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# 125th Anniversary Review: Bacteria in brewing: The good, the bad and the ugly

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**Beer is a beverage that is produced in a multistage process, where some stages of that process are intentionally influenced by microorganisms, while at other stages of the production process microorganisms are actively discouraged. Most of the intentional microbial activity is facilitated by yeast; however bacteria also play an influential role in beer production. This paper will describe the beneficial role of bacteria in the beer production process (the Good), but will also pay due attention to the negative influences bacteria might have on the quality of beer as a commodity (the Bad), and the properties of beer that have given it the status of an inherently safe food for human consumption with regards to disease-causing bacteria (the Ugly).** 

**Keywords:** beneficial bacteria; spoilage bacteria; pathogenic bacteria


## Bacteria in brewing: the Ugly

### Hurdle technology

The microbial safety and stability, the sensory and nutritional quality, and the economic potential of many foods are maintained using a combination of preservative factors (hurdles), termed hurdle technology *(1)*. By employing numerous hurdles at reduced levels, rather than one single hurdle at an intense level, a product with an extended shelf-life can be produced with more desirable organoleptic properties. Beer is intrinsically resistant to the growth of spoilage and pathogenic (disease-causing) microorganisms owing to a combination of inhibitory factors (hurdles) (see Fig. 1). The presence of ethanol [up to 10% (v/v), typically 3.5–5.0% (v/v)], hop (*Humulus lupulus*) bittering compounds (approximately 17–55 ppm iso-$\alpha$-acids), low pH (approximately 3.9–4.4), elevated carbon dioxide (approximately 0.5%, w/w), low oxygen ($<$0.1 ppm) and the professed lack of nutritive substances protect beer from infection by most microorganisms. In addition to these intrinsic antimicrobial factors, many stages of the brewing process reduce the potential for contamination or the proliferation of bacteria. These extrinsic antimicrobial processes include acidification of malt, mashing, wort boiling, pasteurization, filtration and cold storage.

A classical example of the (intentional or unintentional) applied use of hurdle technology in beer is India pale ale (IPA). During the 1700 s, the British Empire controlled India by maintaining a large contingent of troops, whose needs included the provision of British brewed ales. This required the shipment of British brewed beers on long ocean voyages. In the late 1700 s, some ales bound for British troops in India spoilt very quickly during this long sea journey. The beers that showed little or no deterioration of drinking quality were those that were brewed at an elevated original gravity, were well-attenuated and had higher hopping rates with fresh hops – all factors that have antimicrobial properties associated with them *(3)*.

Beer is more susceptible to undesirable microbial growth when one or more of these antimicrobial hurdles are absent or present at a reduced level. For example, Vaughan *et al*. *(4)* noted that beers with elevated pH levels, low ethanol and low $CO_2$, and those with added sugar (increased nutrients) were more prone to spoilage. According to the work of Fernandez and Simpson *(5)*, levels of nitrogen (free amino and total soluble), amino acids, maltotriose, beer pH and colour significantly affected the resistance of beers to spoilage by lactic acid bacteria (LAB).

Table 1 summarizes the primary targets and mode of inhibition of many of the antimicrobial hurdles of beer, which are discussed in more detail below. Although these hurdles are considered in reference to preventing the survival and growth of pathogens, it is important to note that these principles can also be applied to reducing the incidence of beer spoilage bacteria.

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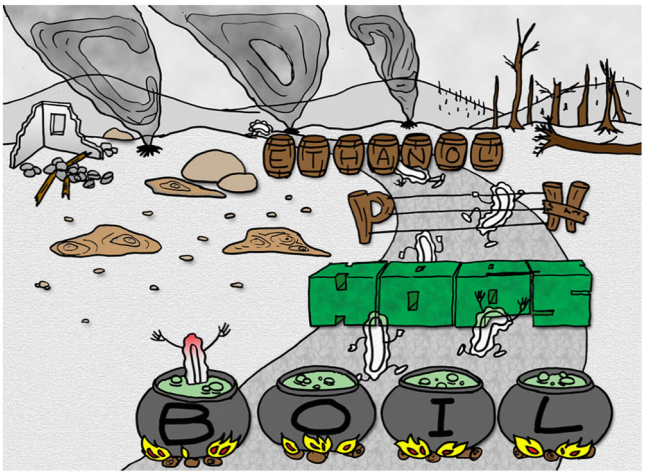


**Figure 1**. Pathogens cannot survive in beer owing to the antimicrobial 'hurdles', including the kettle boil, hop bitter acids, low pH, ethanol, carbon dioxide ($CO_2$) and the lack of nutrients and oxygen (depicted by the wasteland). Artwork by Ms Peggy Hsu. Reproduced with permission from Elsevier *(5)*.

