together rather than form curds. The milk is then cooled to about 45 °C. The bacteria culture is added, and the temperature is maintained for 4 to 7 hours to allow fermentation.
- **Dairy yoghurt is produced using a culture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus salivarius subsp. thermophilus* bacteria.** In addition, *Lactobacillus acidophilus*, *Lactobacillus bifidus* and *Lactobacillus casei* are also sometimes used in culturing yoghurt.
- **The total organisms in a refrigerated yogurt should be at least  $10^7$  cfu/g upon consumption.**

Wikipedia

# Yogurt (2)

- Yogurt fermentation is a homolactic fermentation
- Lactose utilization involves lactose transport into the cells via permeases.
- Lactose is hydrolysed by  $\beta$ -galactosidase to glucose and galactose.
- Glucose is metabolized by *Lactobacillus delbrueckii* and *Streptococcus thermophilus* bacteria (and bifidobacteria?)
- Glucose is catabolized and galactose is secreted from the cells
- **Advantages:**
  - *Lactobacilli* have proteolytic activity to hydrolyse proteins into free amino acids and peptides
  - This stimulates the growth of *Streptococcus*
  - On the contrary, *Streptococcus* form formic acid, pyruvate and CO<sub>2</sub>, which stimulates the *Lactobacilli*
  - Since the *Lactobacilli* are more resistant to low pH, it is key to stimulate the *Streptococcus* early in the process

# Kefir

- Kefir from either the Arabic "keyf" (joy/pleasure) or the Turkic "köpür" ((milk) froth, foam)
- Fermented milk drink that originated with shepherds of the North Caucasus region, who discovered that fresh milk carried in leather pouches would occasionally ferment into a sparkling beverage.
- Kefir grains are a combination of bacteria and yeasts in a matrix of proteins, lipids, and sugars. This symbiotic matrix forms "grains" that resemble cauliflower. Many different bacteria and yeasts are found in the kefir grains, which are a complex and highly variable community of lactic acid bacteria and yeasts.
- Traditional kefir is fermented at ambient temperatures, generally overnight. Fermentation of the lactose yields a sour, carbonated, slightly alcoholic beverage, with texture similar to thin yoghurt

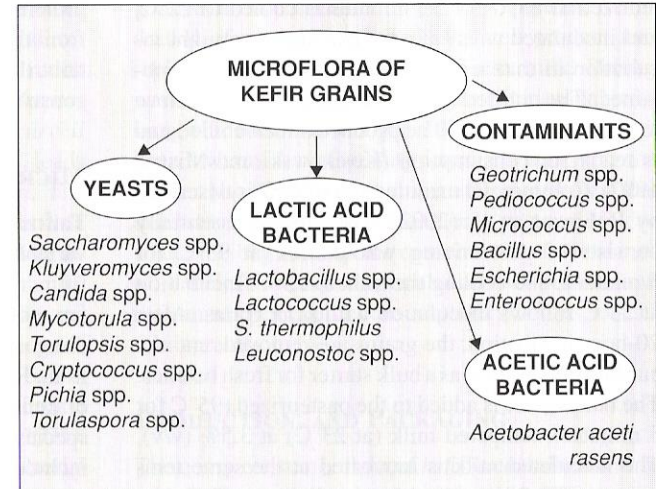


*Wikipedia*



# Kefir - Microbiology

- Microorganisms in the grains include LABs, *Lactococcus lactis subsp. lactis*, *Streptococcus thermophilus*, *Lb delbrueckii subsp. bulgaricus*, *Lb helveticus*, *Lb casei subsp. Pseudo-plantarum* and *Lb brevis*, a variety of yeasts, such as *Kluyveromyces*, *Torulopsis*, and *Saccharomyces*, acetic acid bacteria
- Lactose broken down to lactic acid by LABs. Slow-acting yeasts, late in the fermentation process, break lactose down to ethanol & CO<sub>2</sub>
- They give kefir excellent keeping qualities by keeping away putrifying bacteria that might otherwise colonise the milk. They can inhibit *Salmonella* and *E. Coli*.



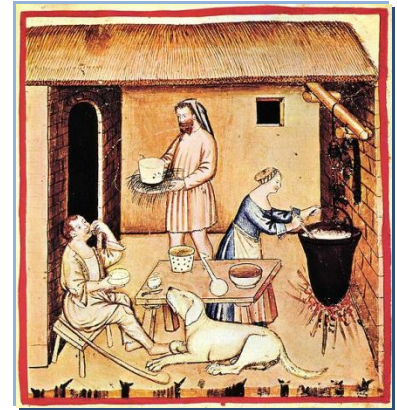
*Manufacturing yogurt and fermented milks, ed. RC Chandan, Blackwell publishing, 2006*



<http://en.wikipedia.org/wiki/Kefir>

# Cheese

- Cheese can be considered a concentrate of the valuable nutrients of milk in a stable form
- Origin of cheesemaking around 8000 BCE (when sheep were first domesticated) to around 3000 BCE. First cheese may have been made in Middle East or by nomadic Turkic tribes in Central Asia.
- A required step in cheesemaking is separating milk into solid curds and liquid whey. Usually this is done by acidification and adding rennet. Acidification can be done directly by addition of an acid like vinegar, but usually starter bacteria are employed.
- The starter bacteria convert milk sugars into lactic acid. The same bacteria (and the enzymes they produce) also play a large role in flavor of aged cheeses. Most cheeses are made with starter bacteria from the *Lactococci*, *Lactobacilli*, or *Streptococci* families. Swiss starter cultures also include *Propionibacterium freudenreichii*, which produces carbon dioxide



Wikipedia

# Rennet

- Complex of enzymes produced in mammalian stomach to digest the mother's milk, and is often used in the production of cheese.
- Rennet contains many enzymes, including a protease that coagulates the milk, causing it to separate into solids (curds) and liquid (whey). The active enzyme in rennet is called chymosin or rennin (EC 3.4.23.4). Other important enzymes: e.g., pepsin, lipase.
- Some molds, *Rhizomucor miehei* produce proteolytic enzymes. Produced in fermenter and concentrated and purified to avoid contamination with unpleasant by-products of the mold growth.
- Chymosin produced by GM organisms was the first GM produced enzyme registered and allowed by US Food and Drug Administration. In 1999, 60% of US hard cheese was made with GM chymosin with up to 80% of global market share for rennet. By 2008, approx. 80-90% of commercial cheeses in US were made with GM rennet.



Wikipedia

# Emmentaler cheese

- Emmentaler is a cheese from Switzerland. It is sometimes known as Swiss cheese in North America, Australia and New Zealand
- The cheese originally comes from the Emme valley in the canton of Bern. Unlike some other cheese varieties, the denomination "Emmentaler" was not protected ("Emmentaler Switzerland" is, though). Hence, Emmentaler of other origin, especially from France and Bavaria, is widely available and even Finland is an exporter of Emmentaler cheese.
- Emmentaler is a yellow, medium-hard cheese. Failure to remove CO<sub>2</sub> bubbles during production, due to inconsistent pressing, results in the large holes ("eyes") characteristic of this cheese. Historically, the holes were a sign of imperfection, and until modern times, cheese makers would try to avoid them. It has a piquant, but not very sharp, taste. Three types of bacteria are used in the production of Emmentaler: *Streptococcus thermophilus*, *Lactobacillus*, and *Propionibacterium freudenreichii*. In the late stage of cheese production, *P. freudenreichii* consumes the lactic acid excreted by the other bacteria, and releases carbon dioxide gas, which slowly forms the bubbles that make holes.



