

Microbial update

brewing

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The history of brewing and fermentation is deeply entrenched in a period of cutting edge scientific discovery. Not least, French scientist Louis Pasteur who considered it a matter of national pride to discover the underlying reasons behind the widely held belief that French beer was inferior to German Beer! The outcome of this work was the discovery and isolation of a number of different spoilage bacteria, including *Acetobacter* spp. and *Lactobacillus* spp., from beer and malt wort and established Pasteur as the 'father' of brewing bacteriology.

The effects of Pasteur's studies were groundbreaking; Whitbread Brewery purchased a microscope on Pasteur's personal advice and J. C. Jacobsen, the owner of Carlsberg brewery, established the Carlsberg laboratory as a tribute to his work.

Today brewing microbiology owes much to the practices and discoveries of Pasteur and his contemporaries. However, the legacy of Pasteur for the brewery microbiologist is not merely the discovery of particular micro-organisms but more their direct effect on beer and thus the principles of product quality and the basis of our understanding of hygiene practice and control in the brewing industry today.

Brewery micro-organisms

The micro-organisms encountered within the brewery environment can be split into those that affect beer quality and those that have no spoilage potential. However, this is becoming a more indistinct categorisation and many of the bacteria that would once

Table 1. Examples of yeast most likely to be seen in breweries and their effects on beer quality.

Yeasts	Effects on beer
<i>Brettanomyces</i>	Production of acetic acid Growth in filtered beer
<i>Saccharomyces cerevisiae</i> var. <i>diastaticus</i>	Phenolic off flavours Altered fermentation characteristics
<i>Pichia</i>	Pellicle forming Yeasty or estery off flavours
<i>Zygosaccharomyces</i>	Turbidity, off flavours

Bacteria	Effects on beer	Isolated from
<i>Lactobacillus</i> and <i>Pediococcus</i>	Butterscotch flavour Silky turbidity Formation of rope	Pitching yeast, fermenters and final package
<i>Acetobacter</i> and <i>Gluconobacter</i>	Vinegar flavour Formation of rope	Enter casks in cellars
<i>Pectinatus</i>	Strict anaerobes Sulphur aroma	Package beer
<i>Megasphaera</i>	Cheesy or sour aroma	Package beer
<i>Obesumbacterium proteus</i>	Blackcurrant or parsnip aroma	Pitching yeasts
<i>Zymomonas</i> (mainly ales)	Rotten apple aroma and taste	Priming sugars, dirty cask brushes, building structure

have been classified as non-spoilage are now recognised as having undesirable effects.

These are exhibited either through the formation of sulphur taints or aromas or via the alteration of expected yeast performance during fermentation.

Wild yeasts

The most diverse of the beer spoilage micro-organisms are 'wild yeasts'. This phrase is applied to any yeast that is different from the brewing strain but can include brewers yeast; i.e. when ale yeast is found in a lager fermentation. As spoilage micro-organisms, wild yeasts are split into two groups: *Saccharomyces* wild yeast (closely related to brewing strains), and non-*Saccharomyces* wild yeast. Breweries will have repeated problems with specific wild yeast types but less commonly for isolated outbreaks involving different species.

Contamination can originate from a number of sources, such as the air, water supply and process equipment and can be further propagated via serial re-pitching. The presence of wild yeasts (Table 1) are indicated

by alterations in the standard fermentation characteristics, or changes in the physical quality of the beer via production of off-taints and aromas (phenolic, acidic).

Wild yeasts are initially able to grow at a faster rate than pitching yeast and may utilise the non-fermentable sugars. Due to the higher concentration of pitching yeast, any contamination by wild yeast is usually out grown early in fermentation. However, such contamination may remain at low levels within the pitching yeast stock. If the contaminating yeast is extremely prevalent during fermentation, the cells may persist throughout maturation and storage and even into final package. Some species of wild yeast may survive filtration or pasteurisation by the formation of heat resistant spores.

Flocculent yeast strains may also survive pasteurisation into final package by forming clumps of cells called flocs. Cells at the centre of the floc will be protected from the harsh conditions of pasteurisation by the cells on the outer layers.

Occurrences of wild yeast can be avoided through good standards of hygiene. If contamination is found within the pitching yeast and is in sufficient numbers, the only solution is to replace the pitching stock.

Bacterial contamination

New species of bacteria have been isolated and identified as spoilage agents, notably *Megasphaera* and *Pectinatus* species.

However, most brewers are still concerned with the same bacteria as their predecessors were in the 19th century. There are few species of bacteria that are adapted

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to survive in beer and this is due to its growth limiting environment. After fermentation, the following conditions inhibit the growth of most bacteria:

- Low pH.
- Lack of available nutrients.
- Presence of anti-microbial hop compounds.
- Alcohol.
- Low oxygen content.

Beer can therefore be described as a spent medium in which only a limited number of specialist bacteria are able to survive (Table 2). Many bacteria responsible for beer spoilage are also suited to the oxygen deficient environment found within the brewing process.