## Ethanol

The conversion of carbohydrates to ethanol [0.5–10% (v/v), typically 3.5–5.0% (v/v)] by yeasts during the fermentation of wort provides one of the major antimicrobial hurdles. The antimicrobial properties of ethanol in beer were described as early as in 1935 by Shimwell *(6)*, who showed that beers with a higher ethanol content were more resistant to spoilage by *Saccharobacillus pastorianus* (now *Lactobacillus brevis*) than those of lower ethanol content. Similar observations have recently been shown for a range of pathogenic bacteria *(7)*. In general, ethanol inhibits cell membrane functions *(8)*, and inactivates bacteria by inducing cell membrane leakage *(9)*. Exposure to 5% (v/v) ethanol has been shown to increase cell membrane permeability, which heightens the sensitivity to low pH by allowing an increased passage of protons into the cytoplasm, leaving bacterial cells unable to maintain pH homeostasis *(10)*. As a result of damage to the cell by ethanol, morphology and a range of cell functions may be affected *(11–13)*.

At concentrations typical of beer, ethanol exerts only a limited effect on enzyme activity. Few of the glycolytic enzymes studied by Scopes *(14)* showed any substantial changes in activity at ethanol concentrations up to 5.8% (v/v), while membrane-bound enzymes in *E. coli* were reported to be relatively insensitive to inhibition by ethanol [NADH oxidase, D-lactate oxidase, and ATPase were inhibited by less than 10% by the presence of 3.9% (ABV) *(9)*]. A dose-dependent inhibition of the lactose permease of *E. coli* by ethanol was reported by Ingram *et al. (15)*.

The consumption of alcoholic beverages has been reported to enhance a person's resistance to infection by pathogens. Intake of alcohol during or after consumption of contaminated food may protect against *Salmonella* spp. *(16)*, and consumption of beverages with >10% alcohol was reported to provide a protective effect against Hepatitis A from oysters *(17)*. Furthermore, moderate alcohol consumption suppresses *Helicobacter pylori* infection *(18–20)*.

## Low pH

Most beers have a relatively low pH (range 3.4–4.8) *(2)*. Low pH values enhance the entry of weak organic acid into cells, leading to intracellular acidification, the destruction of enzyme systems and reduction in nutrient uptake, and result in metabolic exhaustion. For example, Neal *et al. (21)* reported that low pH values (4.0) impacted on alcohol dehydrogenase, aldolase and pyruvate decarboxylase. Microorganisms attempt to maintain a steady, close to neutral intracellular pH, in spite of the pH of the external environment *(22)*. The ability of a cell to maintain a desired intracellular pH is limited and varies between species and strains within species, being primarily driven by the controlled ATP-consuming movement of cations across the membrane *(22)*. When the mechanisms of passive and active pH homeostasis are overwhelmed, starvation ensues, leading to cell death.

In addition to its direct action, the low pH of beer exhibits a synergistic effect with the antimicrobial properties of hop compounds, as hop exhibit increased antibacterial activity at lower pH values *(23–26)*. Simpson and Hammond *(27)* reported that a decrease in the pH of 0.2 can increase hop antibacterial activity by up to 50%.

Figure 2 plots the minimum growth pH for many food borne bacterial pathogens against the typical pH range of beer. The pH values are for growth, not survival, as sufficient comparable data is not available in the literature. The cited minimum pH values are under optimal conditions for each organism, which is not the case for beer, as it contains other inhibitory factors. Many pathogens are unable to grow at typical beer pH levels, and only *Yersinia enterocolitica*, *Staphylococcus aureus*, *Clostridium botulinum* and *Salmonella* spp. have been reported to grow at the pH levels of the majority of beers (Fig. 2). Even though these pathogens can grow at these low pH values, other hurdles in beer (such as ethanol, hops and $CO_2$) provide extra barriers to growth.

