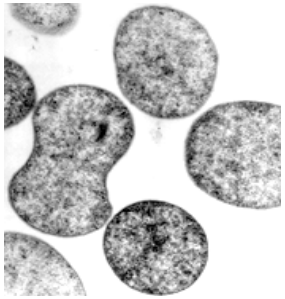
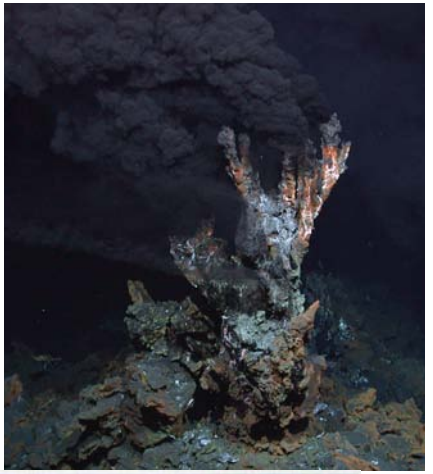
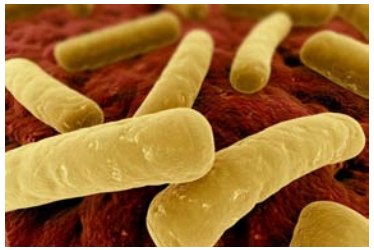
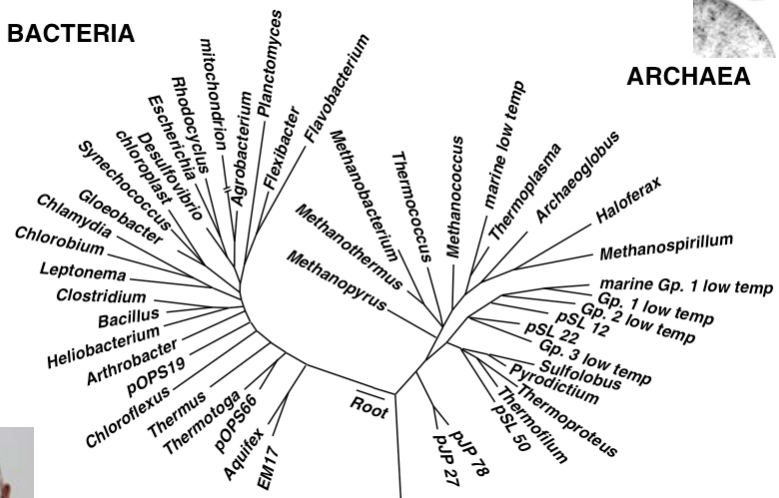
Yeast and Homebrewing

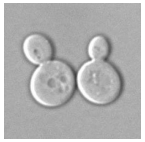
Jasper Akerboom

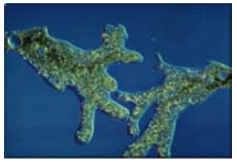






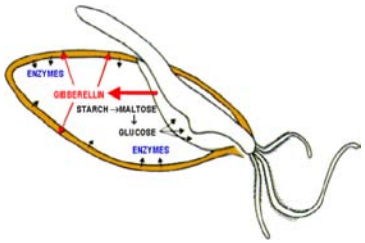




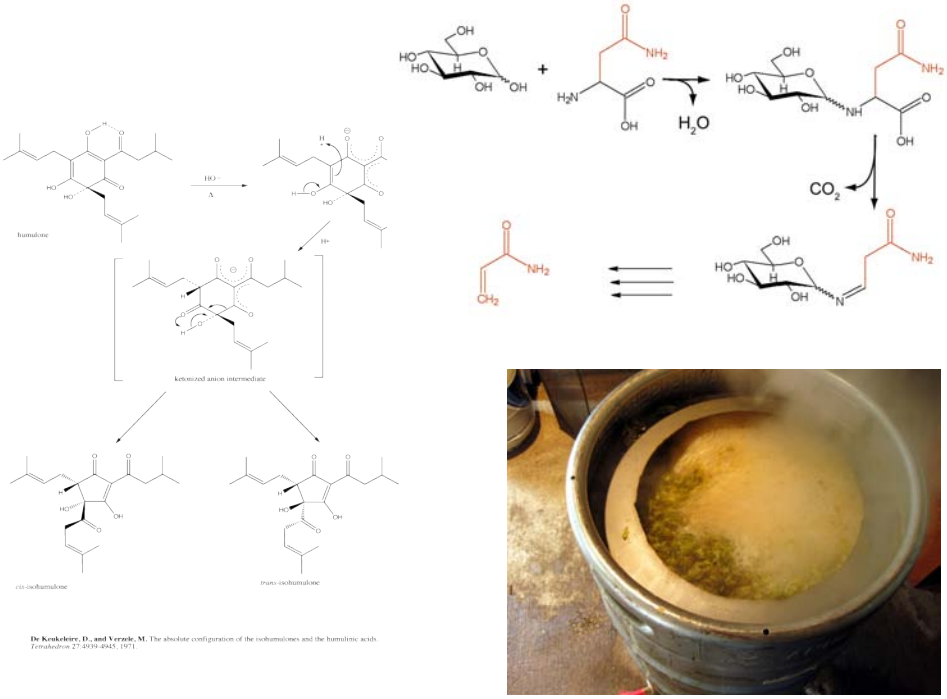


Pace, NR. *Science* 276: 734-740 (1997)

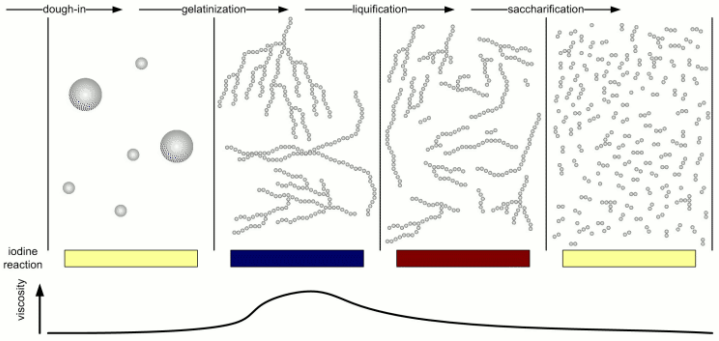
Malting



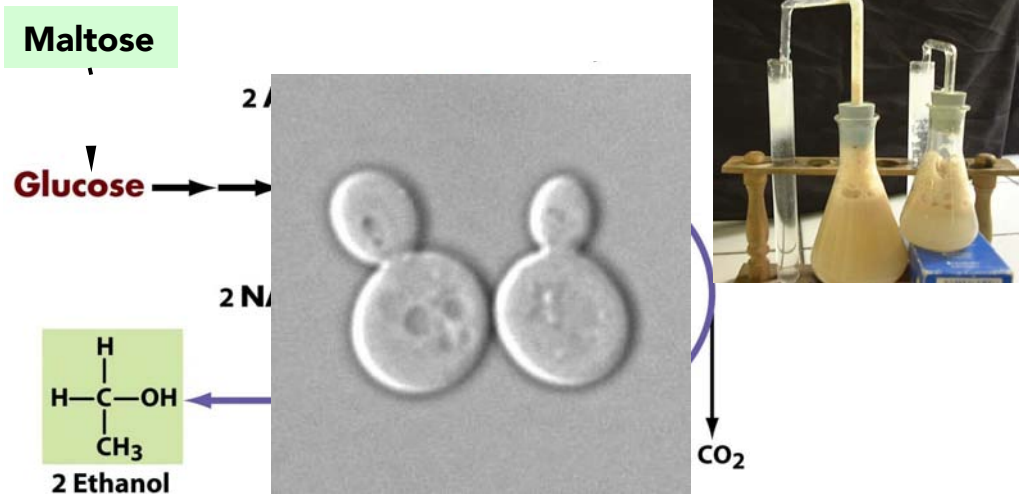
Boiling



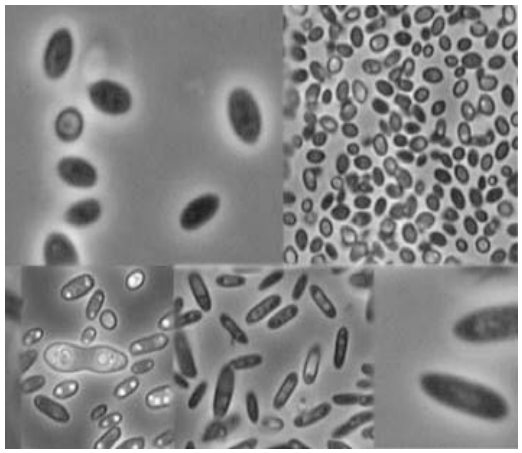
Mashing



Fermentation



Yeasts: Single cell Fungi - Over 1500 species known – Number will increase



Different cell sizes and morphologies

Different colors and colony shapes

For single-strain brewing, mainly three genera are used:

Saccharomyces, Torulaspora delbrueckii, Dekkera/Brettanomyces

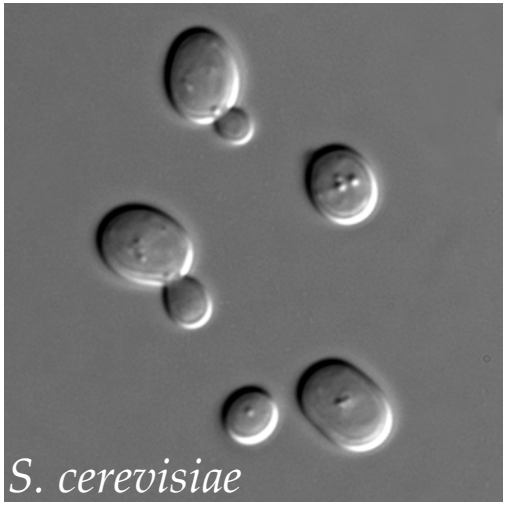


TABLE 61.3 Fermentation Power (w/v) of Some Yeast Species[1]

<2%	2–5%	5–8%	>8%
Candida ernobii	*Hanseniaspora uvarum*	*Candida stellata*	*Saccharomyces bayanus/*
C. guilliermondii	*Pichia fermentans,*	*Lachancea thermotolerans*	*uvarum S. cerevisiae*
C. melinii	*Lachancea kluyveri*	*Saccharomyces kudriavzevii*	*S. pastorianus*
C. parapsilosis	*Schwannyomyces*	*S. mikatae*	*S. paradoxus*
C. sake	*occidentalis*	*Saccharomycodes ludwigii*	
C. tropicalis	*Torulaspora pretoriensis*	*Schizosaccharomyces pombe*	
C. valida	*Zygotorulaspora mrakii*	*Torulaspora delbrueckii*	
Debaryomyces castellii		*Zygosaccharomyces bailii*	
Lindnera saturnus		*Z. rouxii*	
Metschnikowia pulcherrima		*Zygotorulaspora florentinus*	
M. reukafii			
Pichia membranifaciens			
Schwanniomyces polymorphus			
+>300 ca. fermenting species, including about 50 that exhibit slow or retarded fermentation			

H. Phaff, A. Martini – 1980's

"strains of *S. cerevisiae* are rarely if ever present on the fruits and berries of wild species of plants"

The Life of Yeasts - Phaff et al. 1978

S. cerevisiae must be associated with some other ecological niche …

Peynaud and Domerq.1959. A review on microbiological problems in wine making in France

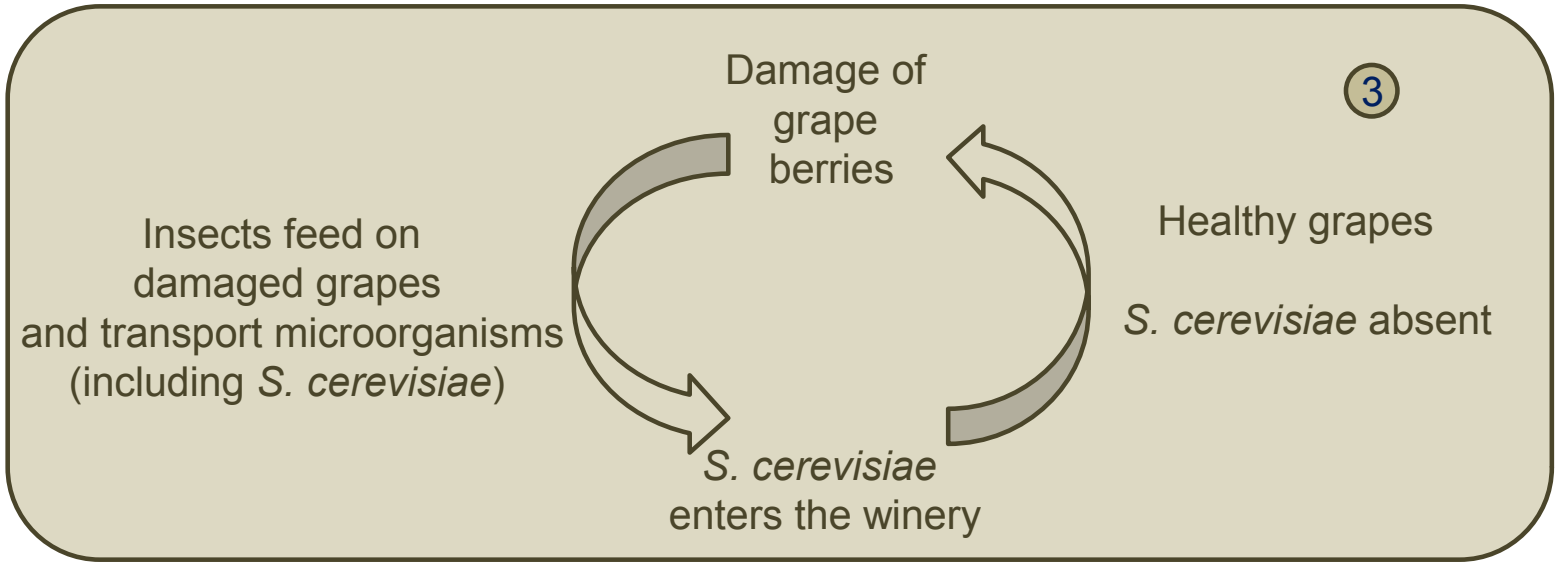
Ale yeast, pure strain, can be isolated from Oak bark (17%), forest soil (14%), rotten wood (9.2%), orchard soil (9.1%), fruit samples (6%) (grapes are the lowest success rate!!)

Wang, QM *et al.*, Mol. Ecol. (2012)

Mortimer and Polsinelli 1990's

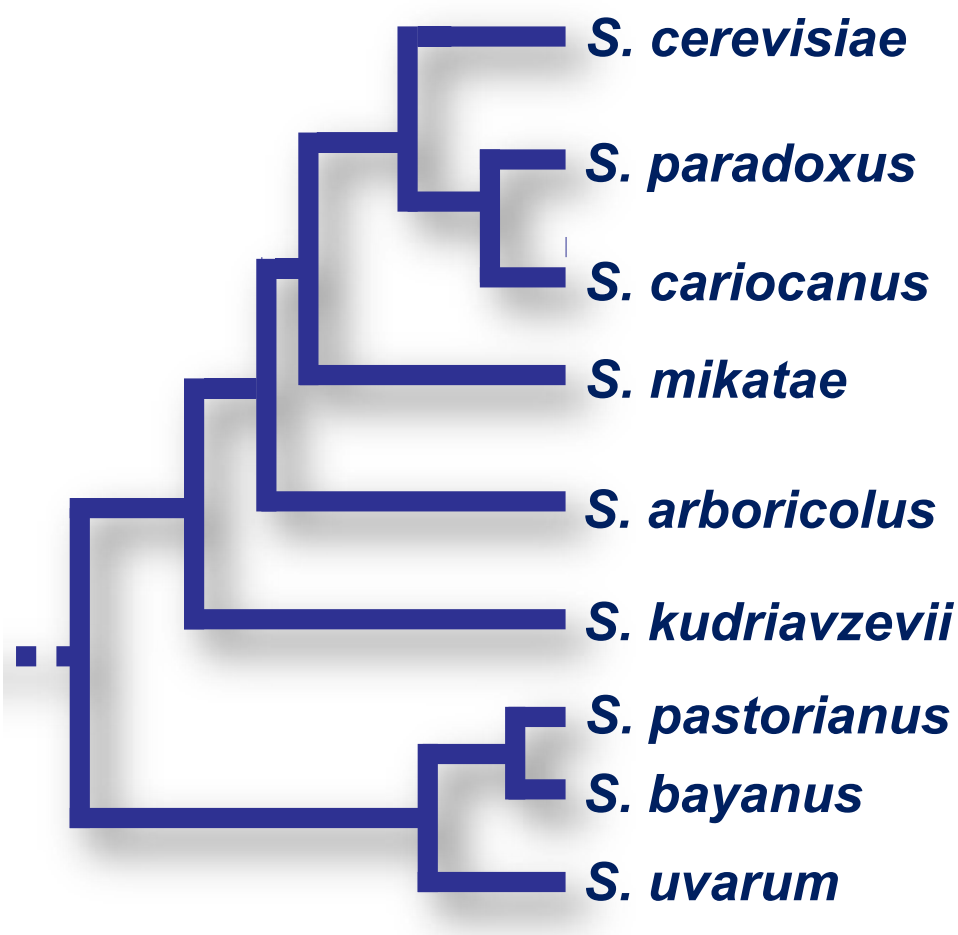
1. Most grape berries do not have *S. cerevisiae* (1/1000)