Wikipedia



Wikipedia



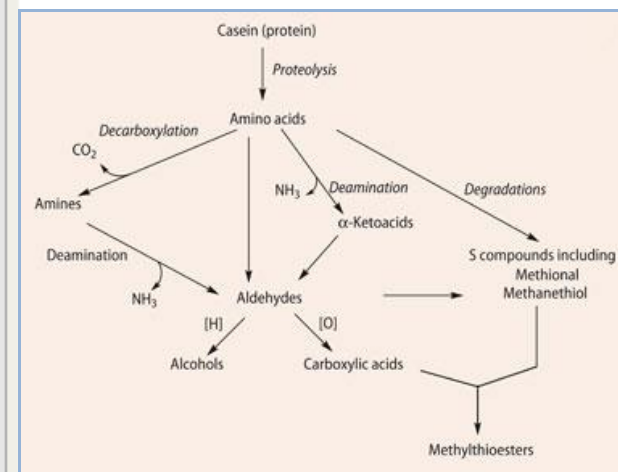
# Cheese flavour biochemistry (1)

- The molecules come from breakdown of the three types of chemical in the milk - **the protein casein, lipids in milk fat and lactose.**
- Lipolysis yields carboxylic acids**, the source of a range of aroma molecules (Table 1). By themselves, carboxylic acids would not give a cheese a wholesome aroma, so the presence of other molecules is necessary. The fatty acids are the source of the methyl ketones that give 'blue cheese' notes, and also react with alcohols, especially ethanol, to afford a range of aromatic esters.
- Proteolysis breaks down proteins like casein** - first into peptides and then into amino acids (Scheme 2). These contribute taste to cheese but more importantly undergo a wide range of transformations - decarboxylation, deamination, oxidation and reduction - again affording a whole range of short-chain volatiles.

<http://www.rsc.org/Education/EiC/issues/2011January/ReallyCheesyChemistry.asp>

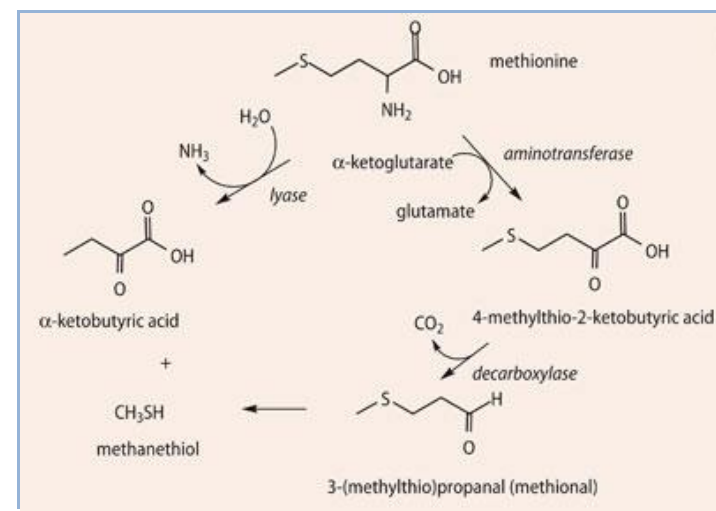
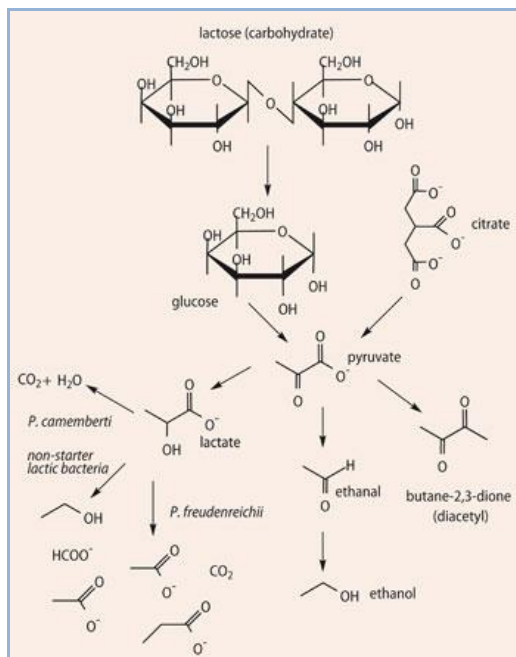
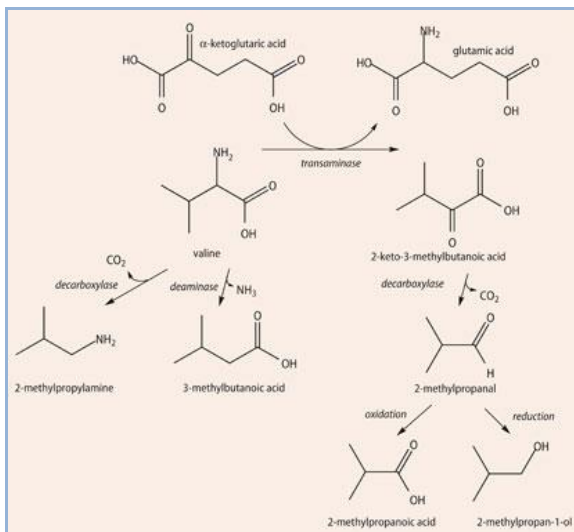
**Table 1** Breakdown products of major milk components.

Casein	Milk fat	Lactose
Ammonia	Carboxylic acids	Butane-2,3-dione
Ethanoic acid	$\beta$ -hydroxy acids	Ethanal
Aldehydes	$\beta$ -keto acids	Ethanoic acid
Alcohols	Methyl ketones	Ethanol
Carboxylic acids	Lactones	
Sulfur compounds		



# Cheese flavour biochemistry (2)

- Examples of transformations possible with **valine** are shown in Scheme 3.
- Breakdown of **lactose and citrate** produces important molecules like **diacetyl** (a 'buttery' taste), ethanal and ethanol. Butane-2,3-dione is a metabolite of pyruvate (Scheme 4)
- **Methional** is formed from the amino acid methionine (Scheme 5).
- No single molecule dominates the aroma, which is perceived due to a balanced blend of components with various notes identified - sour, salty, nutty, fatty acid, cowy, brothy, buttery and sweet

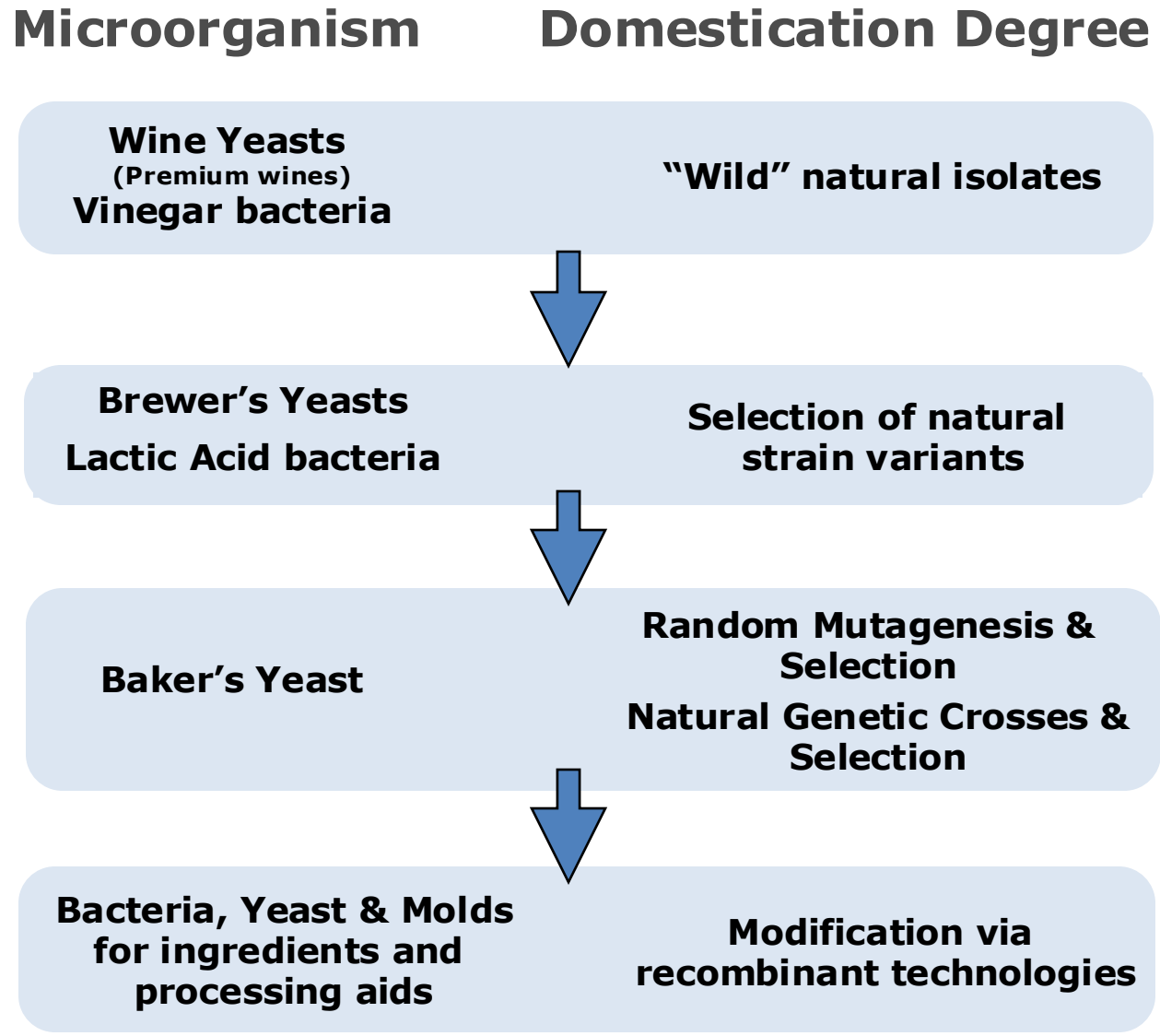


[http://www.rsc.org/Education/Ei/issues/2011 January/ReallyCheesyChemistry.asp](http://www.rsc.org/Education/Ei/issues/2011%20January/ReallyCheesyChemistry.asp)