**Table 1.** Primary targets and mode of inhibition of both the intrinsic and extrinsic (processing) antimicrobial hurdles of beer

| Antimicrobial hurdles | Mode of inhibition |
|---|---|
| *Intrinsic hurdles* | |
| Ethanol | Inhibits cell membrane functionality |
| Low pH | Affects enzyme activity |
| | Enhances inhibitory effects of hops |
| Hops | Inhibits cell membrane functions |
| | Affects Gram-positive bacteria only |
| Carbon dioxide | Creates anaerobic conditions |
| | Lowers pH |
| | Affects enzyme activity |
| | Affects cell membrane |
| Low oxygen levels | Creates anaerobic conditions |
| Lack of nutrients | Starves cells |
| Sulphur dioxide[a] | Affects various metabolic systems |
| *Processing (extrinsic) hurdles* | |
| Mashing | Causes thermal destruction of cells |
| Kettle boil | Causes thermal destruction of cells |
| Pasteurization[a] | Causes thermal destruction of cells |
| Filtration[a] | Removes cells by physical size exclusion |
| Bottle conditioning[a] | Creates anaerobic conditions |

[a]Not applicable to all beers.

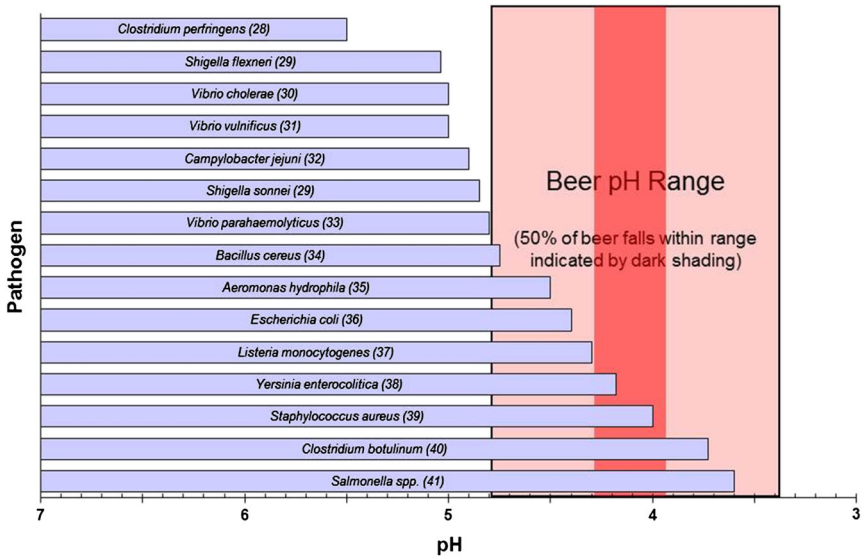


**Figure 2**. Minimum growth pH of pathogens under optimal conditions, compared with the typical pH range of beer. Beer pH range excludes outliers, data obtained from analysis of 444 beers of various styles *(42)*. Reproduced with permission from Elsevier *(5)*.

## Hops

Hops are primarily added to beer to impart a characteristic bitterness and aroma, although their antimicrobial properties have long been recognized. Hop compounds can be divided into two fractions: the total resins and the essential oils. Of most significance are the total resins, which include the $\alpha$-acids (humulone and its isomers) and $\beta$-acids (lupulone and its isomers). The $\alpha$-acids are isomerized during wort boiling to the more soluble iso-$\alpha$-acids, which impart bitterness and antimicrobial properties to the beer. Early reports of these chemical changes were documented by Hayduck *(29)*. Although the $\beta$-acids show increased antimicrobial action *(30)*, they have low solubility in wort *(31)* and are therefore of little significance in the resistance of beer to pathogens. Hop aroma is provided to the beer from the essential oils.

The undissociated forms of hop and hop-derived compounds are antimicrobial, whereas their ionized forms have negligible activity *(32)*. Hop compounds (lupulone, humulone, isohumulone and humulinic acid) have been shown to induce leakage of the cell membrane of *Bacillus subtilis (33)*. This breakdown of the cell membrane led to inhibition of the active transport of sugars and amino acids across the membrane, thus respiration and the synthesis of protein, DNA and RNA were interrupted. Further studies determined that hop bitter acids act as mobile-carrier ionophores and cause complete dissipation of the transmembrane pH gradient of sensitive cells *(34)*. The reduction in intracellular pH leads to inhibition of nutrient transport, and ultimately starvation of the cell *(32)*. Recently, hops have been shown to not only effect proton motive force depletion, but also cause divalent cation (e.g. $Mn^{2+}$) limitations in hop sensitive cells, further inhibiting metabolism *(35)*. An excellent detailed overview of the action of hops and the interaction between hops and LAB was recently published by Suzuki *(26)*.

