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The Concept of Nature-Nurture Applied to Brewer's Yeast and Wort Fermentation

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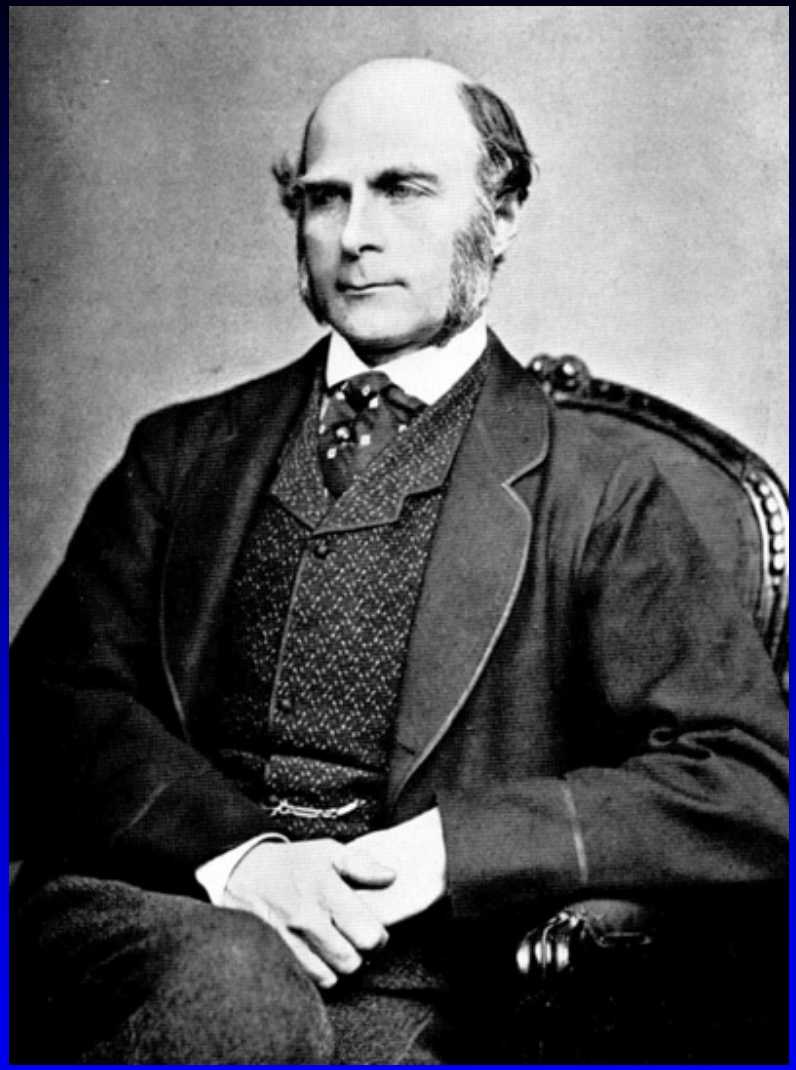

Sir Frances Galton, 1822-1911

- Concept of nature-nurture described in “Hereditary Genius” by Francis Galton and published in 1869.

Galton's Controversial Theories

- He adopted the terms eugenics and nature-nurture.
- He advocated the improvement of human genetics through reproduction of people with desirable traits and reduced reproduction of people with less desirable traits.
- He devised a method for classifying fingerprints – advent of forensic science.
- He conducted research on the power of prayer!

Definition of Nature-Nurture

A traditional and long-standing discussion over whether heredity (nature) or the environment (nurture) is more important in the development of living things, for example, brewer's yeast species and strains.

Nature-Nurture Anomalies—Further Studies in Yeast Flocculation[1]

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ABSTRACT

The relative contributions of phenotype (*i.e.*, the nurture effect) and genotype (*i.e.*, the nature effect) to flocculation in a number of yeast strains have been assessed. It is quite apparent from these studies that both factors play a vital role. It appears, however, that the genetic influences are paramount. The flocculation characteristics of certain yeast strains are susceptible to alterations in such environmental conditions as incubation temperature and wort gravity, whereas other strains have a uniform flocculation over quite a wide spectrum of such conditions. The flocculation characteristics of all strains studied are affected by such environmental conditions as wort adjunct level, yeast pitching rate, and wort oxygen level at pitching. Yeast flocculation is under genetic control and both dominant and recessive genes for this trait have been identified. It also appears that the expression of the flocculation genes is modified in many hybrids. This modification could either be genetic or cytoplasmic in origin.

Key words: ***Brewing, Fermentation, Flocculation, Genetics, Yeast.***

The relative contributions of heredity and environment to the flocculation characteristics of a particular yeast culture have been matters of considerable discussion and dispute (12, 30). Yeast flocculation has been regarded by some researchers as primarily genotypic (48) with the environment playing an insignificant role. However, studies in this laboratory, described in this paper, tend to indicate that there is a very fine balance between the role played by the genetic makeup of the strain and the environmental conditions in which it finds itself. Francis Galton (1822-1911) first used the term *nature-nurture* to represent these two types of influence.

The studies described in this paper are far from complete and are only an introduction to nature-nurture effects as they influence the use of yeast strains in the brewery. It is hoped that this paper will be regarded in this light and will serve to encourage polemists to begin a debate of the whole question of yeast genetics and its application in the brewing industry because, as will become clearer in the **DISCUSSION** section, the authors are of the opinion that the time is now opportune for intensive and meaningful research to be conducted on this subject.

EXPERIMENTAL

A number of flocculent and nonflocculent strains of *Saccharomyces cerevisiae* and *S. carlsbergensis* were studied; they are referred to in the text by their number in the Labatt Culture Collection. A number of strains with known genetic markers have been used in this work, the origins of which are described in the **RESULTS** section.

Media

The following media were used routinely:

a) Wort. Unless otherwise stated, 11.8° P hopped wort was used (30% corn adjunct).

b) Malt-yeast extract-glucose-peptone (MYGP) (46).

c) Peptone-yeast extract-nutrient medium (28).

d) Defined medium: 6.7 g yeast nitrogen base (Difco), 20 g glucose, 40 g maltose, 3.3 g citric acid, and 5.2 g sodium citrate dissolved in 1 l. distilled H_2O.

Flocculation Tests

Two *in vitro* tests for flocculence were used:

a) The Helm Sedimentation Test (17). This test was performed in distilled H_2O at pH 4.0 containing 3.6 mmol calcium chloride.

b) A method adapted from the technique of Gilliland (14) was used to screen for the flocculating ability of a large number of yeast strains. This method allowed the assessment of flocculation in a growth medium. A small inoculum of the yeast strain was seeded into 20-ml bottles containing 10 ml of medium (wort, MYGP, or defined medium). After 3 and 5 days of incubation at 25°C, the flocculation characteristics of the cultures were distinguished first by the appearance of their sediment and secondly by the nature of the flocs subsequent to the sediment being brought back into suspension by shaking the bottle.

The degree of flocculation was quantitatively expressed by applying the subjective gradation used in previous publications from this laboratory (41), namely:

5 - Extremely flocculent, 4 - Very flocculent, 3 - Moderately flocculent, 2 - Weakly flocculent, 1 - Rough, 0 - Nonflocculent.

To study the flocculation characteristics of strains under conditions more closely related to the static fermentation encountered in a brewery, *i.e., in vivo* conditions, the yeast was precultured in wort and then inoculated into 16 l. of hopped wort in a 20-l. unstirred glass fermentor unless otherwise stated. An inoculation level of 0.25% wet weight of cells (1.35×10^7 viable cells/ml) was used in all experiments together with an incubation temperature of 21°C unless otherwise stated. Sequential wort samples were taken throughout fermentation and the specific gravity and concentration of yeast in suspension were determined on each sample.

Fermentability of the wort with each yeast was determined by incubating 200 ml of wort with 4 g of the yeast strain in a 300-ml flask on a New Brunswick Gyrotory shaker at 160 rpm at 21°C for 72 hr. The difference between the original gravity and the gravity after shaken fermentation represents 100% fermentability for the wort and yeast strain in question.