# Extra Slides on yeast, cultures and some fermented foods

# Domestication evolved differently for different food microorganisms

- Depending on the application field, food micro-organisms have undergone from no to extensive domestication
- The degree of domestication was limited mainly by consumer perception & acceptance and legal frameworks, but only to a smaller degree by technical limitations



# “Protective” microorganisms

- Protective (“dominant”) microorganisms have ability to kill, suppress or retard multiplication of potentially harmful contaminating microorganisms.
- The following factors play a role:
  - Production of anti-microbial compounds, which inhibit or kill other microorganisms (organic acids, aldehydes, alcohols, bacteriocins)
  - Capacity to out-compete other microorganisms via exclusive or more rapid nutrient consumption or e.g. via capacity to multiply in high salt or low pH
- For food fermentation applications, protective microorganisms are mainly lactic acid bacteria, propionibacteria and yeasts.
- In many applications, protective microorganisms are not able to deploy their effect alone. They may be used to increase the “robustness” of a fermentation process in combination with a reduction of the initial contaminating flora and/or with relevant process parameters (e.g. salt, low pH, low temperature).

# Preservation:

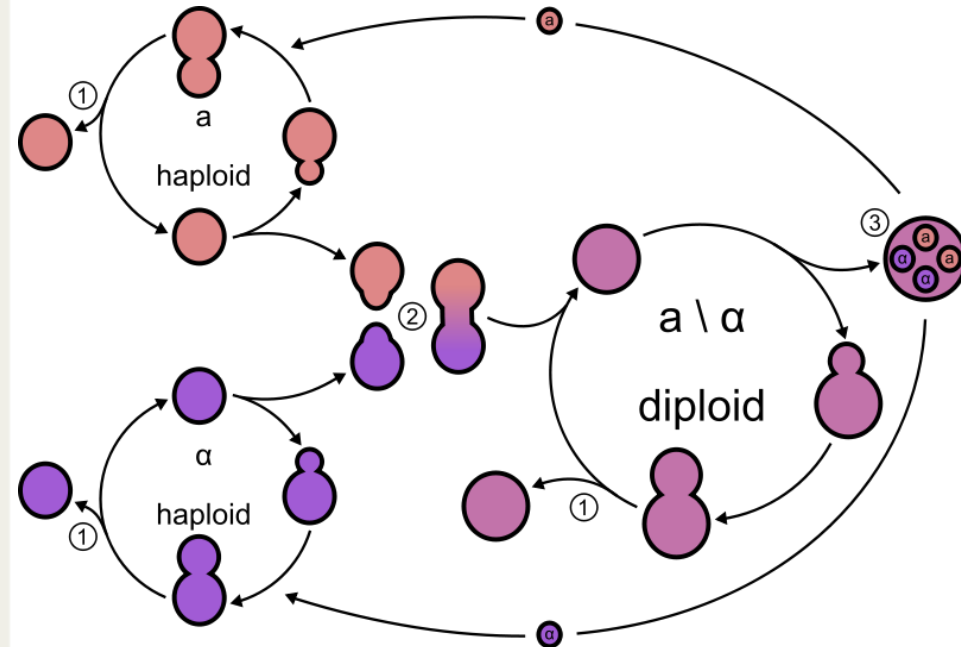
## Fermentation used for centuries to preserve food

Product	Raw material	Starter culture
Beer	Cereals	Yeast
Wine	Grape juice	Yeast, lactic acid bacteria
Vinegar	Wine	Acetic acid bacteria
Bread	Grains	Yeast, lactic acid bacteria
Soy sauce	Soybeans	Mold, lactic acid bacteria
Sauerkraut, kimchi	Cabbage	Lactic acid bacteria
Fermented sausages	Meat	Lactic acid bacteria
Pickled vegetables	Cucumbers, olives a.o.	Lactic acid bacteria
Fermented milks	Milk	Lactic acid bacteria
Cheese	Milk	Lactic acid bacteria, yeast, mold

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)

# Yeast (reproduction)

- Asexual and sexual reproductive cycles. Most common mode of vegetative growth is asexual reproduction by budding. A small bud (daughter cell) is formed on the parent cell. The nucleus of the parent cell splits into a daughter nucleus and migrates into the daughter cell. The bud continues to grow until it separates from the parent cell, forming a new cell.
- *Schizosaccharomyces pombe*, reproduce by mitosis instead of budding.
- Under high stress, haploid cells will generally die; under the same conditions, however, diploid cells can undergo sporulation, entering sexual reproduction (meiosis) and producing a variety of haploid spores, which can go on to mate (conjugate), reforming the diploid



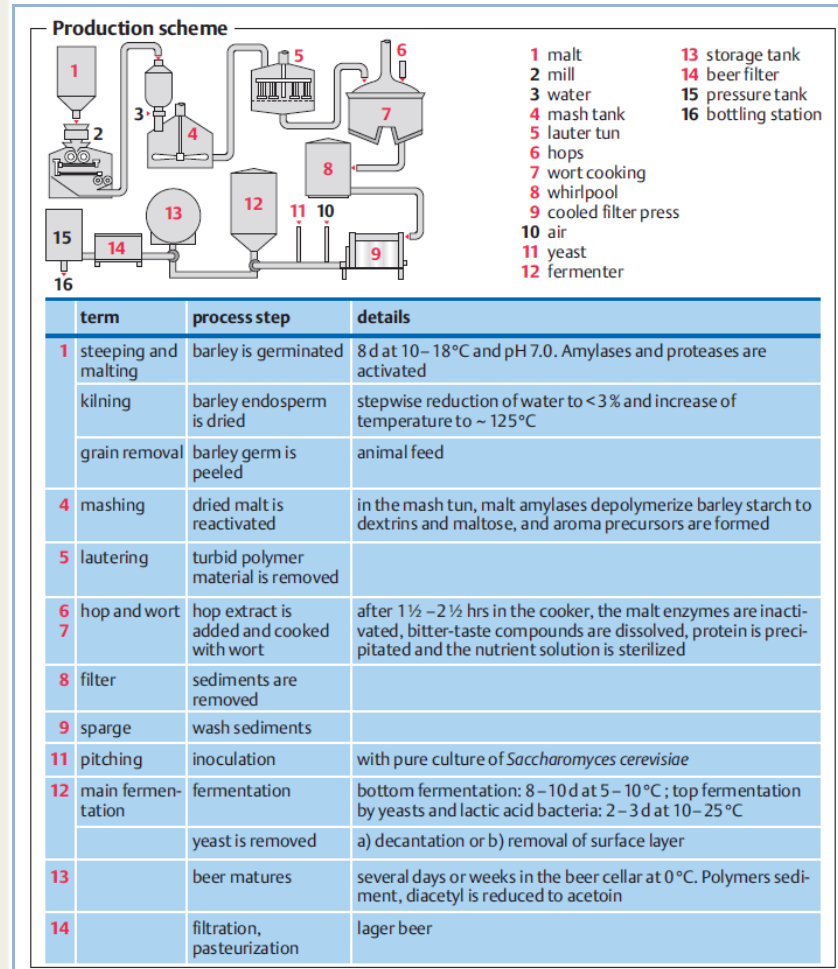
The yeast cell's life cycle:

1. Budding
2. Conjugation
3. Spore

[http://en.wikipedia.org/wiki/File:Yeast\\_lifecycle.svg](http://en.wikipedia.org/wiki/File:Yeast_lifecycle.svg)