2. Damaged berries have much higher frequencies of *S. cerevisiae* (1/4)



Origin of the yeasts vectored by insects is unknown

The tree bark system harbors multiple (all?) *Saccharomyces* species



	Localities	Samples
115	Europe (Portugal, Germany)	164
22	North America (Canada)	96
15	South America (Argentina)	52
1	Oceania (Tasmania, New Zeal.)	64
1	Asia (Japan)	155
47	Total	531
10	*Quercus pyrenaica* 73%	
42	*Quercus faginea* 71%	

Sampaio and Gonçalves. 2008. Appl Env Microbiol 74: 2144-2152
and unpublished work

Louis Pasteur

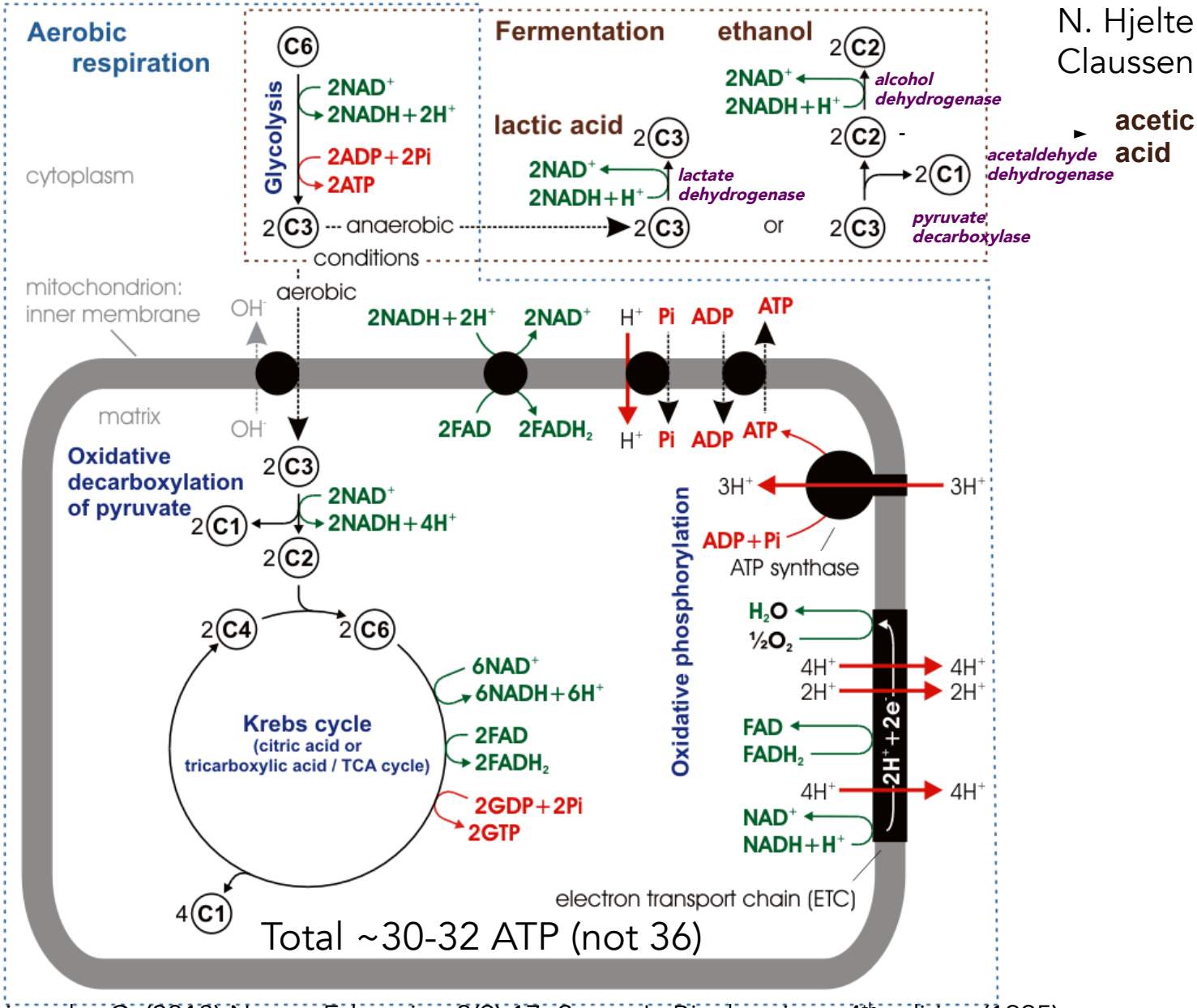
Emil Christian Hansen

Joseph Gay-Lussac

Brettanomyces
Saccharomyces
Homo sapiens

Brettanomyces
Saccharomyces

N. Hjelte Claussen



Alba-Lois, L. & Segal-Kischinevzky, C. (2010) Nature Education 3(9):17, Stryer, L. Biochemistry, 4th edition (1995)

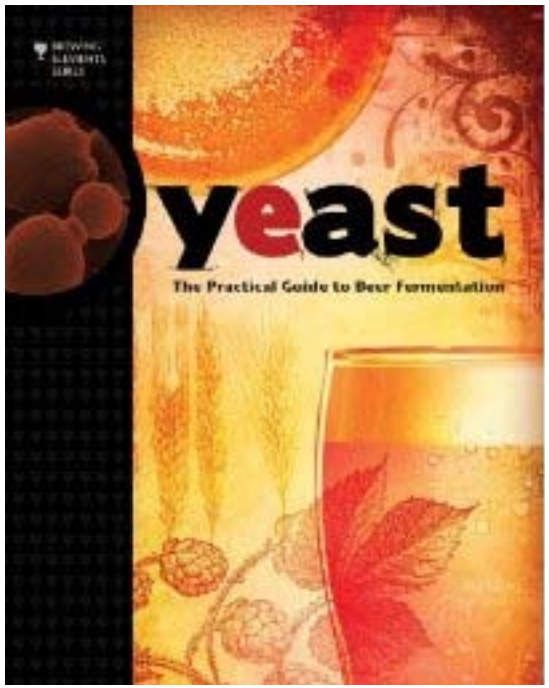
There is a wealth of information out there for (home)brewers to use

Why do this?

- Homebrewers want to push the envelope
- Control over your fermentations
- Isolate your own strains from cool bottles
- Save some $$
- Keep your own stocks
- Confidence in your own work
- *No need to be an expert/microbiologist*

What do you need? Where to start?

-Yeast by Jamil Zainasheff and Chris White (9 dollars on Am)
-http://www.maltosefalcons.com/tech/
yeast-propagation-and-maintenance-principles-and-practices
http://homebrewtalk.com
http://realbeer.com/spencer/yeast-culturing.html
http://www.themadfermentationist.com/
http://www.brettanomycesproject.com/
http://jaapie.org
Etc….



Ok…we have the info. What do we really need?

1. Notebook 5 dollar

The most important piece of your laboratory. Keep good notes – you will come back To them.