However, the facultative anaerobes; *Lactobacillus* spp. and *Pediococcus* spp., are the most commonly identified spoilage micro-organisms in bottled, canned and keg beer with *Acetobacter* spp. being largely responsible for spoilage of cask beer.

Brewery microbiota

Breweries are not sterile environments with micro-organisms found in raw materials and on surfaces and equipment (Table 3).

Traditional detection techniques encourage spoilage micro-organisms to grow using a variety of synthetic agars for different groups of micro-organisms (Table 4).

It is also essential to suppress the growth of brewing yeast by addition of cyclohex-

Media	Conditions	Organisms detected
WLD (WLN + Cycloheximide)	Aerobic	Acetobacter/Gluconobacter
Raka Ray No3	Anaerobic	Lactobacillus/Pediococcus
YM + copper sulphate	Aerobic	Saccharomyces wild yeast i.e. <i>Saccharomyces pastorianus</i>
Lysine medium	Aerobic	Non-Saccharomyces wild yeasts i.e. <i>Pichia anomala</i>

Table 4. Media used for detecting spoilage by bacteria and yeasts.

imide to nutrient medium. This will encourage the growth of contaminating bacteria which would commonly be found in reduced numbers when compared with the amount of brewing yeast found naturally.

The most commonly used techniques used to detect and enumerate spoilage bacteria are:

- Membrane filtration (100ml of sample).
- Pour plating (5ml of sample).
- Spread plating (100-250µl of sample).

These tests offer both a flexible approach to enumeration and detection based on the nature of the sample itself and the level of sensitivity of the method.

Novel microbiology

Traditional detection techniques are specific and sensitive, but slow and time consuming with results generally available in three to seven days. There is however a growing need for faster detection of spoilage micro-organisms within the process and final product in combination with already established

and widely practised rapid hygiene monitoring of the plant.

The most commonly adopted test for hygiene monitoring is ATP Bioluminescence. This rapid test allows 'on the spot' results for cleanliness and hygiene and can identify food and bacterial residues in liquid rinses and upon surfaces. Although it does not replace traditional microbiology it is a rapid indicator of the success of cleaning regimes.

The next generation of microbiological detection arguably still lies with PCR. By targeting and amplifying the unique sections of DNA within the microbial genome it is possible to identify micro-organisms at either a non-specific, genus level or specific, species levels. In addition to PCR, screening probes and gene chips have been developed to detect either different groups of bacterial species at one time or to give an indication of the presence of a number of different spoilage bacteria in a single sample.

However, PCR is still not capable of providing a complete alternative to traditional microbiology and in order to significantly decrease the detection times associated with such methods a growth based approach is not suitable.

The challenge today therefore is to provide a 'real-time' molecular alternative that allows pro-active responses to quality issues related to microbiological stability that perhaps even Dr Pasteur would not have dreamed possible!

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Table 3. Where do brewery micro-organisms originate?

- **Barley.** Heavy contamination with moulds can lead to the gushing of beer, yeast and bacteria are also present.
- **Malting.** Whilst steeping will remove most of the micro-organisms at the surface of the grain, conditions during germination are excellent for growth of the remaining microorganisms. In many cases the levels of bacteria, yeast and mould found on the kilned grain are similar, and in some cases higher, than those found on the barley.
- **Hops.** These contain a wide variety of micro-organisms, but as hops are boiled during brewing the effects to beer are negligible. The antimicrobial compounds associated with hops are produced during the wort boil but they are ultimately only effective against Gram positive bacteria.
- **Water.** Only if the water supply becomes contaminated with beer can water contain spoilage bacteria. *Bacillus* spp., *Enterobacteriaceae* spp. and yeast spores may prove problematic if they gain entry to the process via the water supply.
- **Brew house.** Most raw ingredients support some form of microbial growth, the low pH at mashing and high temperatures during boiling reduce this contamination. However, *Lactobacillus* and *Bacillus* species are able to tolerate low pH and high temperatures and may be found in both the mash tuns and sweet wort. *Bacillus* spores will survive the boiling stage but will not usually germinate in hopped beer due to the low pH.
- **Fermentation.** Generally cooled wort will not contain any viable bacteria, but can quickly become contaminated with bacteria from the plant or pitching yeast. These are known as wort bacteria. The most common example is *Obesumbacterium proteus* which may be found in the pitching yeast. The low pH and presence of alcohol will restrict their growth during the fermentation. *Pediococcus* and *Lactobacillus* species are capable of growth in the primary fermentation and maturation stages, and can cause serious spoilage. *Saccharomyces* wild yeast are also able to grow during the fermentation, but are usually out competed by the brewing strain. The serial re-pitching of yeast may lead to 'wild yeast' becoming dominant over time.

Campden & Chorleywood Food Research Association (CCFRA) and Brewing Research International (BRI) have very recently merged to become Campden-BRI. The new company brings together two client focussed, highly successful world class organisations with proven track records of delivering relevant, timely and efficient services. Campden-BRI employs over 380 highly skilled staff and offers the widest range of R&D, technical services, analysis and training activities for the food and drink sector.

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