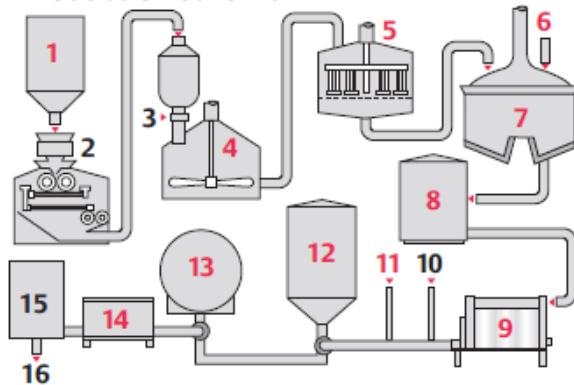
# Yeast (Beer)

- Brewing yeasts: "top cropping" (top fermenting) and "bottom cropping" (bottom-fermenting)
- Top cropping yeasts form a foam at the top of the wort during fermentation, e.g. by *Saccharomyces cerevisiae*, ("ale yeast")
- Bottom cropping yeasts used to produce lager-type beers, though they can also produce ale-type beers. These yeasts ferment well at low temperatures. Example: *Saccharomyces pastorianus*, formerly known as *S. carlsbergensis*
- Most common top cropping brewer's yeast, *S. cerevisiae*, is same species as baking yeast
- Baking and brewing yeasts belong to different strains to favor different characteristics: baking yeast strains are more aggressive, to carbonate dough in shortest amount of time; brewing yeast strains act slower, but tend to produce fewer off-flavors and tolerate higher alcohol concentrations (up to 22%).



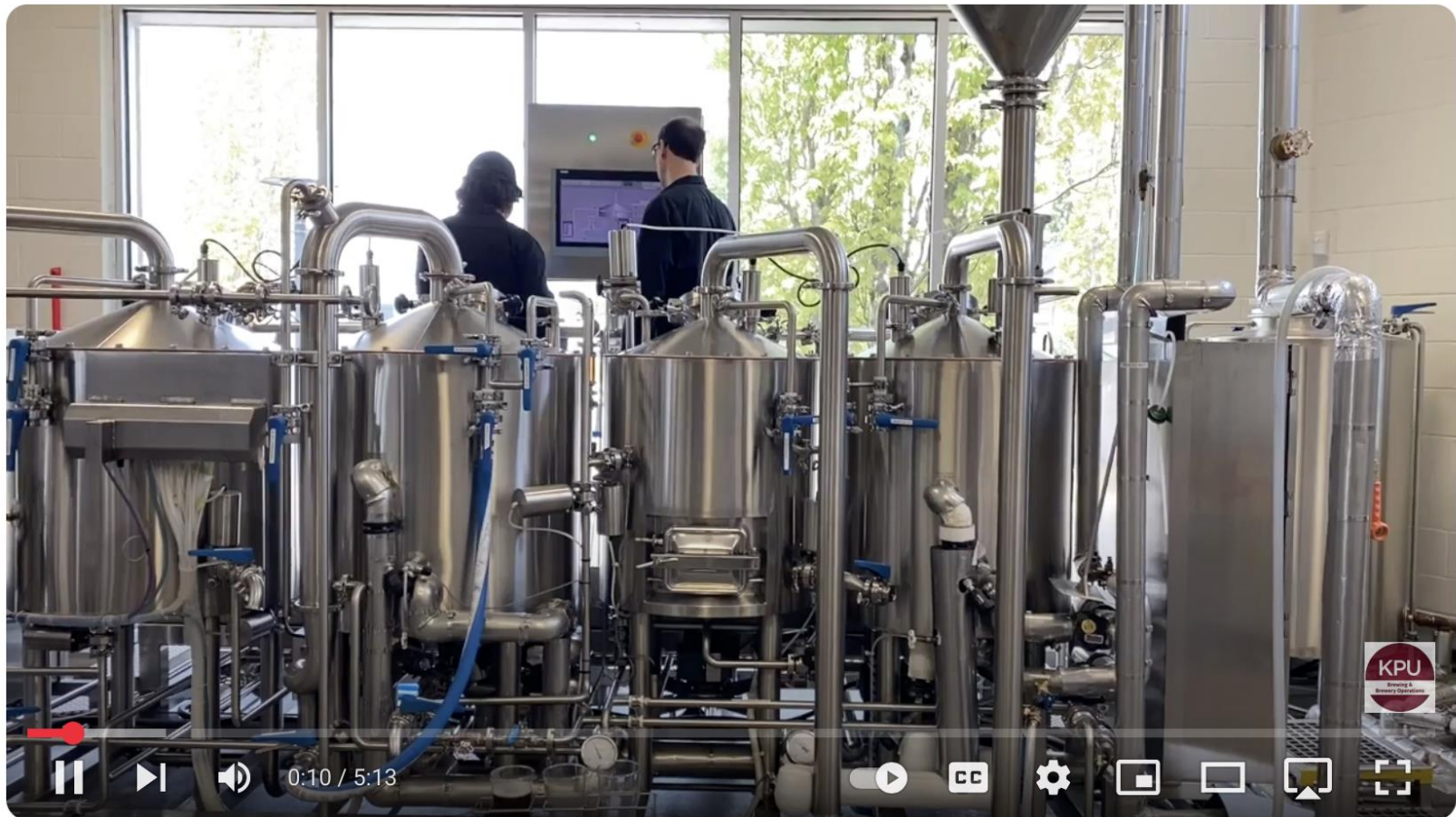
*Pocket Guide to Biotechnology and Genetic Engineering*  
(ed. R. Schmid, Wiley-VCH, 2016)

## Production scheme



- 1 malt
- 2 mill
- 3 water
- 4 mash tank
- 5 lauter tun
- 6 hops
- 7 wort cooking
- 8 whirlpool
- 9 cooled filter press
- 10 air
- 11 yeast
- 12 fermenter
- 13 storage tank
- 14 beer filter
- 15 pressure tank
- 16 bottling station

	term	process step	details
1	steeping and malting	barley is germinated	8 d at 10–18°C and pH 7.0. Amylases and proteases are activated
	kilning	barley endosperm is dried	stepwise reduction of water to <3% and increase of temperature to ~125°C
	grain removal	barley germ is peeled	animal feed
4	mashing	dried malt is reactivated	in the mash tun, malt amylases depolymerize barley starch to dextrins and maltose, and aroma precursors are formed
5	lautering	turbid polymer material is removed	
6	hop and wort	hop extract is added and cooked with wort	after 1½–2½ hrs in the cooker, the malt enzymes are inactivated, bitter-taste compounds are dissolved, protein is precipitated and the nutrient solution is sterilized
8	filter	sediments are removed	
9	sparge	wash sediments	
11	pitching	inoculation	with pure culture of <i>Saccharomyces cerevisiae</i>
12	main fermentation	fermentation	bottom fermentation: 8–10 d at 5–10°C; top fermentation by yeasts and lactic acid bacteria: 2–3 d at 10–25°C
		yeast is removed	a) decantation or b) removal of surface layer
13		beer matures	several days or weeks in the beer cellar at 0°C. Polymers sediment, diacetyl is reduced to acetoin
14		filtration, pasteurization	lager beer



**Brewing Beer - Basic Processing Steps (How is beer made) #beerbrewing #craftbeer**

<https://youtu.be/MJ7Uj4Q9GRs?feature=shared>

# Yeast (Wine)

- Ambient yeasts are naturally present in wine cellars, vineyards and on the grapes themselves
- Cultured yeast are isolated and inoculated
- Wild yeasts in winemaking include *Candida*, *Klöckera/Hanseniaspora*, *Metschnikowiaceae*, *Pichia*, *Zygosaccharomyces*. Produce high-quality, unique-flavored wines. Can be unpredictable and may introduce less desirable traits to the wine, and can even contribute to spoilage. Traditional wine makers use ambient yeast as a characteristic of the region's terroir
- Cultured yeasts belong to the *Saccharomyces cerevisiae* species. Several hundred different strains can be used to affect the heat or vigor of the process and enhance or suppress certain flavor characteristics. The use of different strains of yeasts are a major contributor to the diversity of wine, even among the same grape variety