2. **Pressure cooker** 50 dollar

A pressure cooker is also really handy in the kitchen!

3. **Bunsen-burner** 2.30 dollar

Do not use an alcohol lamp. The flame is too slow. Hook up to a small propane burner.

4. **Glassware**

Spend as little money as possible. Reuse sauce jars, jam jars, ball jars. Buy some glass tubes with black screwcap, and some erlenmeyers (total ~30 dollar).

5. **Stirplate** 12 dollar (+5 dollar stirbar)

http://www.homebrewtalk.com/f51/my-shamelessly-cheap-12-diy-stir-plate-338695/

6. **Inoculation loop** 5 dollar for 3

7. **Petri dishes** 10 dollar for 5, glass

8. **Agar** Telephone-brand, Asian supermarket

9. **Spray bottle** 5 dollar

10. **<u>Stuff you already own</u>**:
    - Microwave
    - Aluminium foil
    - Lighter

11. **<u>Microscope and accessories</u>**:
    - Phase contrast if possible, 50 - 1000 dollar, ebay etc…

Yeast Starters: Aerobic conditions result in greater cell mass (and good for fatty acids sterols)

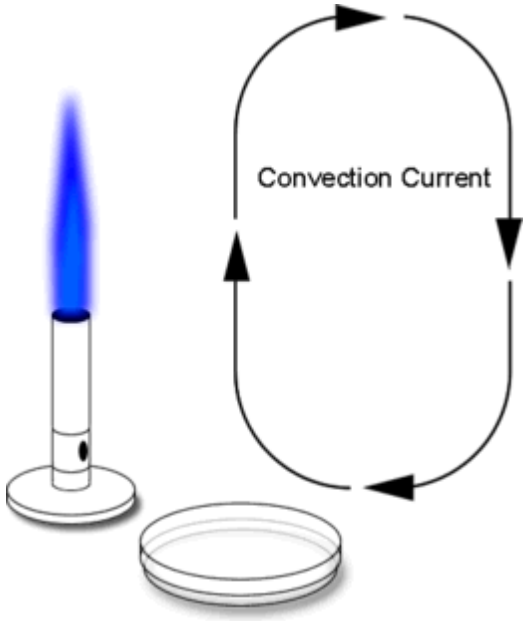
9 grams per liter of glucose: Crabtree effect will force cells into fermentation mode

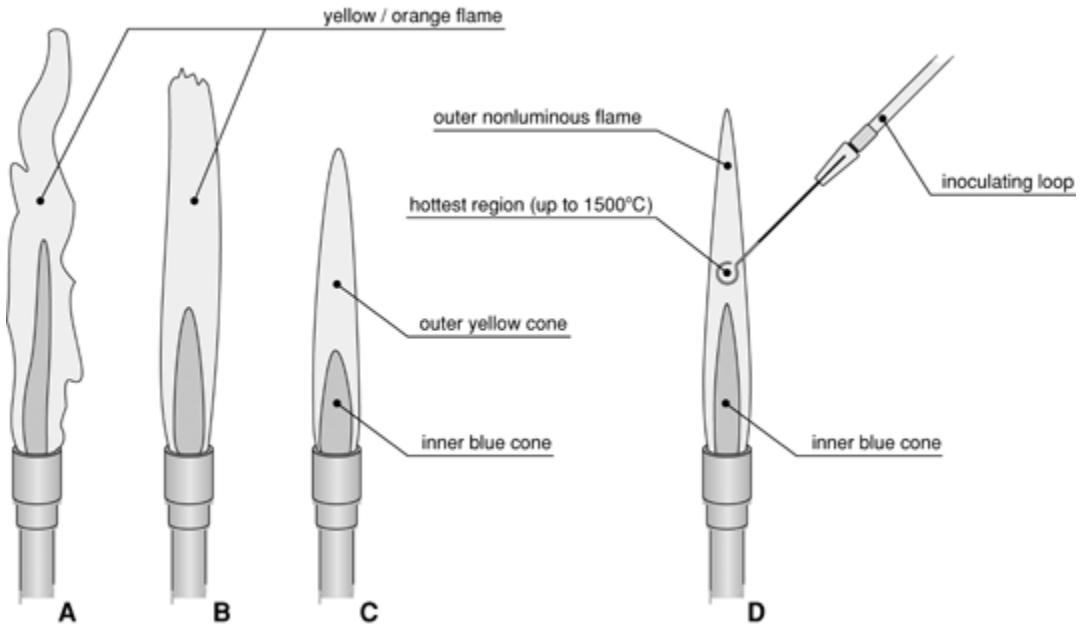
9 grams/liter = 1.0035 = 0.9 °P

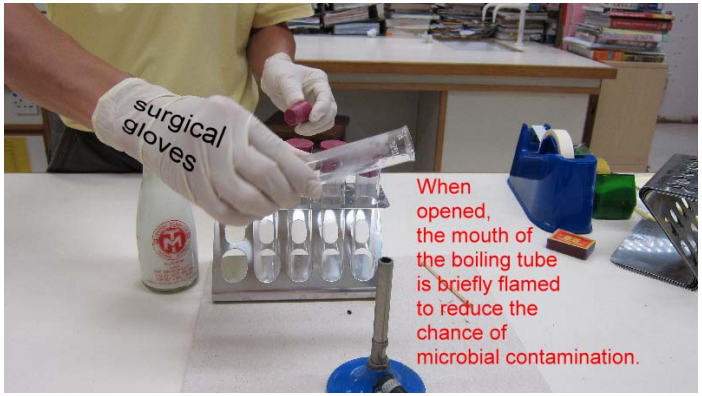
- Use low gravity wort for starters
- Aerate continuously (aeration stone, shaker, no pure O_2)
- Add yeast extract (servomyces/boiled yeast cells)
- add protein source (nitrogen/amino acids)
- its ok to grow the cells at 30 °C (86 °F)

Work Sterile

Work “under a flame”, do not rush, make sure everything is close, flame everything