Induction of Sporulation (10)

a) Presporulation agar: Yeast strain was inoculated onto a medium of the following composition: 20 g glucose, 2 g potassium dihydrogen phosphate, 2 g ammonium sulfate, 5 g yeast extract, and 15 g agar dissolved in 1 l. distilled H_2O. After 2 days' incubation at 30°C, the yeast was subcultured onto the sporulation agar.

b) Sporulation agar: 1 g glucose, 1.8 g potassium chloride, 2.5 g yeast extract, 8.2 g sodium acetate, and 20 g agar dissolved in 1 l. distilled H_2O. Cultures were examined for spores after 5 and 14 days of incubation at 30°C.

c) Spore stain (32): Cultures were heat-fixed on a glass slide and stained with 5% malachite green with heating; the slide was washed with water and counterstained with 0.5% safranin. Microscopic examination of the stained preparation revealed the asci to be green and the vegetative cells to be red.

Isolation of Spores

Asci off the sporulation agar were suspended in sterile distilled water to which was added a few drops of a 1:40 dilution of glusulase (Endo Labs Inc., Garden City, N.Y.). After 20 min of treatment, the asci suspension was spread onto agar slabs and the spores isolated by micromanipulation using a de Fonbrune micromanipulator according to the Fowell method (10). The agar slabs containing the isolated spores were incubated at 30°C for 2–4 days. When the germinated colony from the spore had become large enough, it was subcultured onto fresh agar medium.

[1]Presented at the 41st Annual Meeting, Kiamesha Lake, N.Y., May 1975.

Nature-Nurture Effects during Wort Fermentation

- Many published examples illustrate that during wort fermentation by brewer's yeast species (and strains) both the genetic make-up of the organism (nature) and the fermentation characteristics (nurture) contribute to overall fermentation performance and beer quality.

Examples to Illustrate the Nature-Nurture Concept

- Ale and lager yeast species.
- Uptake of wort maltose.
- Ester formation.
- Yeast flocculation.

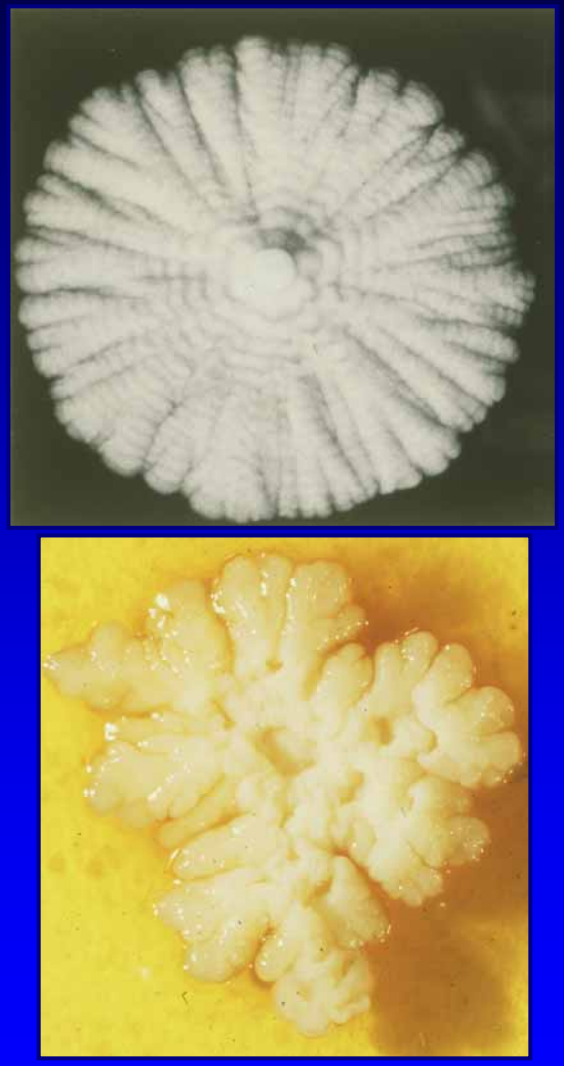
ALE AND LAGER

YEAST SPECIES

Differences between Ale and Lager Yeast Strains

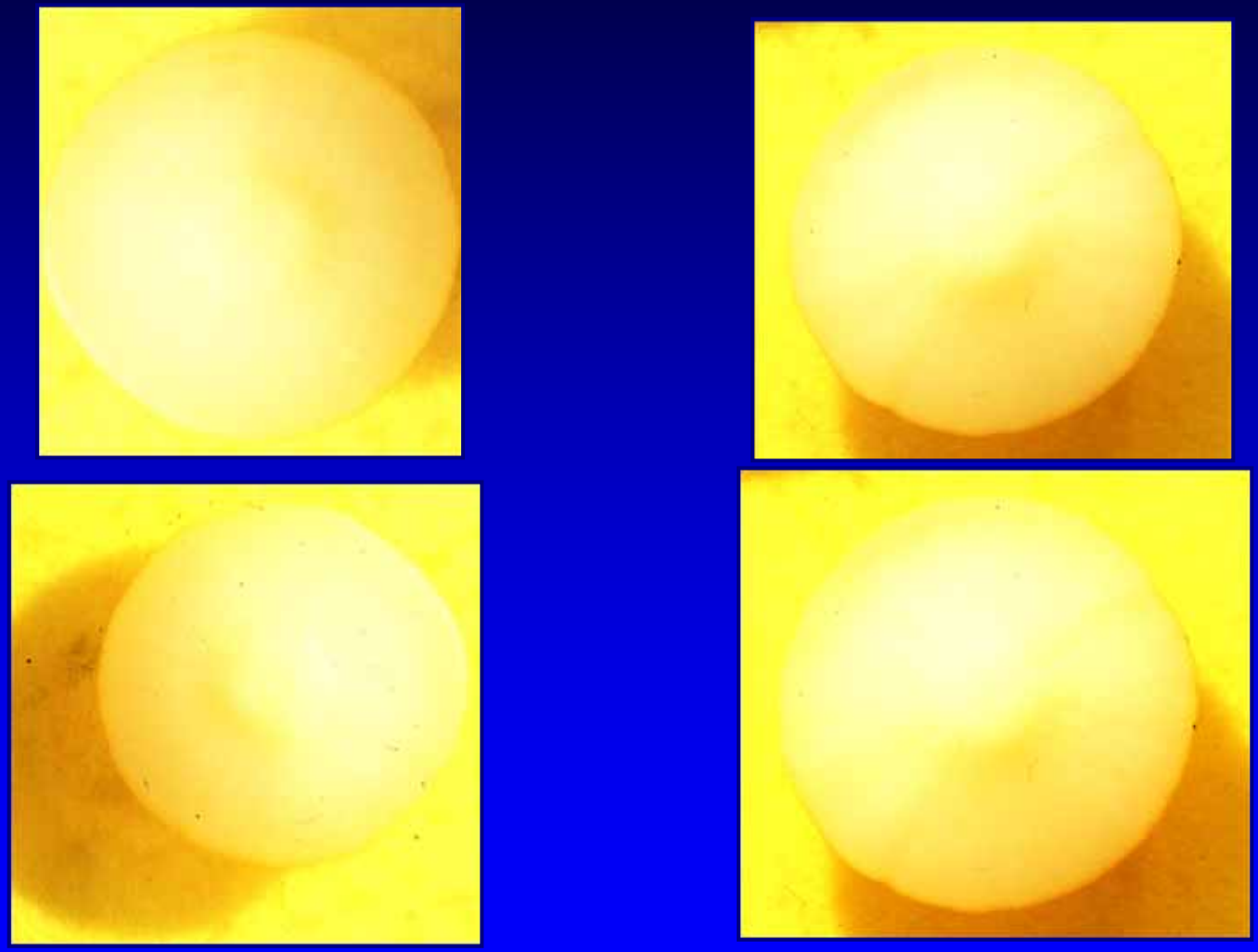
Ale Yeast	Lager Yeast
Saccharomyces cerevisiae (ale type)	*Saccharomyces carlsbergensis*
Saccharomyces cerevisiae (ale and distillers yeast)	*Saccharomyces uvarium* (*carlsbergensis*)
	Saccharomyces cerevisiae (lager type)
	Saccharomyces pastorianus (current taxonomic name)
Fermentation temperature (18-25ºC)	Fermentation temperature (8-15ºC)
Cells can grow at 37ºC or higher	Cells cannot grow above 34ºC
Cells cannot ferment the dissacharide melibiose	Ferments melibiose
Strains with distinctive colonial morphology on wort-gelatin medium	Strains do not have a distinctive morphology on wort-gelatin medium
“Top” fermentation	“Bottom” fermentation