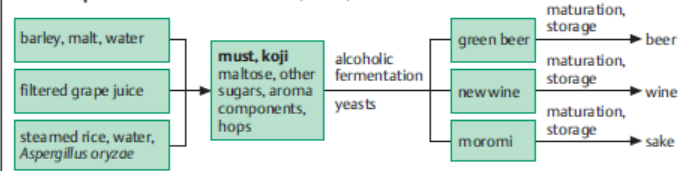
## Alcoholic beverages

beer	starch from barley is degraded to sugars by barley amylases. The sugar solution is fermented by yeast to ethanol, in the presence of hop extracts
wine	grape juice is fermented by yeast
champagne	sugar and yeast is added to wine, followed by a second fermentation
cider	apple juice is fermented by yeast
sake	rice starch is depolymerized by amylases from <i>Aspergillus oryzae</i> , and sugars are fermented by yeast
whisky	extracts of barley, yeast, rye, or corn are fermented by yeast and distilled
vodka	extracts of potatoes or wheat are fermented by yeast and distilled

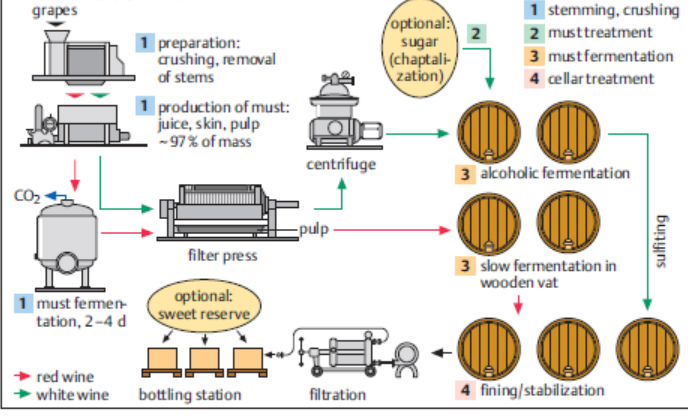
## Production figures

beer		wine		sake
total (2001)	1 420 million hL	total (1999)	281 million hL	
USA	231 million hL	France	60 million hL	Japan (1997) 9,1 million hL
China	227 million hL	Italy	58 million hL	
Germany	109 million hL	Spain	33 million hL	
Brazil	84 million hL	USA	20 million hL	
Japan	71 million hL	Argentina	16 million hL	
Russia	63 million hL	Germany	12 million hL	
United Kingdom	56 million hL	Australia	9 million hL	

## General production scheme for beer, wine, sake

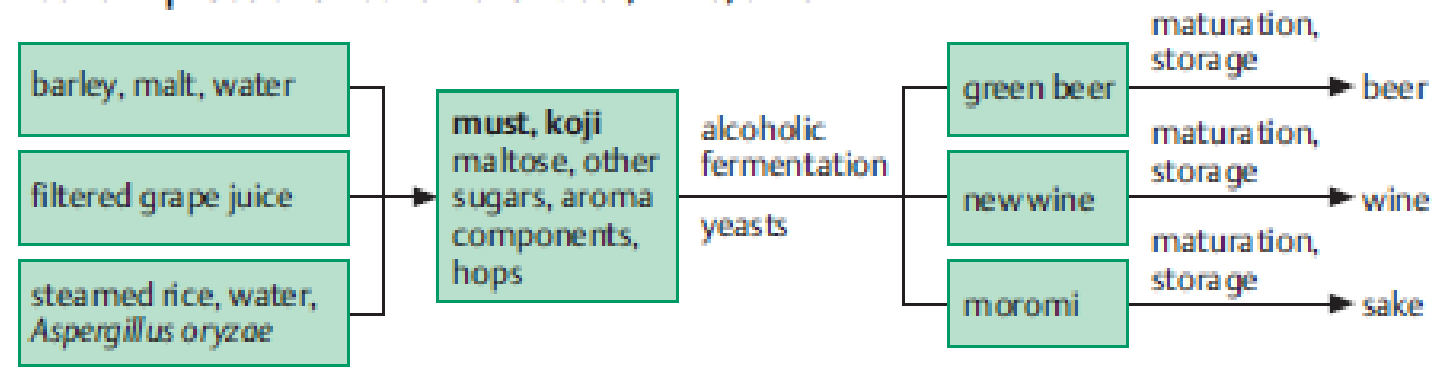


## Manufacture of wine

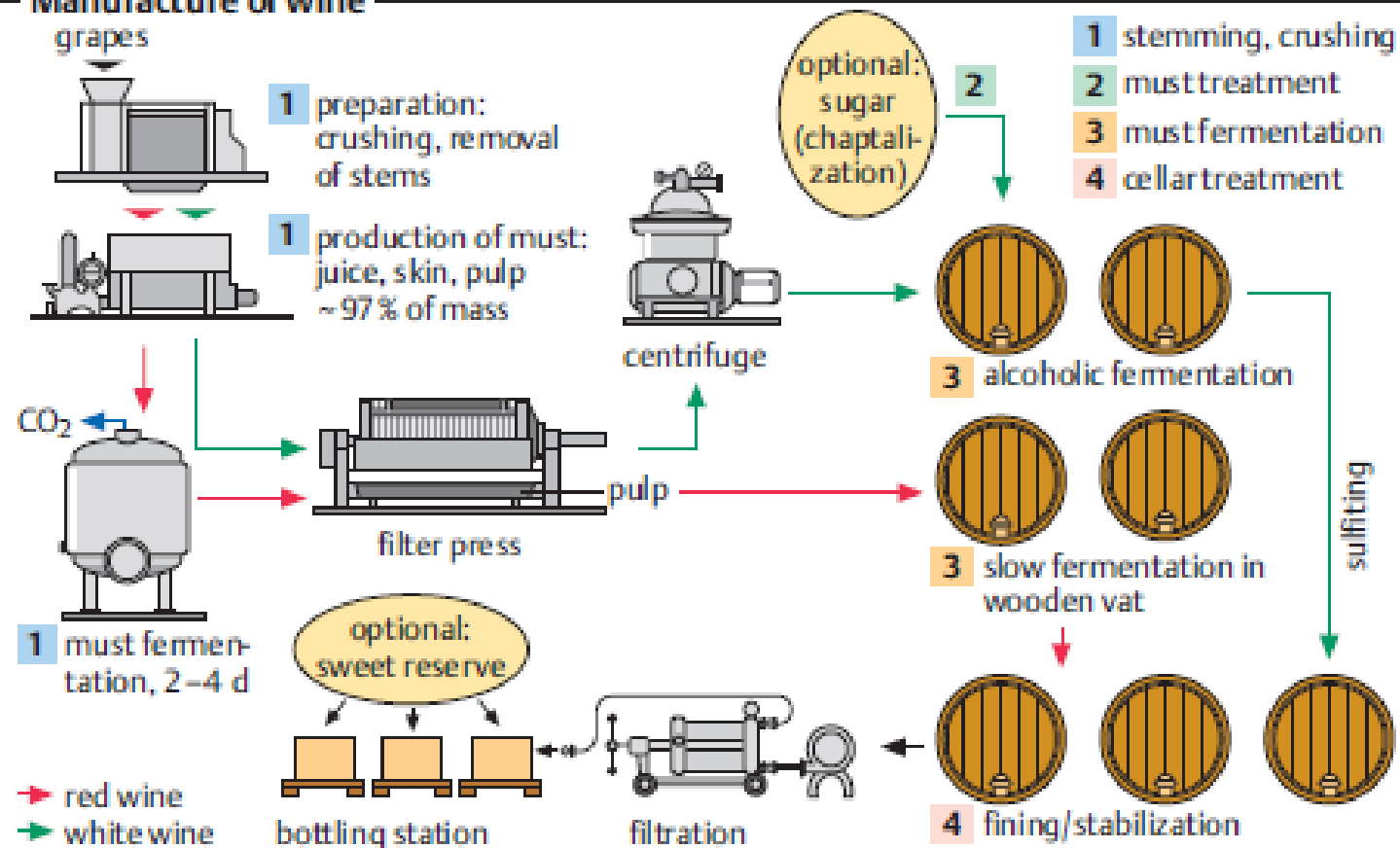


Pocket Guide to Biotechnology and Genetic Engineering  
(ed. R. Schmid, Wiley-VCH, 2003)