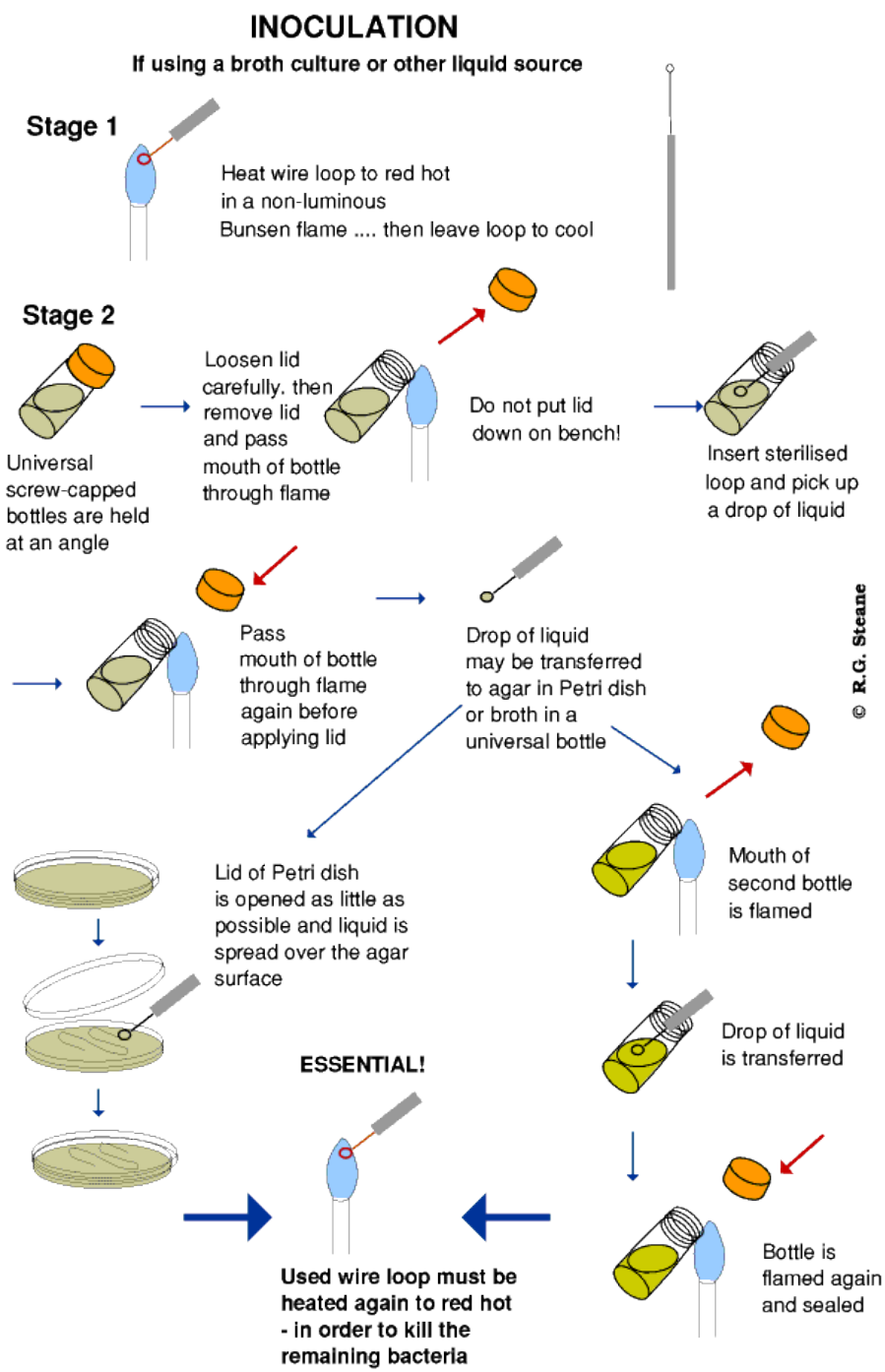




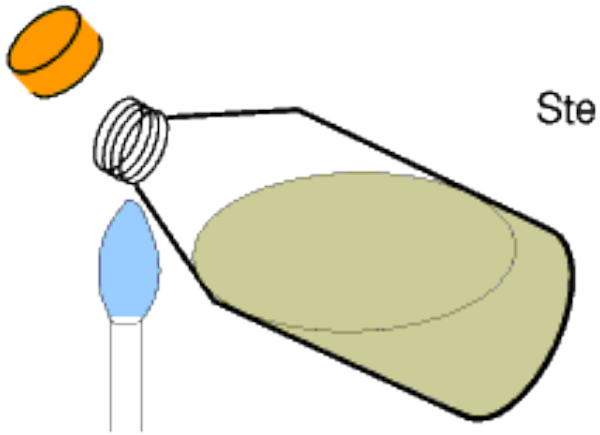
Do not wear gloves like these …people.

INOCULATION

If using a broth culture or other liquid source

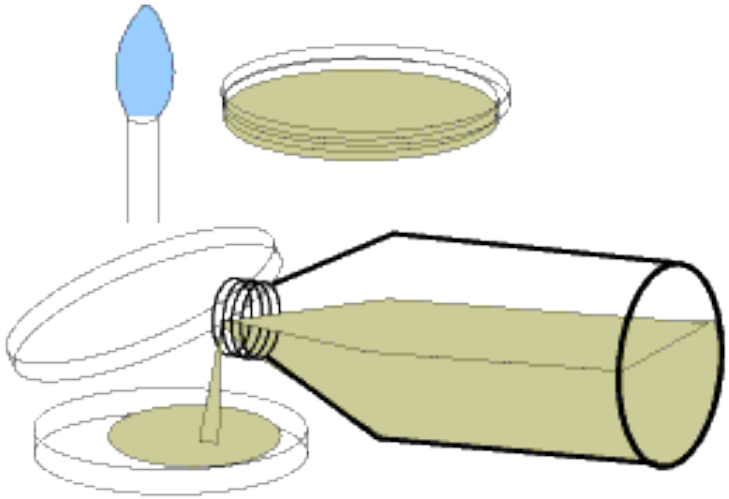


"Pouring a Plate"



Neck of agar bottle is passed through flame

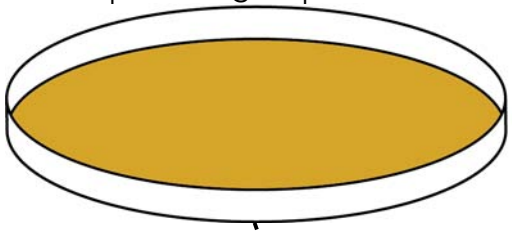
Sterilised molten agar is poured in and left to set.



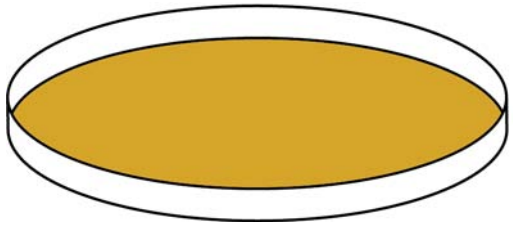
Petri dish lid is opened as little as possible, angled and kept over the base.

Each Petri dish holds about 20 ml, so 200ml will do for 10.

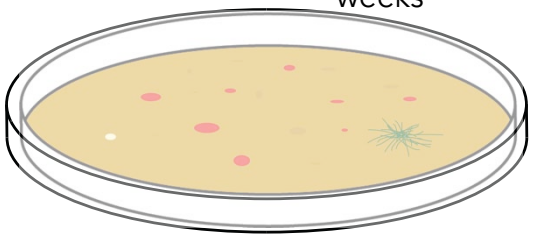
Solid Media
(2% peptone, 1% yeast extract, 2% maltose - agar plates and potato-agar* plates)



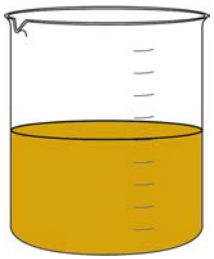
"Air incubate" for 24-48 h



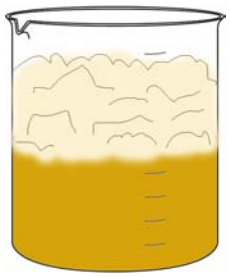
Incubate at 25 °C (77 °F) for 2-3 weeks



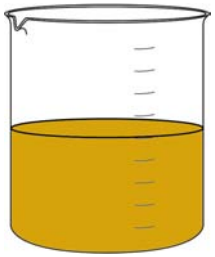
Low Gravity Liquid Media
(2% peptone, 1% yeast extract, 2% maltose)



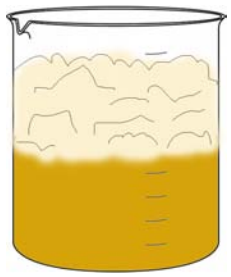
"Air incubate" for 1-6 weeks



High Gravity Liquid Media
(2% peptone, 1% yeast extract, 30% maltose**)



"Air incubate" for 1-6 weeks

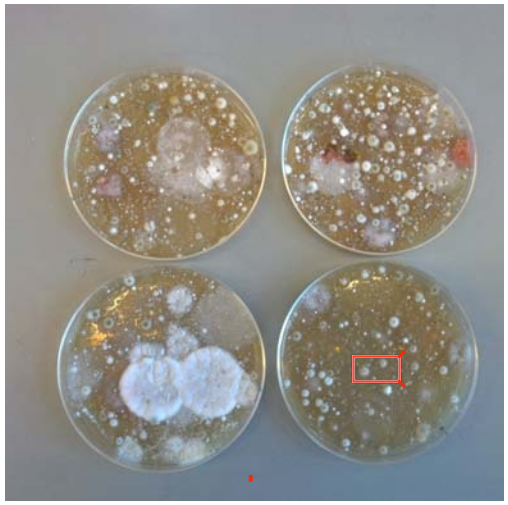


Ferment and isolate organisms

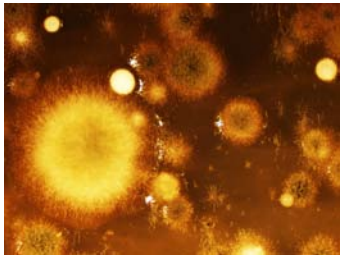
Streak fermenting liquid/ colonies on YPM agar Supplemented with 100µg/ml ampicillin