## Dissolved gases

The presence of carbon dioxide ($CO_2$) and the lack of oxygen ($O_2$) enhance the microbial resistance of beer. $CO_2$ is produced during the primary fermentation of beer, and the beer is further carbonated by the direct addition of $CO_2$ or via secondary fermentation, to give final dissolved $CO_2$ concentrations of approximately 0.5%. Carbonation and modern bottling techniques limit the amount of dissolved $O_2$ available for growth in the bottled product. In addition to improving the chemical stability of the beer, decreased $O_2$ levels reduce the potential for the growth of many pathogenic microorganisms *(36)*.

Carbon dioxide inhibits pathogens by a variety of mechanisms: $CO_2$ creates an anaerobic environment to exclude the growth of aerobic pathogens; causes a lowering of the pH; influences carboxylation and decarboxylation reactions; and exerts a direct inhibitory effect on growth. Hammond *et al. (37)* reported that beers with low levels of dissolved $CO_2$ are at a heightened risk of undesirable microbial growth. This study supported the work of Šavel and Prokopová *(38)*, who documented that a decrease in the dissolved $CO_2$ level of beer reduced its shelf life (changes in $CO_2$ levels showed a larger impact than variations in dissolved $O_2$) *(38)*. Dissolved $CO_2$ in raw milk is inhibitory to bacteria *(39)*, increasing the lag phase and the generation time of microbes *(40)*. More recent studies have demonstrated the inhibitory effect of $CO_2$ on the growth of *B. cereus*, *Enterococcus faecalis*, *E. coli*, *L. monocytogenes* and *Pseudomonas fluorescens* in milk *(41,42)*.

## Lack of nutrients

The concentrations of nutritive substances available for the growth of pathogenic microorganisms in beer, such as carbohydrates, amino acids and some B-vitamins, are very low in most beers as the majority of these compounds have been metabolized by the yeast during fermentation (*45*; also Hucker, B. and Vriesekoop, F., unpublished results). Thus, well attenuated beers (those with minimal residual nutrients) are the least prone to microbial spoilage *(43)*. The effect of a lack of nutrients on the resistance of lager beers to undesirable microbial growth was studied by Fernandez and Simpson *(5)*. Increased levels of free amino nitrogen, total soluble nitrogen, amino acids and maltotriose were correlated with an increased incidence of bacterial growth *(5)*.

### Additional hurdles

In addition to the chief hurdles detailed above, there are a number of other compounds that may increase the antimicrobial nature of beer. Hammond *et al*. *(37)* demonstrated the antimicrobial effects of phytic and ferulic acid on *Lactobacillus* spp., although at much higher levels than typically found in beer. Various specialty beers are brewed with the addition of known antimicrobial compounds such as honey and various spices, which would slightly reduce the product's susceptibility to infection. At levels approximately 100 times higher than those found in beer, diacetyl has been shown to inhibit *Salmonella typhimurium (44)*.

### Processing hurdles

As mentioned earlier, beer contains several intrinsic antimicrobial hurdles that prevent the growth or survival of bacteria. In addition to these, various processing steps add further barriers (Table 1). Some of the first physical barriers are the use of heat applied during mashing. Gram-negative bacteria, yeasts and moulds are rapidly killed in the mash; however, LAB and spore-forming bacilli are able to survive the mashing process *(45)*. During the kettle boil, the wort is boiled for at least 45 min, destroying vegetative cells and their spores.

A number of craft and microbreweries carbonate their products by bottle or cask conditioning (secondary fermentation), and there is evidence that bottle conditioning reduces a beer's susceptibility to microbial attack, as the fermenting yeast reduces the $O_2$ content in the bottle headspace by approximately one-third *(46)*. Dolezil and Kirsop *(47)* reported that bottle conditioning appeared to be a factor in the production of contamination-resistant beer.

Many breweries employ post fermentation treatments such as filtration (physical exclusion), pasteurization (heat treatment) and cold storage to further protect the microbial stability of their beers. However, many beers from smaller breweries (such as brewpubs and microbreweries) and all cask beers are unpasteurized and unfiltered beer, thus extra care should be taken to ensure that the intrinsic hurdles are adequate, and that hygiene and sanitation regimes are well maintained.