## General production scheme for beer, wine, sake



## Manufacture of wine



# Yeast and Moulds commonly used in food fermentation

**Table 2** Yeast and Mold Species Commonly Used in Food Fermentations

Genus	Species	Application
<i>Aspergillus</i>	<i>oryzae</i>	Soy sauce
<i>Candida</i>	<i>famata</i>	Meat
<i>Candida</i>	<i>kefyr</i>	Fermented milk
<i>Candida</i>	<i>krusei</i>	Fermented milk
<i>Candida</i>	<i>lipolytica</i>	
<i>Candida</i>	<i>Parapsilosis</i>	
<i>Candida</i>	<i>valida</i>	
<i>Geotrichum</i>	<i>candidum</i>	Cheese, fermented milks
<i>Penicillium</i>	<i>album</i>	
<i>Penicillium</i>	<i>camemberti</i>	Cheese, meat
<i>Penicillium</i>	<i>chrysogenum</i>	Meat
<i>Penicillium</i>	<i>nalgiovense</i>	Meat
<i>Penicillium</i>	<i>roqueforti</i>	Cheese, meat
<i>Saccharomyces</i>	<i>bayanus</i>	Fermented milks
<i>Saccharomyces</i>	<i>cerevisiae</i>	Baker's yeast, brewing, wine-making, cheese, fermented milks, meat, vegetables, and probiotics

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»

# Bacterial species commonly used in food fermentation

**Table 3** Bacterial Species Commonly Used in Food Fermentations

Genus	Species	Application
<i>Acetobacter</i>	<i>acetii</i>	Vinegar production
<i>Bifidobacterium</i>	<i>adolescentis</i>	Probiotics
<i>Bifidobacterium</i>	<i>animalis</i> <sup>a</sup>	Cheese, fermented milks, probiotics
<i>Bifidobacterium</i>	<i>bifidum</i>	Cheese, fermented milks, probiotics
<i>Bifidobacterium</i>	<i>breve</i>	Probiotics
<i>Bifidobacterium</i>	<i>infantis</i>	Probiotics
<i>Bifidobacterium</i>	<i>longum</i>	Probiotics
<i>Brevibacterium</i>	<i>casei</i>	Cheese
<i>Brevibacterium</i>	<i>linens</i>	Cheese, bioprotection
<i>Carnobacterium</i>	<i>divergens</i>	Meat, bioprotection
<i>Carnobacterium</i>	<i>piscicola</i>	Meat, bioprotection
<i>Enterococcus</i>	<i>faecium</i>	Cheese, fermented milks, meat, vegetables, probiotics, bioprotection
<i>Kocuria</i>	<i>varians</i> <sup>b</sup>	Meat
<i>Lactobacillus</i>	<i>acidophilus</i>	Probiotics, cheese, fermented milks, meat, vegetables
<i>Lactobacillus</i>	<i>alimentarius</i>	Meat
<i>Lactobacillus</i>	<i>brevis</i>	Probiotics, vegetables, bioprotection
<i>Lactobacillus</i>	<i>casei</i>	Probiotics, cheese, fermented milks, meat, vegetables
<i>Lactobacillus</i>	<i>coryniformis</i>	Cheese
<i>Lactobacillus</i>	<i>crispatus</i>	
<i>Lactobacillus</i>	<i>curvatus</i>	Meat
<i>Lactobacillus</i>	<i>delbrueckii</i> subsp. <i>bulgaricus</i>	Fermented milks, cheese, probiotics
<i>Lactobacillus</i>	<i>delbrueckii</i> subsp. <i>delbrueckii</i>	Cheese, vegetables
<i>Lactobacillus</i>	<i>Delbrueckii</i> subsp. <i>lactis</i>	Fermented milks, cheese
<i>Lactobacillus</i>	<i>farctiminis</i>	Meat
<i>Lactobacillus</i>	<i>fermentum</i>	Cheese, probiotics
<i>Lactobacillus</i>	<i>gasseri</i>	Fermented milks, probiotics
<i>Lactobacillus</i>	<i>helveticus</i>	Cheese, fermented milks, probiotics, vegetables
<i>Lactobacillus</i>	<i>johnsonii</i>	Fermented milks, probiotics, probiotics
<i>Lactobacillus</i>	<i>kefiri</i>	Fermented milks
<i>Lactobacillus</i>	<i>panis</i>	Sourdough, bread
<i>Lactobacillus</i>	<i>pentosus</i>	Meat
<i>Lactobacillus</i>	<i>plantarum</i>	Bread, meat, wine, vegetables, bioprotection
<i>Lactobacillus</i>	<i>reuteri</i>	Bioprotection, probiotics
<i>Lactobacillus</i>	<i>rhamnosus</i>	Probiotics
<i>Lactobacillus</i>	<i>sakei</i> subsp. <i>carneus</i>	Meat
<i>Lactobacillus</i>	<i>sakei</i> subsp. <i>sakei</i> <sup>c</sup>	Meat, vegetables, bioprotection
<i>Lactobacillus</i>	<i>sanfranciscensis</i>	Bread
<i>Lactococcus</i>	<i>lactis</i> subsp. <i>cremoris</i>	Cheese, fermented milks, bread, meat, vegetables, probiotics, bioprotection
<i>Lactococcus</i>	<i>lactis</i> subsp. <i>lactis</i>	Cheese, fermented milks, bread, meat, vegetables, probiotics, bioprotection

**Table 3** Continued

Genus	Species	Application
<i>Lactococcus</i>	<i>lactis</i> subsp. <i>lactis</i> biovar <i>diacetylactis</i>	Cheese, fermented milks, bread, meat, vegetables, probiotics, bioprotection
<i>Leuconostoc</i>	<i>carneum</i>	Meat, bioprotection
<i>Leuconostoc</i>	<i>lactis</i>	Cheese, fermented milks
<i>Leuconostoc</i>	<i>mesenteroides</i> subsp. <i>cremoris</i>	Cheese, fermented milks, vegetables
<i>Leuconostoc</i>	<i>mesenteroides</i> subsp. <i>dextranicum</i>	Probiotics
<i>Leuconostoc</i>	<i>mesenteroides</i> subsp. <i>mesenteroides</i>	Cheese, fermented milks, vegetables
<i>Leuconostoc</i>	<i>pseudomesenteroides</i>	
<i>Micrococcus</i>	<i>luteus</i>	Meat
<i>Oenococcus</i>	<i>oeni</i>	Wine
<i>Pediococcus</i>	<i>acidilactici</i>	Meat, probiotics, bioprotection
<i>Pediococcus</i>	<i>damnosus</i>	Meat, bioprotection
<i>Pediococcus</i>	<i>pentosaceus</i>	Meat
<i>Propionibacterium</i>	<i>acidipropionici</i>	Cheese
<i>Propionibacterium</i>	<i>freudenreichii</i> subsp. <i>freudenreichii</i>	Cheese, probiotics
<i>Propionibacterium</i>	<i>freudenreichii</i> subsp. <i>shermanii</i>	Cheese
<i>Staphylococcus</i>	<i>carneus</i> subsp. <i>carneus</i>	Meat
<i>Staphylococcus</i>	<i>carneus</i> subsp. <i>utilis</i>	Meat
<i>Staphylococcus</i>	<i>equorum</i>	Meat
<i>Staphylococcus</i>	<i>xylosum</i>	Meat
<i>Streptococcus</i>	<i>thermophilus</i>	Cheese, fermented milks, bread, meat, vegetables, probiotics
<i>Weissella</i>	<i>confusa</i>	Meat
<i>Weissella</i>	<i>halotolerans</i>	Meat

<sup>a</sup> *Bifidobacterium lactis* is not a separate species but included in *B. animalis*.

<sup>b</sup> *Micrococcus varians* has been renamed *Kocuria varians*.

<sup>c</sup> *Lactobacillus bavaricus* has been included in *L. sakei* subsp. *sakei*.