*http://en.wikipedia.org/wiki/Potato_dextrose_agar
**F. Noé Arroyo-López et al. Int J of Food Microbiol 131 (2009) 120–127

Both YPM and potato agar showed growth

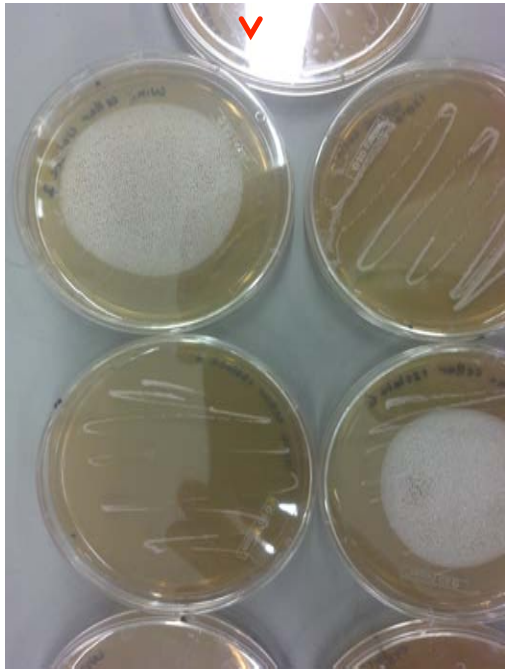


Stereo Microscope

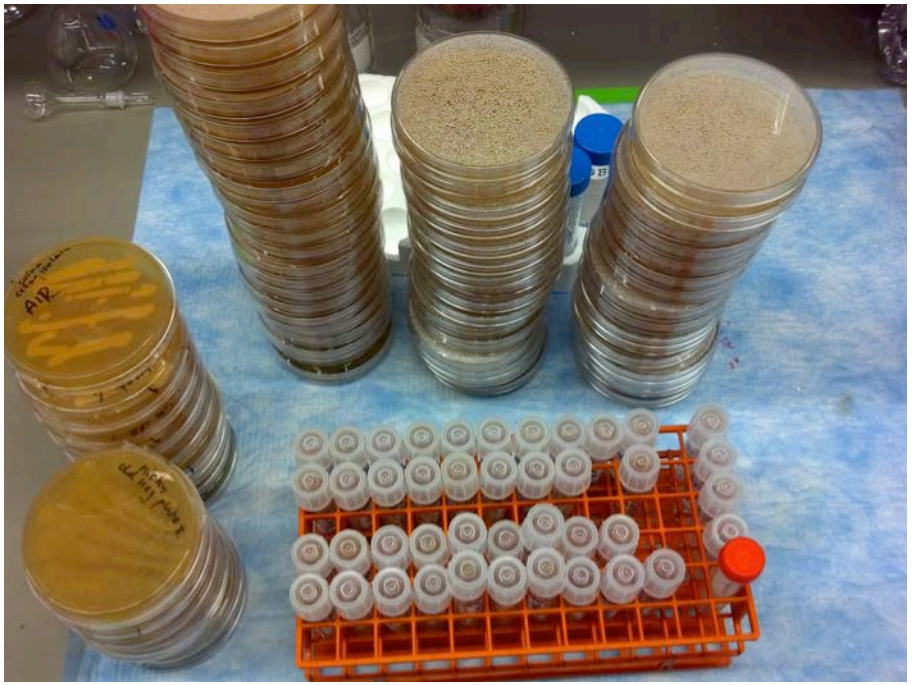


Both high-gravity and low-gravity YPM showed growth

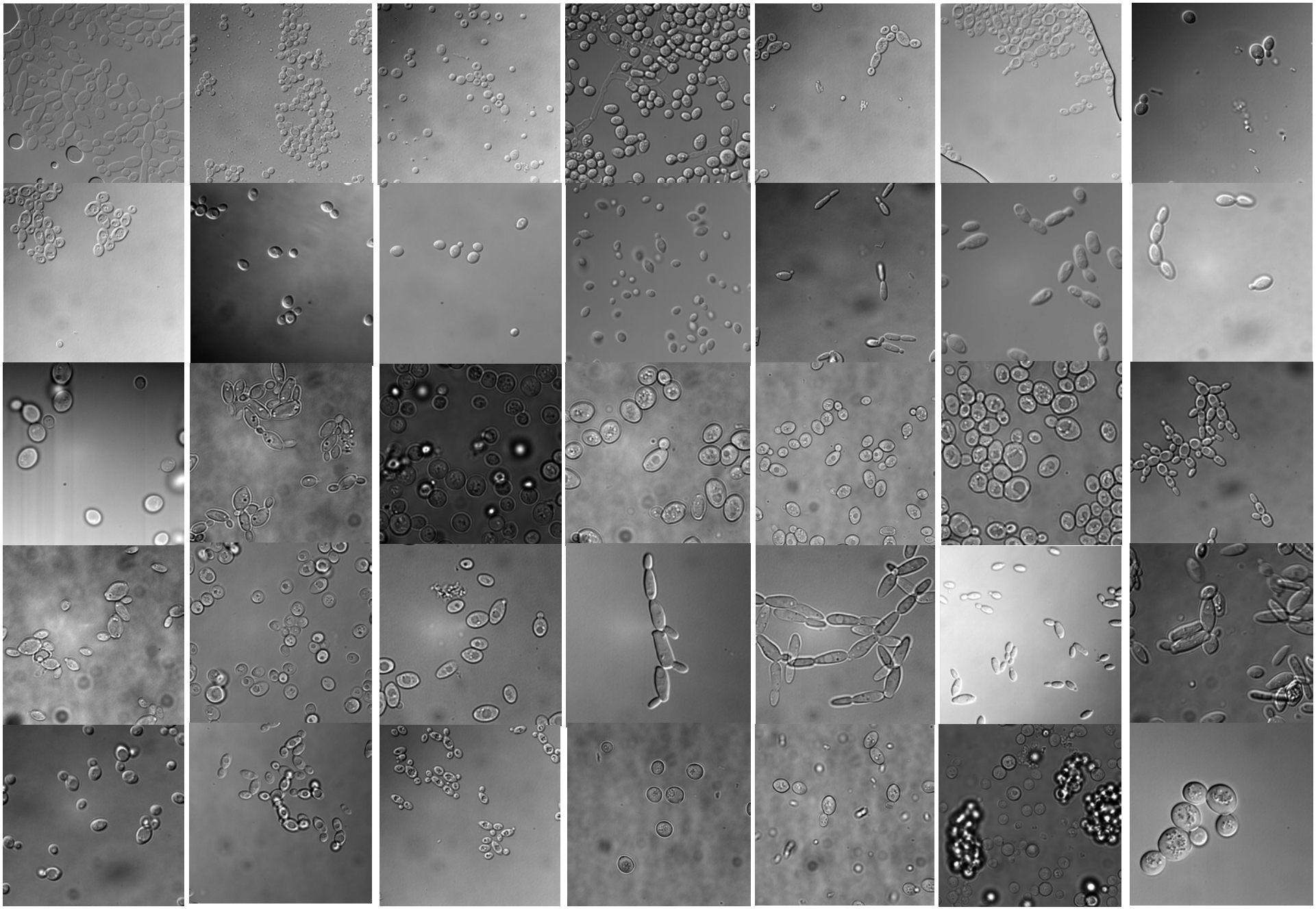
streak and select single colonies



grow small (5ml) cultures



Micrographs phase contrast (FOV 50-72µm)