Giant Colony Morphology of Ale Strains*



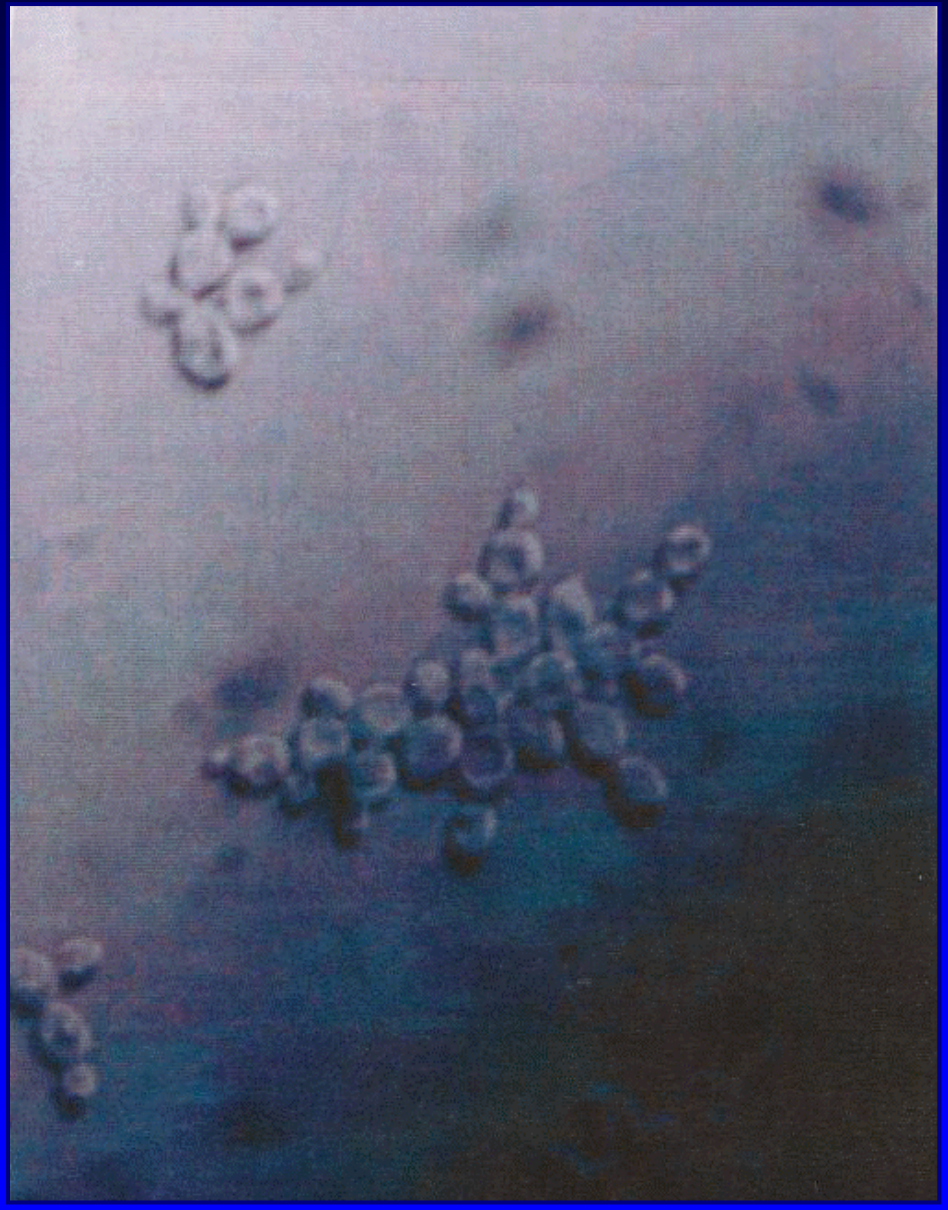
*Cultures grown on wort-gelatin medium at 18°C for three weeks