For a recent update read:

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DOI: 10.1111/1541-4337.12520

COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

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Fermented foods in a global age: East meets West

Jyoti Prakash Tamang<sup>1</sup> | Paul D. Cotter<sup>2</sup> | Akihito Endo<sup>3</sup> | Nam Soo Han<sup>4</sup> | Remco Kort<sup>5,6</sup> | Shao Quan Liu<sup>7</sup> | Baltasar Mayo<sup>8</sup> | Niek Westerik<sup>5,6</sup> | Robert Hutkins<sup>9</sup>

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No need to remember details

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»

# Fermented Dairy Foods consumed in different regions of the world

**Table 1.4.** Major Fermented Dairy Foods Consumed in the Different Regions of the World

Product Name	Major Country/Region
Acidophilus milk	United States, Russia
Ayran/eyran/jugurt	Turkey
Busa	Turkestan
Chal	Turkmenistan
Cieddu	Italy
Cultured buttermilk	United States
Dahi/dudhee/dahee	Indian subcontinent
Donskaya/varenetes/kurugna/ryzhenka/guslyanka	Russia
Dough/abdoogh/mast	Afghanistan, Iran
Ergo	Ethiopia
Filmjolk/fillbunke/fillbunk/surmelk/taettemjolk/tettemelk	Sweden, Norway, Scandinavia
Gioddu	Sardinia
Gruzovina	Yugoslavia
Iogurte	Brazil, Portugal
Jugurt/eyran/ayran	Turkey
Katyk	Transcaucasia
Kefir, Koumiss/Kumys	Russia, Central Asia
Kissel maleka/naja/yaourt/urgotnic	Balkans
Kurunga	Western Asia
Leben/laban/laban rayeb	Lebanon, Syria, Jordan
Mazun/matzoen/matsun/matsoni/madzoen	Armenia
Mezzoradu	Sicily
Pitkapiima	Finland
Roba/rob	Iraq
Shosim/sho/thara	Nepal
Shrikhand	India
Skyr	Iceland
Tarag	Mongolia
Tarho/taho	Hungary
Viili	Finland
Yakult	Japan
Yiaourti	Greece
Ymer	Denmark
Zabady/zabade	Egypt, Sudan

Adapted from Chandan, 2002; Tamime and Robinson, 1999.

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*Manufacturing yogurt and fermented milks, ed. RC Chandan, Blackwell publishing, 2006*

# Milk Fermentation

Product 	Alternative names 	Typical milkfat content 	Typical shelf life at 4°C 	Fermentation agent 	Description
Acidophilus milk	acidophilus cultured milk	0.5-2%	2 weeks <sup>[10]</sup>	<i>Lactobacillus acidophilus</i> <sup>[10][4]</sup>	Thermophilic fermented milk, often lowfat (2%, 1.5%) or nonfat (0.5%), cultured with <i>Lactobacillus acidophilus</i> .
Cheese		1-75%	varies	a variety of bacteria and/or mold	Any number of solid fermented milk products.
Crème fraîche	creme fraiche	30-40%	10 days <sup>[10]</sup>	naturally occurring lactic acid bacteria in cream	Mesophilic fermented cream, originally from France; higher-fat variant of <i>sour cream</i> .
Cultured buttermilk		1-2%	10 days <sup>[10]</sup>	<i>Lactococcus lactis</i> <sup>[4]</sup> ( <i>Lactococcus lactis</i> subsp. <i>lactis</i> *, <i>Lactococcus lactis</i> subsp. <i>cremoris</i> , <i>Lactococcus lactis</i> biovar. <i>diacetylactis</i> and <i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i> ) <sup>[10]</sup>	Mesophilic fermented pasteurized milk.
Cultured sour cream	sour cream <sup>[4]</sup>	14-18% <sup>[4]</sup>	4 weeks <sup>[10]</sup>	<i>Lactococcus lactis</i> subsp. <i>lactis</i> <sup>[4]</sup>	Mesophilic fermented pasteurized cream with an acidity of at least 0.5%. Rennet extract may be added to make a thicker product. <sup>[4]</sup> Lower fat variant of <i>crème fraîche</i> .
Filmjölk	fil	0.1-4.5%	10-14 days <sup>[10]</sup>	<i>Lactococcus lactis</i> * and <i>Leuconostoc</i> <sup>[5][4]</sup>	Mesophilic fermented milk, originally from Scandinavia.
Kefir	kephir, kewra, talai, mudu kekiya, milkkefir, búlgaros	0-4%	10-14 days <sup>[10]</sup>	Kefir grains, a mixture of bacteria and yeasts	A fermented beverage, originally from the Caucasus region, made with kefir grains. Can be made with any sugary liquid, such as milk from mammals, soy milk, or fruit juices.
Kumis	koumiss, kumiss, kymys, kymyz, airag, chigee	4%?	10-14 days <sup>[10]</sup>	<i>Lactobacilli</i> and yeasts	A carbonated fermented milk beverage traditionally made from horse milk.
Vili	filbunke	0.1-3.5%	14 days <sup>[10]</sup>	<i>Lactococcus lactis</i> subsp. <i>cremoris</i> , <i>Lactococcus lactis</i> * biovar. <i>diacetylactis</i> , <i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i> and <i>Geotrichum candidum</i> <sup>[7]</sup>	Mesophilic fermented milk that may or may not contain fungus on the surface. Originally from Sweden but today is a Finnish specialty. <sup>[7]</sup>
Yogurt	yoghurt, yoghourt, yogourt, yogurt	0.5-4%	35-40 days <sup>[10]</sup>	<i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i> <sup>[4]</sup>	Thermophilic fermented milk, cultured with <i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i> .

\* *Streptococcus lactis* has been renamed to *Lactococcus lactis* subsp. *lactis*<sup>[4]</sup>

Wikipedia

# Starter cultures

**Table 1.7.** Starter Cultures Used in the Manufacture of Commercial Fermented Milks

Product	Primary Microorganism(s)	Secondary/Optional Microorganism(s)	Incubation Temperature and Time	Major Function of Culture
Yogurt	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>Strept. thermophilus</i>	<i>Lb. acidophilus</i> , <i>Bifidobacterium longum</i> , <i>Bifidobacterium bifidum</i> , <i>Bifidobacterium infantis</i> , <i>Lb. casei</i> , <i>Lb. lactis</i> , <i>Lb. rhamnosus</i> , <i>Lb. helveticus</i> , <i>Lb. reuteri</i>	43–45°C for 2.5 hours	Acidity, texture, aroma, flavor, probiotic
Cultured butter milk and sour cream	<i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Lc. lactis</i> subsp. <i>cremoris</i> , <i>Lc. lactis</i> subsp. <i>lactis</i> var. <i>diacetylactis</i>	<i>Leuc. lactis</i> , <i>Leuc. mesenteroides</i> subsp. <i>cremoris</i>	22°C for 12–14 hours	Acidity, flavor, aroma
Probiotic Fermented milks	<i>S. thermophilus</i> , <i>Lb. acidophilus</i> , <i>Lb. reuteri</i> , <i>Lb. rhamnosus</i> GG, <i>Lb. johnsoni</i> , <i>Lb. casei</i> , <i>Bifidobacterium longum</i> , <i>Bifidobacterium bifidus</i>	<i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Lc. lactis</i> subsp. <i>cremoris</i>	22–37°C/37–40°C for 8–14 hours	Acidity, flavor, probiotic
Kefir	<i>Lc. lactis</i> subsp. <i>lactis</i> , <i>Lc. lactis</i> subsp. <i>cremoris</i> , <i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>Lb. delbrueckii</i> subsp. <i>lactis</i> , <i>Lb. casei</i> , <i>Lb. helveticus</i> , <i>Lb. brevis</i> , <i>Lb. kefir</i> , <i>Leuc. mesenteroides</i> , <i>Leuconostoc dextranicum</i>	<b>Yeasts:</b> <i>Kluyveromyces marxianus</i> subsp. <i>marxianus</i> , <i>Torulaspora delbrueckii</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida kefir</i> <b>Acetic acid bacteria:</b> <i>Acetobacter aceti</i>	15–22°C for 24–36 hours	Acidity, aroma, flavor, gas (CO <sub>2</sub> ), alcohol, probiotic
Koumiss	<i>Lb. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>Lb. kefir</i> , <i>Lb. lactis</i> <b>Yeasts:</b> <i>Saccharomyces lactis</i> , <i>Saccharomyces carilaginosus</i> , <i>Mycoderma</i> spp.	<b>Acetic acid bacteria:</b> <i>Acetobacter aceti</i>	20–25°C for 12–24 hours	Acidity, alcohol, flavor, gas (CO <sub>2</sub> )

Adapted from Chandan and Shahani, 1995; Hassan and Frank, 2001; Tamime and Robinson, 2002.