GLASS
HANDLE WITH CARE

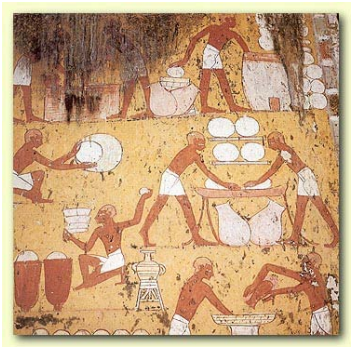
Where are we now

		Initial Growth	Gravity (P) (6 weeks in)	Smell/Taste		
NY State	Air1		No Fermentation	-		
	Air2		No Fermentation	-		
	Air3		13.1	ok		
	Air4		Did not grow	-		
	Air5		Did not grow	-		
	NY13.2		4.5	Grainy, Fruity, Pleasant	<	-
	NY3.1		14.3	Very awful - discard		
Questionable Barrels	W1		5.3	Very Clean, Honey	<	-
	W2		5.6	Tart, Citrus	<	-
	W3		No Fermentation	Very Ropey		
	W4		14.5	ok		
	W5		15.9	ok		
	W6		16.1	Dumped		
	W7		14.7	ok		
	W8		6.8	Grainy, pleasant, fruits	<	-
	W9		16	Discarded		
	W10		No Fermentation			
	W11		No Fermentation			
Ashburn Air	YF1		14.7	Discarded		
	YF2		Did not grow			
	YF3		6.3	ok		
	YF4		12.4	ok		
	YF5		5.6	Rancid butter		
	YF6		4.5	socks		
	YF7		No Fermentation			
	YF8		14.3	ok		
	YF9		Did not grow			
	YF10.1		6.8	apple, fruity loops	<	-
	YF10.2		15.2	Bitter smell/taste		
	YF11		4.6	Socks, sweat, locker room		
	YF12		14.4	ok		
	YF13		Too bad to measure	Discard		
	YF14		6.1			
	YF15		2.6	Sour Apples, Tart	<	-
	YF16.1		15.2	ok		
	YF16.2		5.1	Brett, tart, nice	<	-
	YF17		13.3	Not very great		
	YF18		16.1	Very awful		
	YF19		14.6	Taste is bitter aroma clean		
Controls	WB1		7.1	Aromatic Brett, Fruity		
	WY1		5.1	Clean, ok		
	WY2		5.1	Clean, ok		
	LRB		0.5	Clean, fruity, Brett	<	-
	Fantome		8.05	Nice, belgianesque		

8 out of 41 enjoyable after just 6 weeks

All fermenting ones have been bottled

~4300 BC Babylonians brewed beer

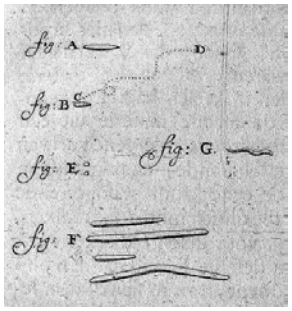


~4000 BC Egyptians Used yeast to rise bread – used it for beer as well.

1516AD: Rheinheitsgebot

1000AD – Hops Enters the scene

1683AD: Antonie van Leeuwenhoek describes bacteria & yeasts



time

Evidence of fermented beverages found in neolithic (~9000 BC) pottery Jiahu, China

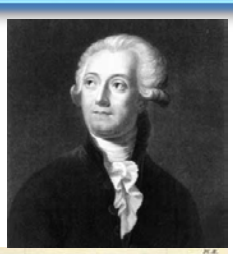
~55 BC Romans introduced beer to Europe (Cerevisia)

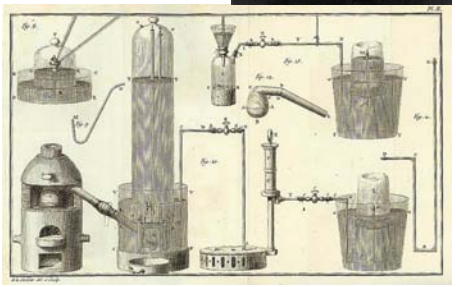
1420AD – Germans Develop lager brewing

1595AD: Hans & Zacharias Janssen develop 1st microscope

The First Compound Microscope (circa 1595)

1789AD: Antoine Lavoisier deciphers mass balance of fermentation

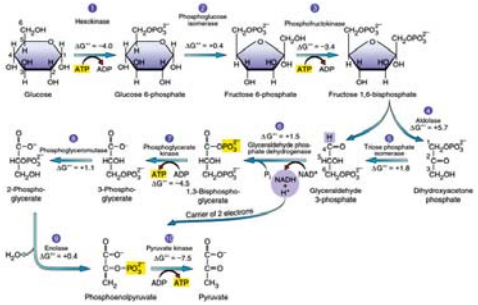




$C_6H_{12}O_6$ + Yeast → 2 C_2H_5OH + 2 CO_2

1815: Gay-Lussac finds that alcohol is formed by yeast without air present

1883: Emil Christian Hansen identifies lager yeast, *Saccharomyces pastorianus*



1940: Glycolysis (glucose to pyruvate) elucidated

1996: Complete genome of *S. cerevisiae* is sequenced.

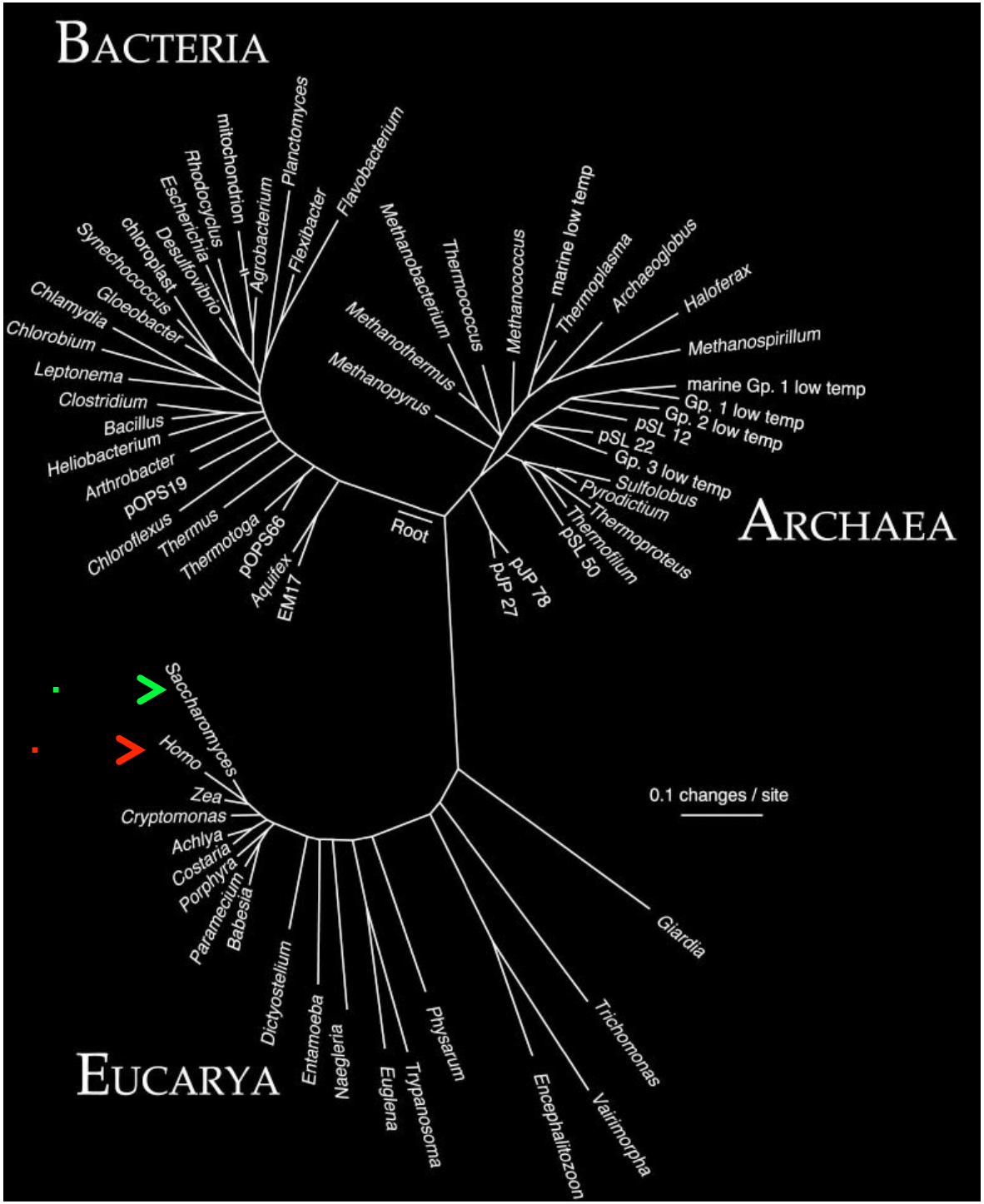
1835AD: Charles Cagniard de la Tour described budding yeast. Yeast are alive.