Giant Colony Morphology of Lager Strains*

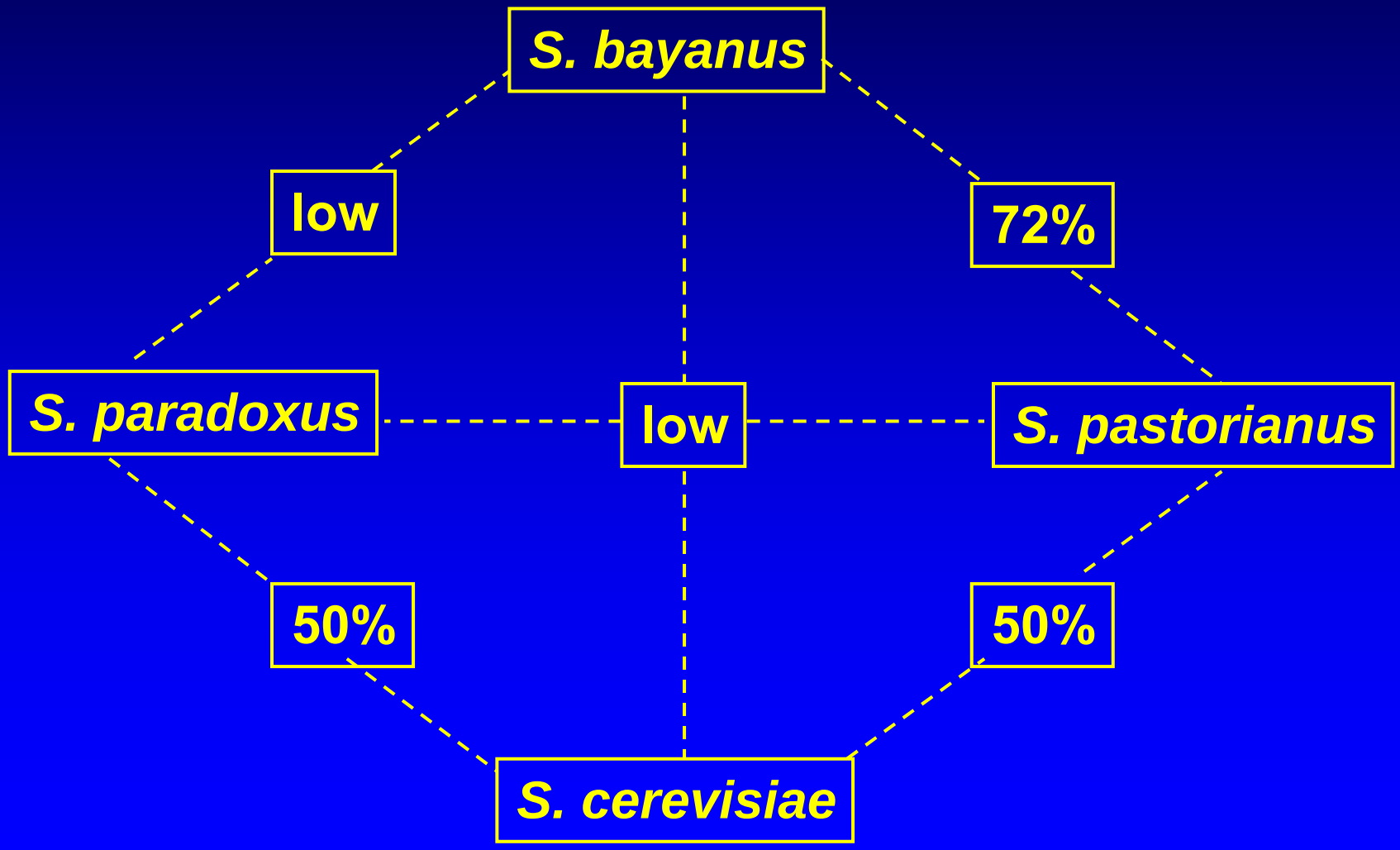


*Cultures grown on wort-gelatin medium at 18ºC for three weeks

Chain Formation in Ale Yeast Strains



The *Saccharomyces sensu stricto* group
Ale and Lager Strains



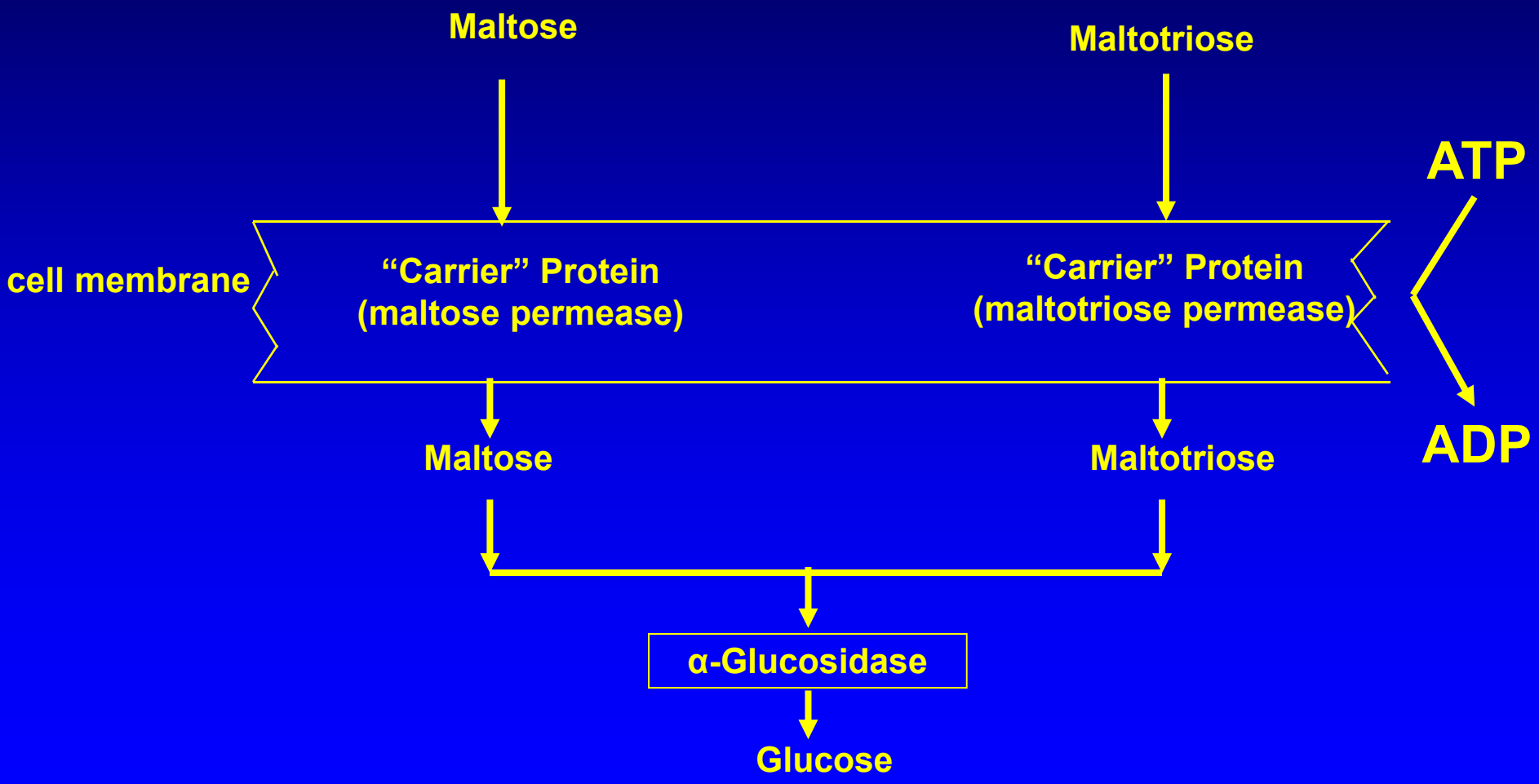
UPTAKE

OF

WORT

MALTOSE

Uptake and Metabolism of Maltose and Maltotriose by the Yeast Cell



Polymeric Gene Cassettes for Maltose Metabolism

MAL1

MAL2

MAL3

MAL4

MAL6

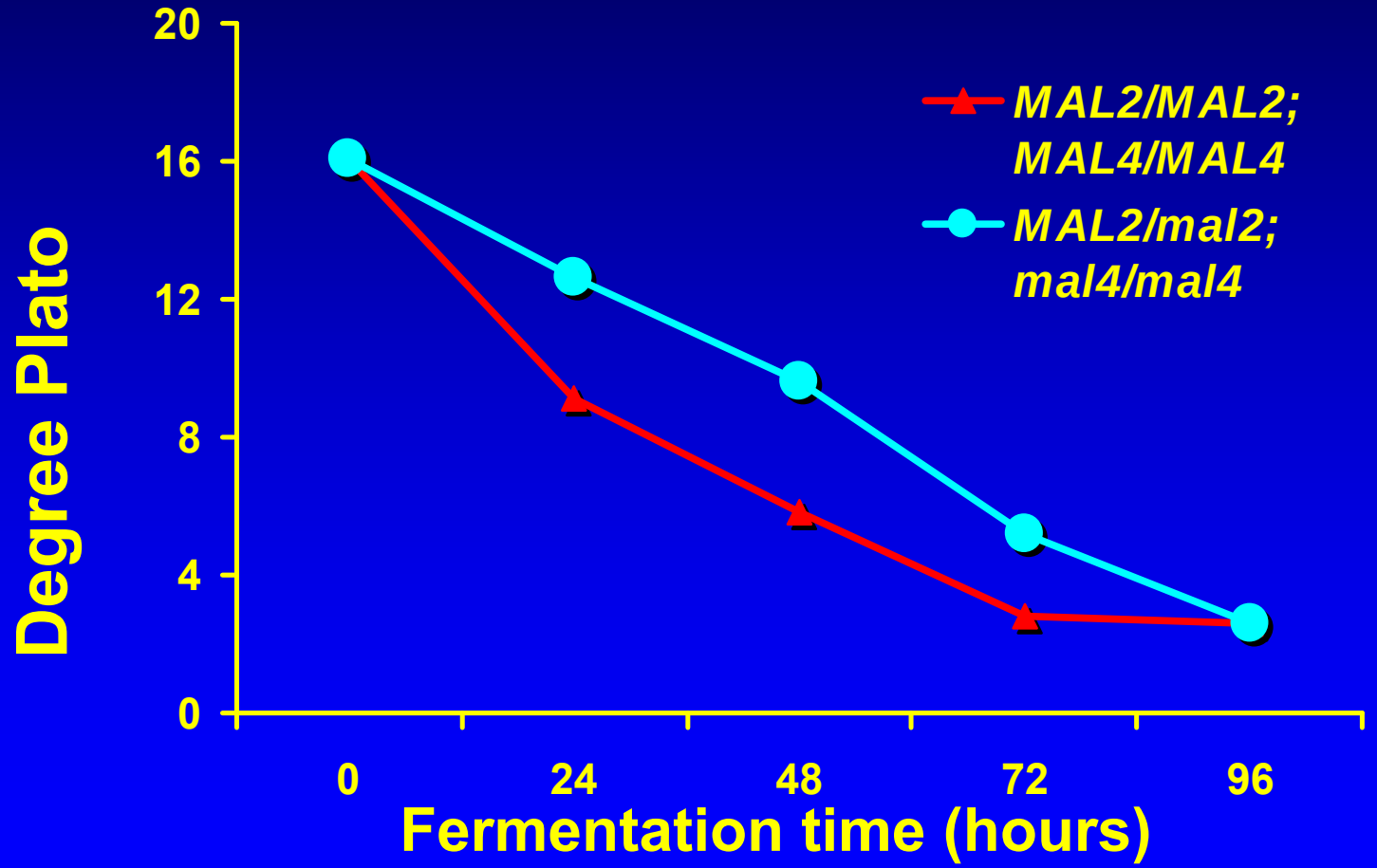
Composition of a Maltose Gene Cassette

MALS – α glucosidase (maltase)