*Manufacturing yogurt and fermented milks, ed. RC Chandan, Blackwell publishing, 2006*

# Cheese ripening

- A newborn cheese is usually salty, bland in flavor and, for harder varieties, rubbery in texture. These qualities are sometimes enjoyed—cheese curds are eaten on their own—but normally cheeses are left to rest under controlled conditions. This aging period (also called ripening, or, from the French, *affinage*) lasts from a few days to several years. As a cheese ages, microbes and enzymes transform texture and intensify flavor. This transformation is largely a result of the breakdown of casein proteins and milk fat into a complex mix of amino acids, amines, and fatty acids.
- Some cheeses have additional bacteria or molds intentionally introduced before or during aging. In traditional cheese making, these microbes might be already present in the aging room; they are simply allowed to settle and grow on the stored cheeses. More often today, prepared cultures are used, giving more consistent results and putting fewer constraints on the environment where the cheese ages. These cheeses include soft ripened cheeses such as Brie and Camembert, blue cheeses such as Roquefort, Stilton, Gorgonzola, and rind-washed cheeses such as Limburger.



*Wikipedia*

# Meat Fermentation

- Salami is cured sausage, fermented and air-dried meat. Historically, salami was popular in Southern European, because it could be stored at room temperature for periods of up to 10 years, supplementing an inconsistent supply of fresh meat.
- Salami are cured in warm, humid conditions to encourage growth of bacteria involved in the fermentation process.
- Lactic acid is produced by the bacteria as waste product; lowering the pH and coagulating and lowering the water-holding capacity of the meat. The acid produced by the bacteria makes the meat an inhospitable environment for other, dangerous bacteria and imparts the flavor that separates salami from machine-dried pork
- Today, starter cultures are used. The whole process takes about 36 weeks, although some age it more for additional taste



*Wikipedia*

# Sausage metabolism

- Acidulation of the sausage matrix is induced by the interactions of salt-solubilizes muscle proteins with both lactic acid and ammonia formed during fermentation.
- Lactate is formed by fermentation
- Ammonia is formed from amino acids.
- The final flavour is a complex mixture of taste and aroma compounds
- Many sausages are ripened with surface molds
- pH is reduced from 6 to 5
- Reduction in water holding capacity
- Starter cultures developed for acidity, flavour, texture and colour
- *Pedococcus*, *Lactobacillus plantarum*, *micrococcus*

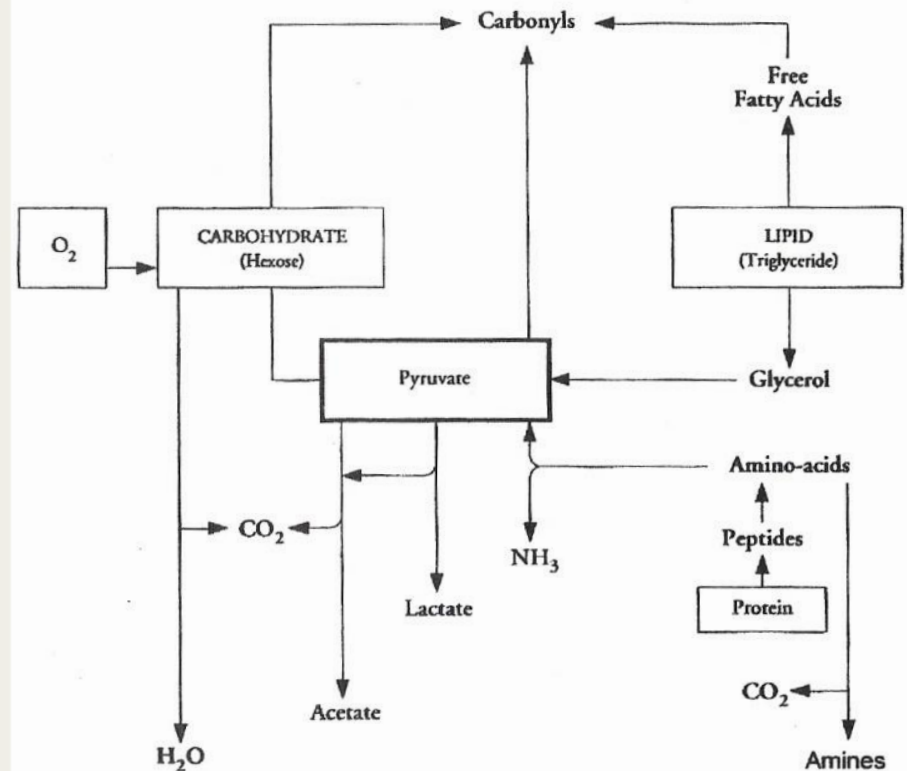
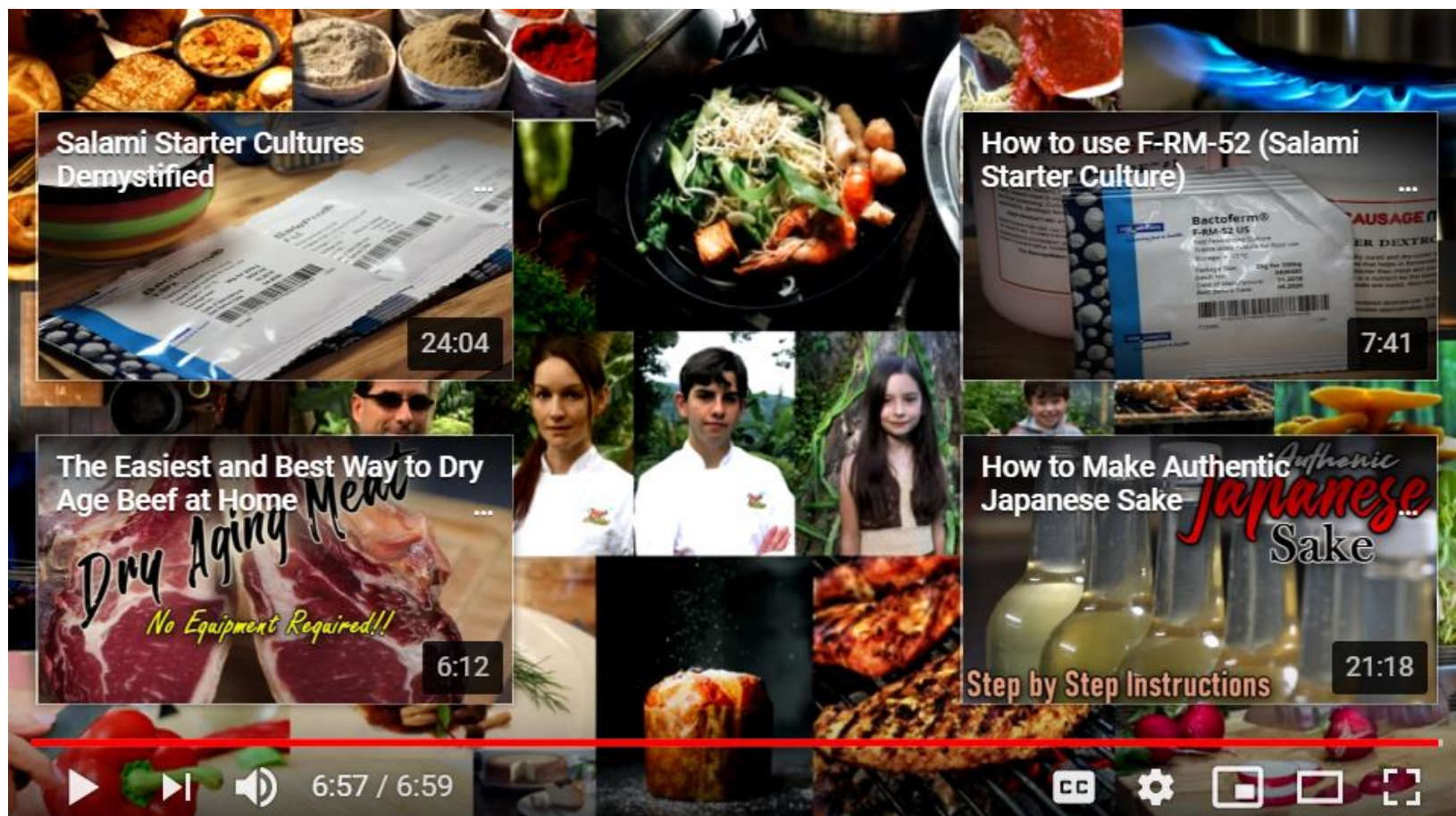


Figure 1 Reaction scheme underlying the stoichiometric model of sausage metabolism.

«Handbook of Food and Beverage Fermentation Technology (ed. YH Hui, Marcel Dekker, Inc. 2004)»



<https://www.youtube.com/watch?v=6L1eD5Qdkt8>

How to make salami