Owing to these aforementioned antimicrobial hurdles, it is widely assumed that food-borne pathogenic microorganisms cannot survive in beer. Whilst several studies have shown that the survival of pathogens in beer is generally poor *(7–27,48–61,28–47,62–68)*, other work has suggested that beer may not be as hostile to pathogens as some have assumed. For example, Hompesch *(69)* showed that *Salmonella paratyphi* could survive in beer for up to 63 days, and it has been reported that pathogens can grow in alcohol-free beer *(70)*, while food-borne pathogens have been reported or inferred in some traditional African beers *(71,72)*.

## Bacteria in brewing: the Bad

### Lactic acid bacteria

Lactic acid bacteria are Gram-positive, non-spore-forming rods or cocci that are strictly fermentative, facultative anaerobes that belong to the order *Lactobacillales (73,74)*. While most Gram-positive bacteria are strongly inhibited by the hops added to the beer production process, a small number of beer-specific LAB have an evolved adaptation to hops and are capable of spoiling beers. Two of the more common beer spoilage LAB are *Lactobacillus brevis* and *Pediococcus damnosus (24,26,31)*. In the vast majority of reports regarding beer spoilage *Lactobacillus brevis* is the main culprit, producing a variety of off-flavours and aromas, and high turbidity to the final product *(75–79)*. The second most prevalent LAB, *Pediococcus damnosus*, has the ability to produce a variety of undesirable flavours and aromas, including diacetyl *(24,31,80)*. Some LAB have been reported to lower the quality of malt *(81–83)*; however, select strains of LAB are used to improve a number of malt characteristics *(84,85)*. They also have the ability to produce a variety of biogenic amines that pose a potential health threat *(86–88)*.

Generally Gram-positive bacteria will not grow in beer owing to the hop content; however, many strains of *Lactobacillus* spp. and *Pediococcus* spp. have obtained the *horA* gene that helps deal with this problem *(26,89–95)*. This review does not aim to cover LAB behaviour in detail; instead readers are referred to an excellent recent review of LAB in beer *(26)*.

### Acetic acid bacteria

Acetic acid bacteria are aerobic, Gram-negative, rod-shaped bacteria that belong to the Acetobacteraceae family *(96)*. There are reportedly 10 species of *Acetobacter* and only one *Gluconobacter* (*Gluconobacter oxydans*) that are associated with the brewing industry *(97)*. Acetic acid bacteria are generally strict aerobes; however, some strains isolated from draught beer have been reported to be micro-aerotolerant *(98)*. Acetic acid bacteria can survive in high levels of ethanol (>10% v/v) and have the ability to oxidize ethanol to acetic acid, producing vinegary off-flavours and aromas *(99,100)*. Various efforts to limit the ingress of oxygen into the beer have reduced the incidence of acetic acid bacteria-related spoilage. However a study of 1203 samples found that 153 samples were positive for acetic acid bacteria *(101)*. Ploss and co-workers *(101)* found that these contaminants were only found in samples from the filling and filtration processes of the brewery, with 70% of the contamination belonging to *A. pasteurianis* sub *pasteurianis*. Van Vuuren *et al*. *(102)* also found that samples from fermentation and storages tanks were occasionally contaminated with *Acetobacter* and *Gluconobacter*. Furthermore, Ingledew *(99)* reported an increased incidence of acetic acid bacterial spoilage in draught beer kegs. Most incidences of *Acetobacter* spp. are typically associated with the ingress of oxygen and are most likely to occur at filling lines in breweries and tap lines in pubs.

### Enterobacteriaceae

Enterobacteriaceae are a family of Gram-negative, rod-shaped facultative anaerobic bacteria that typically include *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Salmonella*, *Serratia* and *Shigella* species *(97)*. However, only a small number of Enterobacteriaceae species are occasionally associated with the brewing industry, such as *Obesumbacterium proteus*, *Rahnella aquatilis* and *Citrobacter freundii (97,103,104)*. The presence of *O. proteus* in beer was first described by Lindner *(105)* as *Termobacterium lutescens*; it has been renamed several times *(104,106,107)*, and this microorganism has recently been renamed as *Shimwellia pseudoproteus (108)*. This bacteria has the ability to spoil beer and wort by producing acetoin, lactic acid, propanol, DMS, isobutanol and 2,3-butandiaol *(104,109)*.

Contamination of this bacterium is generally found in pitching yeasts and may only create a significant effect in the first 24 h of fermentation until the pH drops below pH 4.5 *(110,111)*.