MALT – Maltose permease

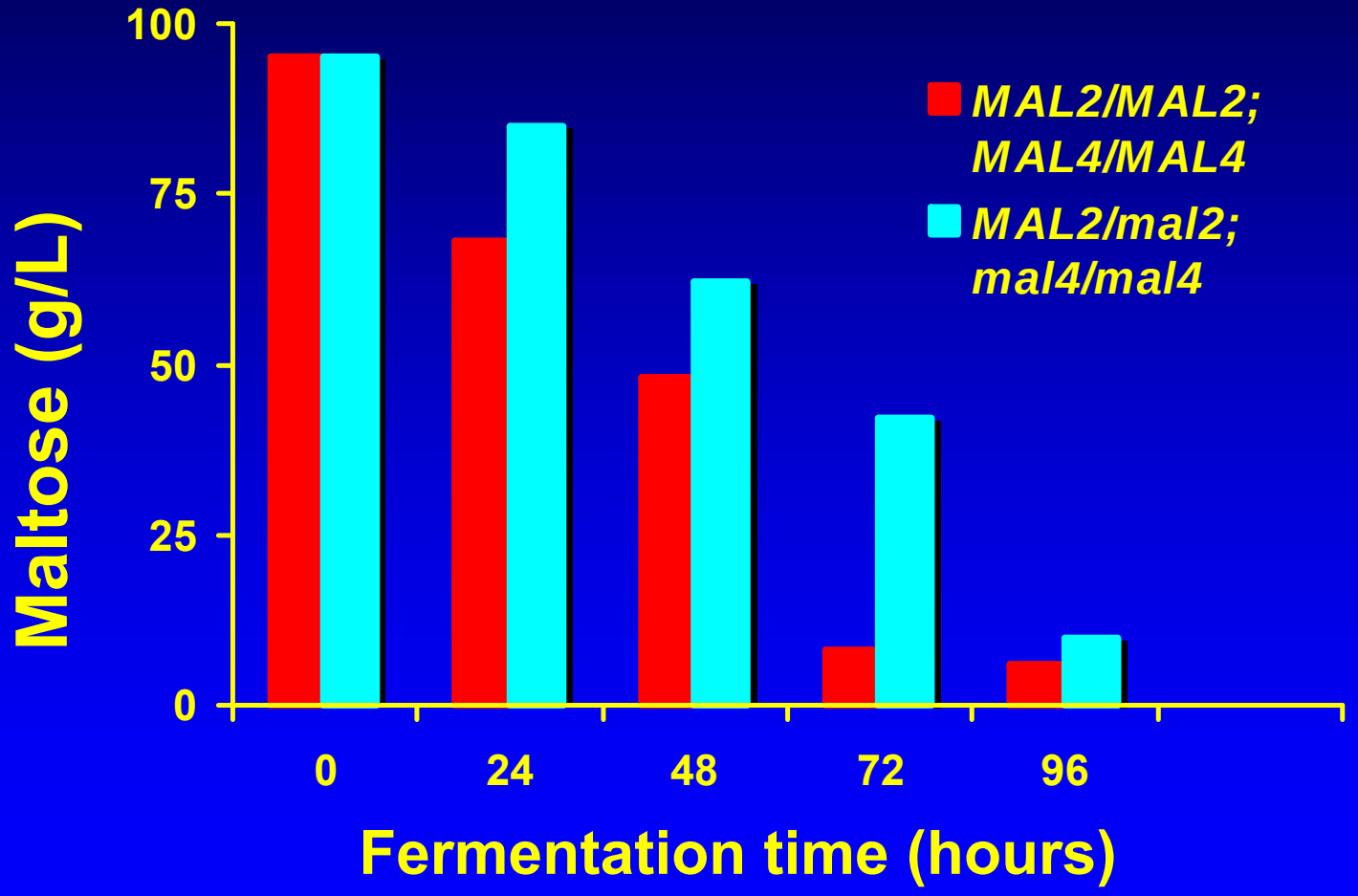
MALR – Regulates the expression of α glucosidase and maltose permease.

Fermentation Profile of a 16°Plato Wort with Diploid Yeast Strains Containing Multiple Maltose (*MAL*) Genes*



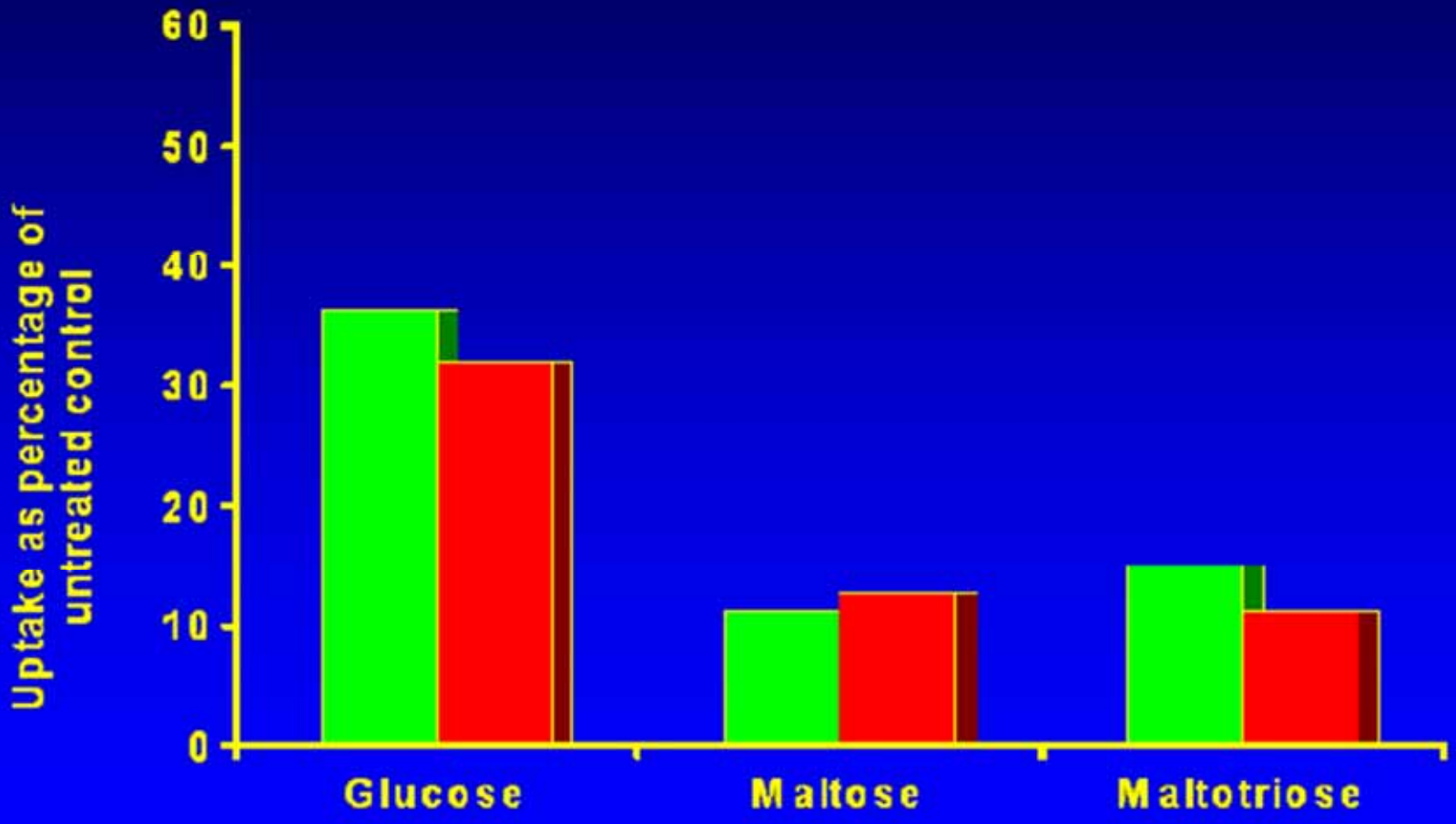
*Shaking fermentations at 21°C

Uptake of Maltose from 16°Plato Wort by Diploid Yeast Strains Containing Multiple Maltose (*MAL*) Genes*



