Beer “Spoilage” Organisms

Brewing Short Course/
Non-Saccharomyces Yeast and Bacteria
in the Beverage Industry
7-11 July 2014
Bacteria

Gram Staining
First stain (crystal violet = purple) stains the thicker peptidoglycan layer of gram + bacteria (cell walls)

Sample is washed with acetone to remove excess stain

Counter stain (safronin = pink) is applied and set

Gram + = Purple
Gram - = Pink
Gram Positive Bacteria

*Lactobacillus*

- 28 species divided into four physiological groups
- Lactobacilli, like most Gram-positive bacteria, are sensitive to hop resins, but selection in the brewery has led to the emergence of resistant strains
- Group I strains are homofermentative i.e. produce lactic acid as virtually the only metabolite from glucose. They are further divided into:
  - IA (thermobacteria) - grow at 45°C but not 15°C; and
  - IB (streptobacteria) - the opposite temp relationships
Gram Positive Bacteria

*Lactobacillus*

- Group II (betabacteria) - hetero-fermentative and metabolize glucose to lactic and acetic acid, $CO_2$, EtOH
- IIA - well-recognized, fermentatively active species
- IIB - inert to most carbohydrates, acidophilic and tolerate ethanol (15%), less well studied and tend to occur in restricted habitats (e.g., malo-lactic wine fermentation)

- The common brewery lactobacillus is the heterofermentative (Group IIA) *L. brevis* (Formerly *L. pastorianus*). The cells exhibit considerable pleomorphism, forming long rod-shaped structures, and they grow optimally at about 30°C and pH 4 to 5
- Brewery strains are typically insensitive to hops but ethanol becomes inhibitory at around 5%
Gram Positive Bacteria

*Lactobacillus*
Some of the species that have been isolated from breweries include:

- *L. lindneri* has a lower optimal temperature for growth (19°C) and was isolated from lager;
- *L. diastaticus*, an *amylolytic* organism, was isolated from superattenuated stout;
- *L. pastorianus var. brownii* produces *extracellular* polysaccharide, thus causing ‘rope’ in beer;
- *L. delbrueckii*, a thermophilic member of group IA that has been found in sweet wort held at 50 – 60°C; and
- the homofermentative (Group 1B) species *L. plantarum* and *L. casei*; and
- *L. fructivorans* from Group IIB.
Gram Positive Bacteria

*Pediococcus*

- Many gram + cocci can be isolated from beer and breweries, but only one species is hop-tolerant and able to proliferate in beer, *Pedicoccus damnosus* (formerly *P. cerevisae*)
- This organism was originally associated with “sarcina sickness” in beer, characterized by turbidity, acidity and diacetyl
- Many varieties of *P. damnosus* that produce ‘rope’ and are amylolytic have been described
Gram Negative Bacteria

Acetic Acid Bacteria
• The most distinctive property of these bacteria is their ability to oxidize ethanol to acetic acid
• Aerobic rods, typically hop and acid-tolerant and cause spoilage of beers stored in the presence of air
• The genus *Acetobacter* comprises the “over-oxidizing” strains that oxidize ethanol to acetic acid, and finally to CO₂ and water
• *Gluconobacter* strains produce only acetic acid from ethanol
• Common brewery species are *A. pastorianus* and *G. oxydans*
• Some strains (particularly *G. oxydans*) can grow in beer in the presence of very limited supplies of oxygen and produce large quantities of rope
Gram Negative Bacteria

*Zymomonas*

- These virtually anaerobic Gram-negative rods ferment glucose and fructose, but not maltose, to alcohol via a unique pathway.
- They grow readily in beer, producing turbidity and serious off-flavors, but fortunately they tend to be uncommon.
- Their habitat includes the soil, water and brewing equipment.
- There is only one species, *Z. mobilis*, which accommodates both motile and non-motile strains.

Two varieties are recognized:
- subsp. *mobilis* includes those strains isolated from breweries; and
- subsp. *pommaceae* comprises strains responsible for “cider sickness”
Gram Negative Bacteria

*Enterobacteriaceae*

- This family includes a variety of genera of Gram-negative, rod-shaped bacteria, all phenotypically similar and differentiated on the basis of sugar fermentations and other metabolic attributes.
- They are aerobic and facultatively anaerobic organisms, typically bile-salt tolerant but sensitive to ethanol and low pH, two factors that restrict their spoilage potential in the brewery.
- They are generally unable to grow in beer but grow rapidly in wort, producing metabolites that can affect beer flavor adversely.
- One example, apparently peculiar to breweries, is *Obesumbacterium proteus* (synonyms: *Flavobacterium proteus* and *Hafnia protea*).
- This bacterium is ubiquitous in top-fermenting yeasts and less common in lager yeasts.
- Other enterobacteria isolated from wort and breweries include *Hafnia alvei, Cirobacter freundii, Klebsiella* and *Enterobacter* species.
- All these organisms develop in wort and during the early stages of fermentation.
Molds

Ascomycetes
The principal distinguishing features of the Ascomycetes are:

a) normally haploid;

b) asexual reproduction by non-motile sores (conidia) formed on hyphae or on modified hyphae (conidiophores);

c) sexual reproduction by fusion of hyphae or some similar process. This gives rise to a diploid ascus and a fruiting body or ascocarp which may develop to enclose several asci

• The number of fungi belonging in the Ascomycetes is stunning (over 15,000!)