1876AD: Louis Pasteur shows yeast ferments anaerobically, and describes lactic fermentation.

1890AD: Hermann Müller ferments wine with pure yeast cultures

1943AD: Carl Lindegren discovers the mating types α and a, and elucidates the yeast life cycle.

2011: Original Lager strain (*S. eubayanus*) isolated from tree bark in patagonia



All life on earth can be divided in three domains:

Bacteria
Archaea
Eukaryotes

Yeast: ~1500 species

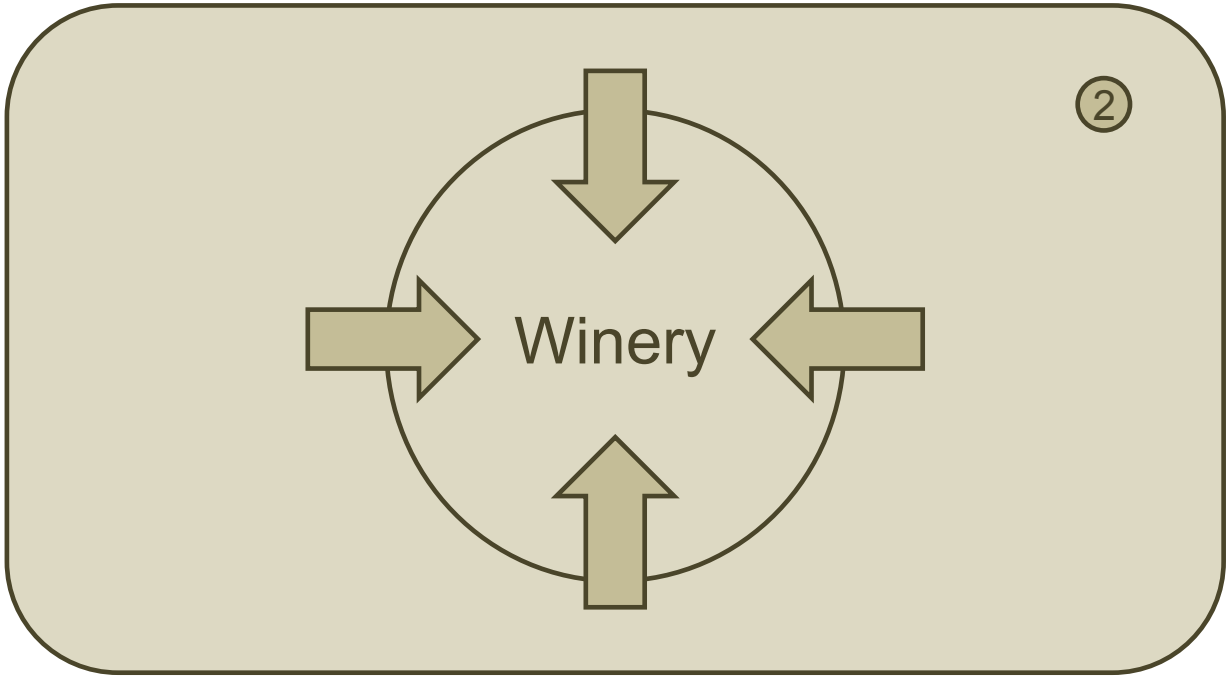
Two species are of interest:
- *Dekkera/Brettanomyces*
- *Saccharomyces*

"Yeast" and Man are very alike

- Similar metabolism
- Cell structure/machinery shows overlap
- Likes to hang out in the same places

Where is this yeast?

The primary and exclusive habitats of *S.cerevisiae* are the various surfaces of the winery



S. cerevisiae does not exist in natural environments – it is therefore a domesticated organism

Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast

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Domestication of plants and animals promoted humanity's transition from nomadic to sedentary lifestyles, demographic expansion, and the emergence of civilizations. In contrast to the well-documented successes of crop and livestock breeding, processes of microbe domestication remain obscure, despite the importance of microbes to the production of food, beverages, and biofuels. Lager-beer, first brewed in the 15th century, employs an allotetraploid hybrid yeast, *Saccharomyces pastorianus* (syn. *Saccharomyces carlsbergensis*), a domesticated species created by the fusion of a *Saccharomyces cerevisiae* ale-yeast with an unknown cryotolerant *Saccharomyces* species. We report the isolation of that species and designate it *Saccharomyces eubayanus* sp. nov. because of its resemblance to *Saccharomyces bayanus* (a complex hybrid of *S. eubayanus*, *Saccharomyces uvarum*, and *S. cerevisiae* found only in the brewing environment). Individuals from populations of *S. eubayanus* and its sister species, *S. uvarum*, exist in apparent sympatry in *Nothofagus* (Southern beech) forests in Patagonia, but are isolated genetically through intrinsic postzygotic barriers, and ecologically through host-preference. The draft genome sequence of *S. eubayanus* is 99.5% identical to the non-*S. cerevisiae* portion of the *S. pastorianus* genome sequence and suggests specific changes in sugar and sulfite metabolism that were crucial for domestication in the lager-brewing environment. This study shows that combining microbial ecology with comparative genomics facilitates the discovery and preservation of wild genetic stocks of domesticated microbes to trace their history, identify genetic changes, and suggest paths to further industrial improvement.

beer yeast | next-generation sequencing | yeast ecology | yeast taxonomy

Saccharomyces pastorianus (syn. *Saccharomyces carlsbergensis*) strains (8); two other cryotolerant *Saccharomyces* spp. have been associated with beer as contaminants (*Saccharomyces bayanus*) and with cider or wine fermented at low temperatures (*Saccharomyces uvarum*) (9). *S. pastorianus* has never been isolated from the wild, depends on humans for its propagation, and appears to be an allotetraploid hybrid species of *S. cerevisiae* and an unidentified species (10, 11). Several hypotheses have been advanced for the source of the non-*S. cerevisiae* genome present in *S. pastorianus*, including the taxonomically and genetically complex species *S. bayanus* (12–14) and an unknown "lager" lineage distinct both from *S. bayanus* and *S. uvarum* (11, 15). Identifying the wild genetic stock of the cryotolerant subgenome of *S. pastorianus* is necessary for resolving the taxonomy and systematics of this important species complex, and for understanding the key events that led to the domestication of lager yeast.

In contrast to extensive investigation into domestication of crops and livestock (2, 16–19), studies of domestication of eukaryotic microbes have been limited (20–24), perhaps because of the inability to conduct direct field studies. Identifying the genetic basis of traits under selection during domestication may clarify the emergence of new traits and show the way toward further improvement. Because domesticated lineages derive from a subset of the original populations, a genetic bottleneck is likely to have caused the disappearance of some alleles (17), especially in microbes, which are often propagated clonally. In an age of accelerated habitat destruction and diminishing biodiversity, discovery of wild genetic stocks of domesticated microbes will facilitate preservation of their genetic resources for strain improvement.

Isolating Yeasts -

Prehistoric Yeast Used to Brew Fossil Fuels Beer

by Sara Bonisteel, Posted Aug 5th 2009 @ 5:30PM

Stumptown's XPort. Photo: Steve Kocino/flickr

Talk about a cold swig of history.

Beer made from a yeast 45 million years old will soon be hitting taps in California.

Northern California microbrewer Stumptown Brewery is already selling two brews made with the yeast, extracted from a prehistoric piece of amber found in Burma. But as early as next month, Fossil Fuels Brewing Co. plans to distribute two others made with the yeast on a larger scale.

Don't expect to be drinking mead when you down one of the company's wheat or "Ancient Ales."

"In the world of microbiology, 45 million years doesn't cause a lot of changes," Chip Lambert, the president of Fossil Fuels Brewing Co., of Oakland, Calif., told Slashfood on Wednesday. "We call this the mother of all modern yeasts, but it's just a 45-million-year-old mom."

Find out where to find the prehistoric brew after the jump.

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Cheers! Scientists resurrect microbe used to ferment alcohol in ancient Indian burial ritual

By TED THORNHILL
UPDATED: 07:20 EST, 22 February 2012

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Yeasts that had lain dormant for over 1,300 years in an Andean tomb have been kick-started back to life - and revealed as an entirely new species.