*Shaking fermentations at 21°C

Effect of Osmotic Pressure [25% (w/v) Sorbitol*] on the Uptake of Glucose, Maltose and Maltotriose by a Lager Strain and an Ale Strain



*Peptone – yeast extract – 2.5% (w/v) sugar medium

ESTER

FORMATION

IMPORTANT ESTERS IN BEER

- ethyl acetate (fruity/solvent)
- isoamyl acetate (banana/apple)
- isobutyl acetate (banana/fruity)
- ethyl caproate (apple/aniseed)
- B-phenylethyl acetate (roses/honey)

Factors (nature and nurture) that Influence the Level of Ester Production During Wort Fermentation

- Yeast characteristics – yeast strain (nature), pitching rate (nature and nurture), physiological state (nurture).
- Wort composition (nurture) – sugar and amino acid spectrum, lipids, vitamins, inorganic nutrients, dissolved oxygen, clarity (trub), original gravity.
- Fermentation conditions (nurture) – temperature, agitation, CO_2 tension, pH, fermenter design, pitching rate.

Ethyl Acetate and Isoamyl Acetate (mg/L) Production by Distiller's and Brewer's Yeast Strains

	Ethyl acetate	Isoamyl acetate
Distillers	46.2	1.3
Distillers	36.2	0.9
Ale	20.6	0.6
Ale	36.2	0.8
Ale	25.6	3.5
Lager	60.2	4.6
Lager	32.6	1.6

Fermentations conducted in 16ºPlato all-malt-wort at 20ºC

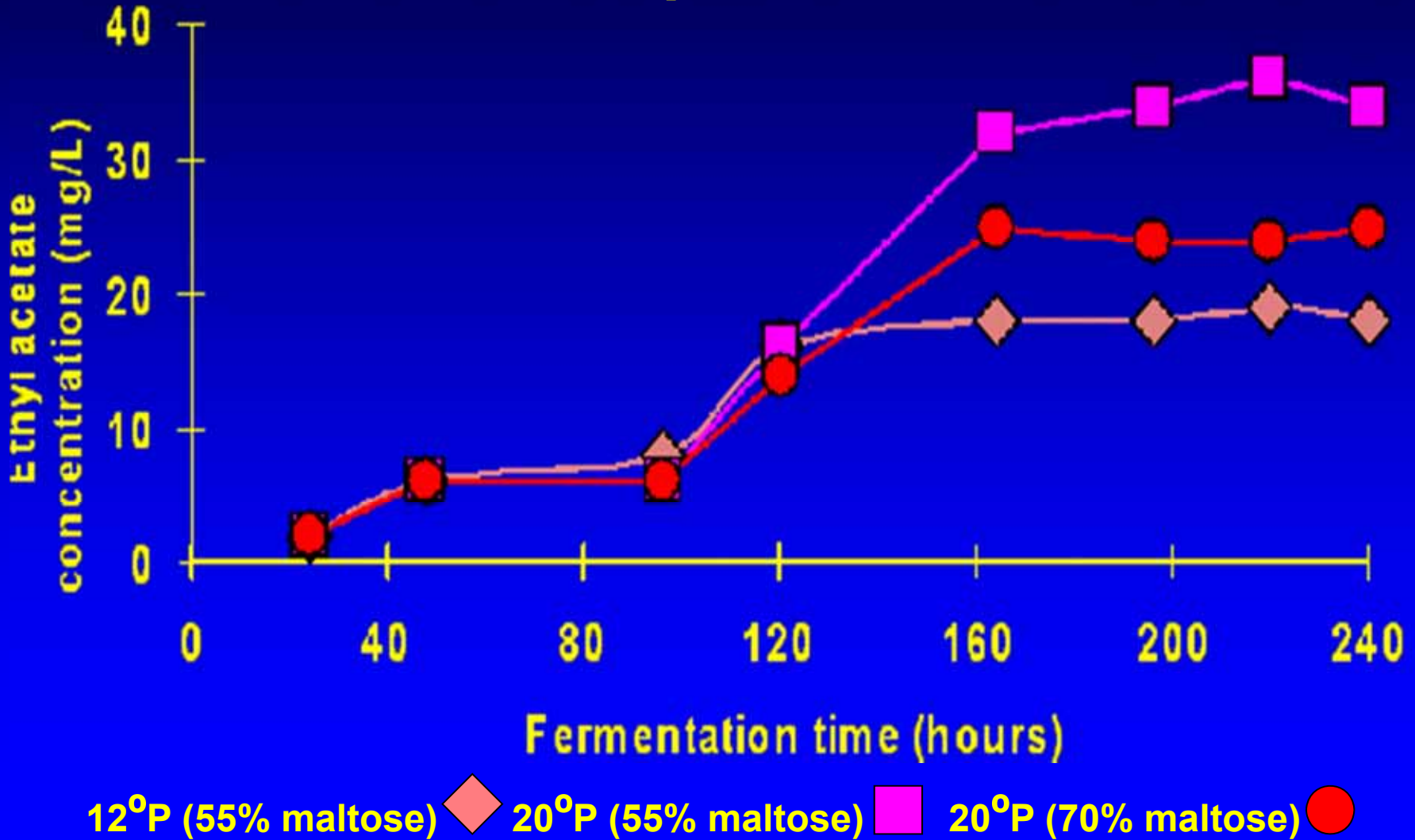
Levels of Ethyl Acetate (mg/L) Produced by Brewer's Yeast Cultures During Fermentation of Synthetic Media containing Either 4% (w/v) Glucose or Maltose

	Glucose	Maltose
Ale	4.13	2.79
Ale	2.97	2.59
Ale	3.13	2.71
Lager	6.02	5.82
Lager	3.75	3.28
Lager	4.62	3.62

Influence of Wort Gravity on Beer Ester Levels

	12° Plato	20° Plato
Ethanol (v/v)	5.1	5.0
Ethyl Acetate (mg/L)	14.2	21.2
Isoamyl Acetate (mg/L)	0.5	0.7