• Fortunately for mushroomers, but not so fortunately for brewers, most of these fungi are yeasts, mildews, molds, and so on

• A mushroom belonging to the Ascomycetes has a fertile surface that is covered with asci--in the morels, for example, this surface is the pitted surface of the cap
Molds

Fungi Imperfecti (Deuteromycetes)

- Where sexual reproduction is rare, absent or unknown, the organisms are placed in the fungi imperfecti group.
- These are generally imperfect forms of Ascomycetes and dual names may be given to the same organism depending on the presence or absence of the sexual stage.
- The fungal flora of barley in the field includes *Fusarium*, *Helminthosporium* and *Alternaria*, and during storage *Aspergillus* and *Penicillium*, all fungi imperfecti.
Molds

*Aureobasidium pullulans*

- A ubiquitous black, yeast-like fungus that can be found in different environments (e.g. soil, water, air)
- One of the earliest contaminants of barley, increases after fruiting
- Notable for its phenotypic plasticity - colony morphology may be affected by carbon source, colony age, temperature, light and substrate, with colonies ranging from homogeneous to sectored, yeast-like to filamentous growth, and from small to large
- Adaptable to various stressful conditions: hypersaline, acidic and alkaline, cold, and oligotrophic - considered to be polyextremotolerant
- Can be cultivated on potato dextrose agar, where it produces smooth, faint pink, yeast-like colonies covered with a slimy mass of spores. Older colonies change to black due to chlamydospore production
- Primary conidia are hyaline, smooth, ellipsoidal, one-celled, and variable in shape and size; secondary conidia are smaller
- The fungus grows at 10–35 °C with optimum growth at 30 °C.
Yeasts

*Saccharomyces*

Wild yeasts, including *Saccharomyces* species, covers everything under the definition “yeasts not deliberately used and under full control.

May include the following species:

- *S. bayanus, S. cerevisiae, S. exigus, S. pastorianus, S. unisporus, S. diastaticus*

- May produce unintended fermentation, producing turbidity and off-flavors.

- Their ability to metabolize a wide range of sugars may lead to outcompeting the intended *Saccharomyces* species.

*S. diastaticus*
Yeasts

*Brettanomyces*
- Aerobic but produce large quantities of acid and may seriously affect flavor profile
- Considered a spoilage microbe, but prescribed use with intended outcomes is a defining factor of many products
- *Dekkera*: spore-forming equivalent of *Brettanomyces* (anamorph)
- Species: *bruxellensis, lambicus, intermedius, schand*
- Boat-shaped, elliptical, variable depending on growth media
- Can be difficult to culture on traditional media
- Can ferment most sugar, some can metabolize ethanol
- Produce high amounts of acetic acid under glucose fermentation
- Produce higher levels of: **4-ethyl-phenol, 4-ethyl-guaiacol, isovaleric acid**
- Fairly pH sensitive, sensitive to SO₂, slower growing the *Saccharamoyces*
Yeasts

**Torulaspora**
- *Torulaspora delbrueckii* is a yeast related to *S. cerevisiae* and used to produce wheat beer
- Also known as *S. delbrueckii* or *S. rosei* (anamorph called *Candida colliculosa*)
- PRELUDE™: a pure strain of *Torulaspora delbrueckii* to be used in co-inoculation with *S. cerevisiae*. Increases the body, softens the palate and rounds the mouth feel of wines.
- Capable of metabolizing maltose in wort
- Fermentation using *T. delbrueckii* takes longer to reach terminal gravity than *S. cerevisiae*
- Produces abundant esters (mainly banana, rose and bubblegum) and 4-Vinyl Guaiacol, which gives a spicy, clove like flavor to beer, especially at higher temps
Yeasts

*Kluyveromyces*

- Cell: multilateral budding; spheroidal to ellipsoidal, occur singly in pairs or short chains or small clusters; simple to complex pseudohyphae may form
- Colony: cream-colored to brownish cream, shiny to dull, forms smooth, white, slightly shiny colonies
- Growth on malt agar forms smooth, cream colored, slightly shiny colonies
- WL: *Kluyveromyces thermotolerans* is not sensitive to actidione and will grow on WL plates
- Fermentation: Glucose, Sucrose, Raffinose
- CONCERTO™: launched in 2012, this pure *Kluyveromyces thermotolerans* strain is ideal for wines produced in warm climate regions. It produces lactic acid giving roundness and balanced acidity to the wines, improving flavor complexity. Definitively a product to use before you inoculate your usual yeast if you produce red or white wines in a warm to hot area.