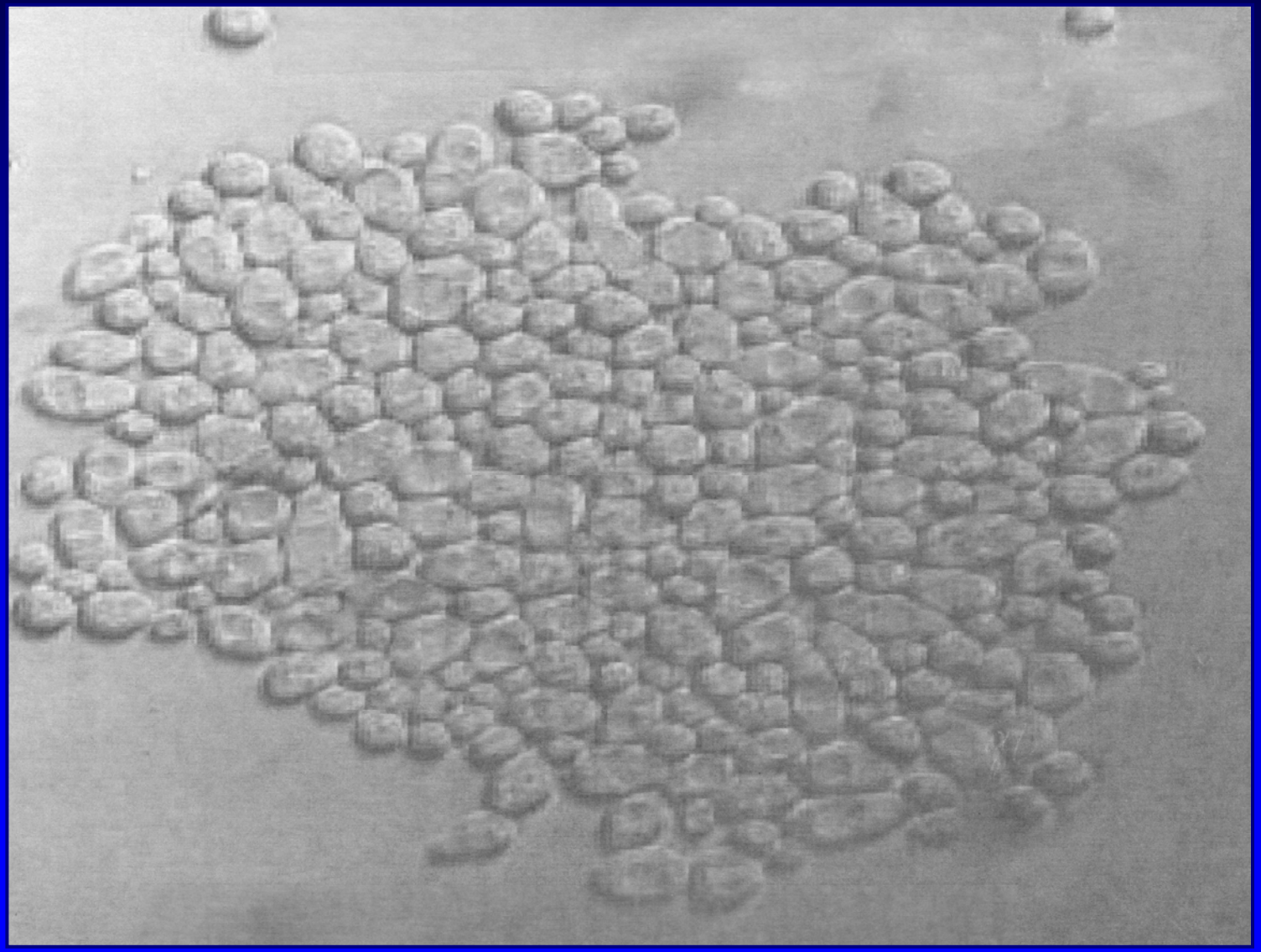
Ethyl Acetate Concentration in Fermenting Worts of Differing Gravities and Sugar Composition



YEAST

FLOCCULATION

Yeast Floc



What is Yeast Flocculation?

Yeast flocculation is a nonsexual, homotypic (involving only one type of yeast cell in the interaction), reversible (flocs can be reversibly dispersed by the action of the chelating agent EDTA or specific sugars such as mannose) and can aggregate into multicellular masses composed of thousands of cells called flocs.

Yeast Flocculation Nature-Nurture Effects

- Formation of yeast flocs is dependent on both genetic (nature) and environmental (nurture) parameters.
- It is a cell surface characteristic.
- Specific lectin-like proteins only present in flocculent cell walls recognize and interact with carbohydrate residues of mannan on neighbouring cells.
- The presence of calcium ions is essential to enable cell-cell interactions.

Dominant Flocculation *FLO* Genes

FLO1
Lg-FLO1
FLO2
FLO3
FLO4
FLO5
FLO7
FLO8
FLO9
FLO10
FLO11

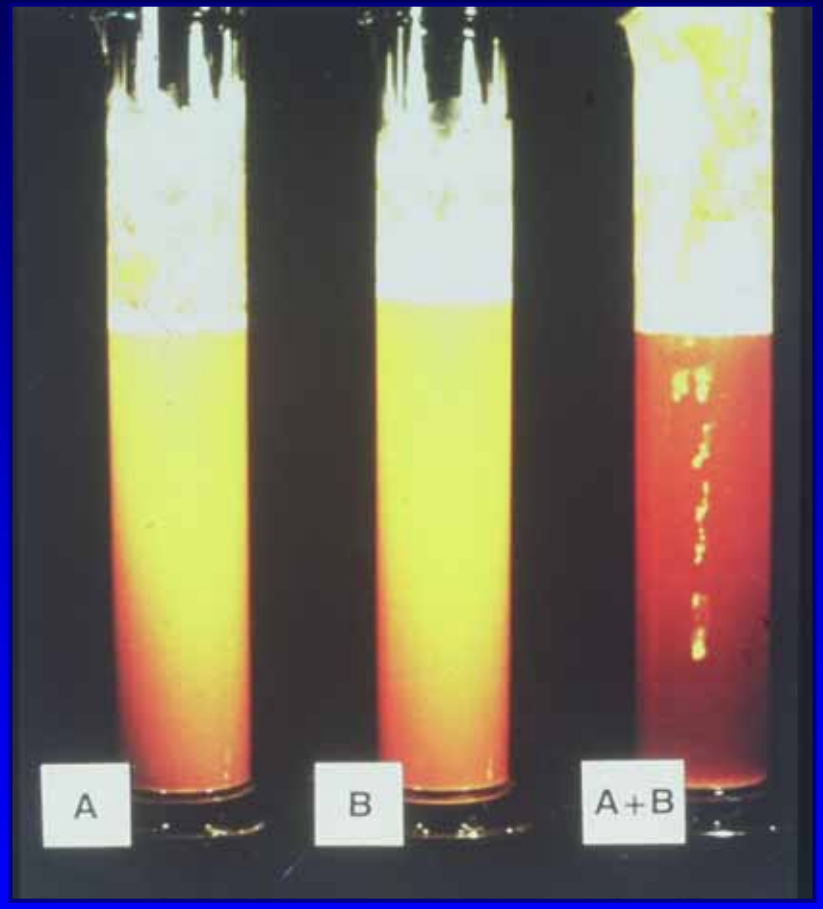
Environmental Factors that Influence Yeast Flocculation

- Medium (wort) pH 2.5-5.5.
- Inducer – acidic peptides.
- Temperatures between 50-60ºC promote floc dispersal of most strains.
- Specific sugars can disperse flocs.
- Ethanol [4-6% (v/v)] has a positive influence whereas ethanol [10% (v/v)] impairs flocculation expression.

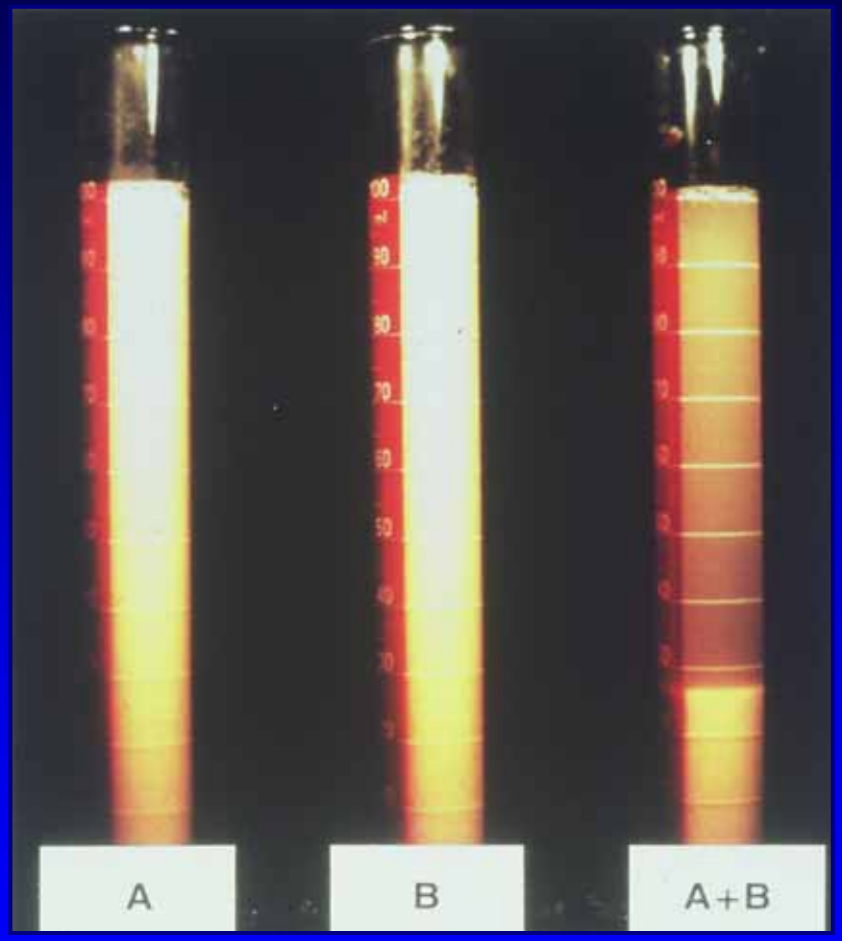
Other Types of Yeast Flocculation

- Co-flocculation between two different yeast strains – acidic peptide and calcium induced.
- Bacteria induced yeast flocculation – independent of calcium.

Co-flocculation between Two Ale Yeast Strains



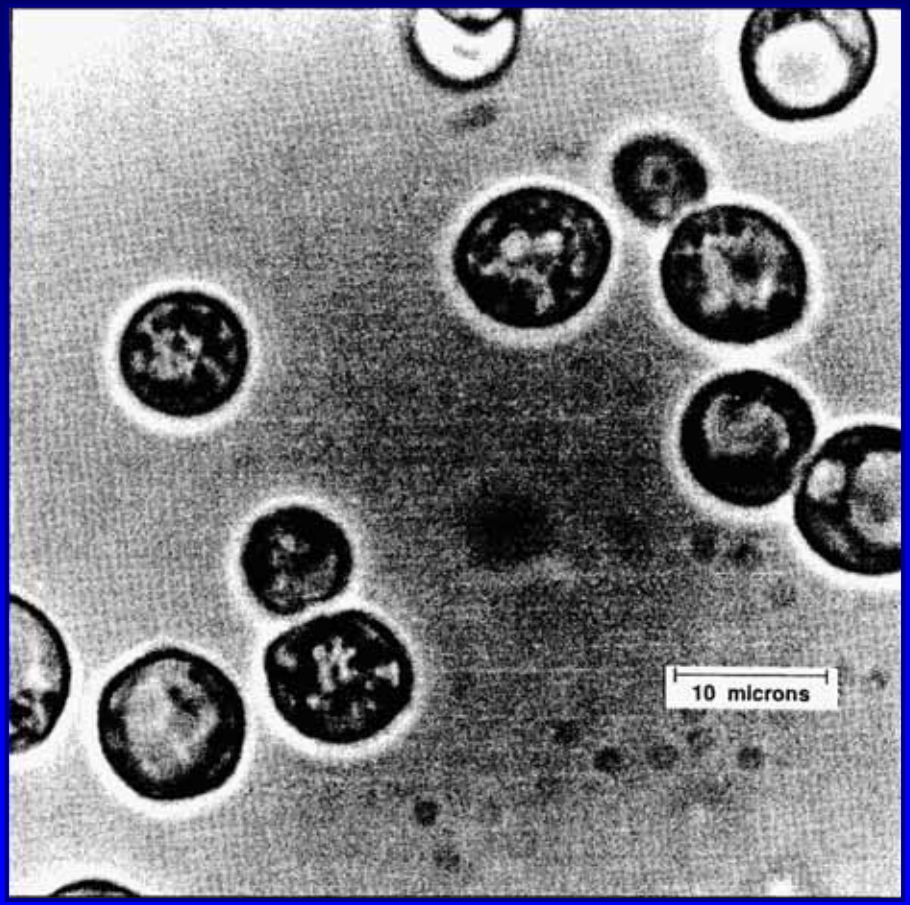
Cylinder wort fermentations
in vivo



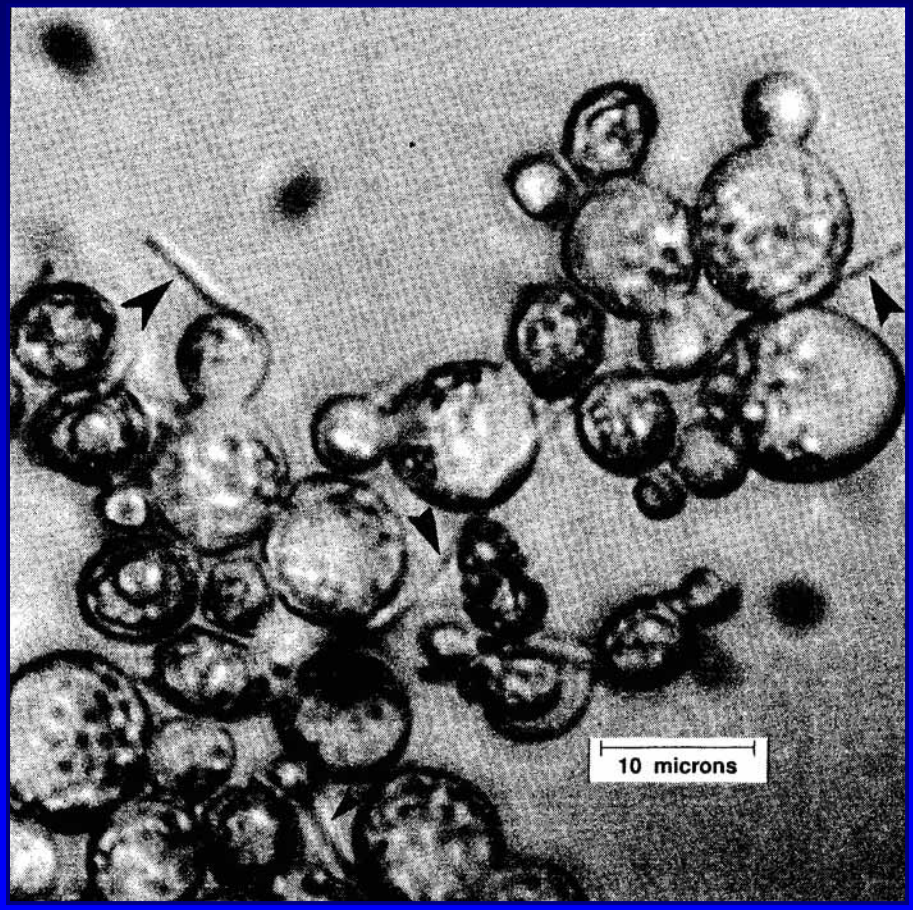
Helm's sedimentation test
in vitro

Bacterial-induced Flocculation

- Bacteria



+ Bacteria



***Lactobacillus fermentum*, strain 125**
Arrows indicate bacterial bridges.

Summary

- Nature-nurture concepts have been an area of discussion recently. It is a concept that was originally proposed in 1869.

- Consideration of nature-nurture ideas are currently relevant and can be applied to wort fermentation by brewer's and related yeast strains.

- Areas that have been reviewed here are:
  - Ale/lager yeast species.
  - Uptake of wort maltose.
  - Ester formation.
  - Yeast flocculation.

- The advent of yeast genetic manipulation has complicated this issue, where the nature of a yeast strain can be modified by nurturing techniques.

Acknowledgments

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