*Citrobacter freundii* and *Rahnella aquatilis* affect the beer by producing a variety of off-flavours and aromas from the production of diacetyl, DMS, acetoin, acetaldehyde, lactic acid and 2,3-butandiol *(107)*. The production of these compounds usually occurs at the start of the fermentation and contamination can be easily avoided by using highly active yeast starter cultures that will rapidly increase the ethanol concentration, which will ultimately slow and even stop the off-flavour production *(74)*.

### Zymomonas

Shimwell *(106)* first isolated *Zymomonas mobilis* (originally named *Achromobacter anaerobium*) from beer. *Z. mobilis* is a Gram-negative, aerotolerant, anaerobic bacterium, which uses the Entner–Doudoroff pathway to ultimately ferment a limited substrate range (glucose, fructose and sucrose) to ethanol *(112,113)*. The fact that this bacterium cannot utilize maltose as an energy or carbon source means that its occurrence as a beer spoilage bacterium is generally limited to beers that use sucrose as an adjunct or priming sugar. This bacterium is quite common in ciders, while in breweries it can be found in the bottling stages of the beer production process *(112,114–116)*. *Z. mobilis* contaminated beers generally have 'fruity' and 'sulphidic' characteristics, owing to the high levels of acetaldehyde and hydrogen sulphide produced during fermentation *(114,117)*.

### *Pectinatus* spp.

*Pectinatus* spp. are Gram-negative, strictly anaerobic bacteria that can produce large amounts of acetic and propionic acids, acetoin and turbid beer, and they were first isolated in 1978 as *P. cerevisiiphilus (118,119)*. This bacterium has been isolated from a variety of breweries with many reports of the beers tasting sour and with a rotten egg aroma owing to hydrogen sulphide and a variety of acids being produced *(120–126)*.

### *Megasphaera* spp.

Beers that are spoiled by *Megasphaera* are generally turbid, contain high levels of hydrogen sulphide, and can contain a range of fatty acids including butyric, caproic and valeric acid *(125,127)*. *M. cerevisiae* is a Gram-negative, strictly anaerobic cocci that is generally not found in finished beers and was first isolated from beer in 1979 *(128)*. *Megasphaera* strains are, however, sensitive to low pH and high alcohol and are therefore typically only present at the start of fermentations, until the ethanol content exceeds 2.8% v/v *(129)*.

### Miscellaneous

Apart from the potential negative effects that bacteria can have on the final product of the brewing process, the grain-microflora can also negatively affect malt and beer quality and contribute to, or even be responsible for, poor germination in water-sensitive grain *(130,131)*. Heavy microbial populations decrease germination rate, extent of germination, rootlet growth and alpha-amylase production. Microbial activity on the surface layers of the grain influences the responsiveness of the barley aleurone layers to a dose of gibberellic acid. Warm storage reduces the microbial population on grain and makes grain tissues less oxygen-dependent *(132,133)*. Soaking injury and induced water sensitivity are probably due to the accumulation of microbes on the grains *(131,133)*.

## Bacteria in brewing: the Good

The beneficial aspects of the use of LAB in food technology for improving food safety as a low-cost method of food preservation and in improving the nutritional quality of the food raw materials have been known to mankind for centuries *(134)*. Bacteria play a number of beneficial roles in the production of beers. LAB play a well-described role in the production of acidulated malt, while some beer styles (e.g. Berliner Weissbier and Lambic beers) are explicitly dependent on the action of bacteria during the production process.

The natural occurrence of LAB in malt mashes was reported in the late 1880 s by Lindner *(105)*, while in 1896 Leichmann *(135)* described LAB in distillery mashes. During the early 1900s many brewers realized the benefits LAB could offer them. Formerly treated as beer spoiling bacteria, some LAB strains provided brewers with a means to improve brewhouse yield and beer quality when lactic acid-fermented unhopped wort was added to mash or wort *(136–138)*. Acidification of mash and/or wort is not an uncommon technique in the production of beer *(139,140)*. The use of mash acidification, however, does not result in a lower beer pH, as the lower mash pH increases the activity of phosphatases and as such provides a buffering effect. The pH of finished beer can only be reduced by wort acidification conducted shortly before the end of the wort boiling process. Acidification of fermenting wort or beer is typically only done with technical acid owing to the high risk of microbiological infections by the non-sterile biological acidified wort.

There are a number of benefits resulting from mash and wort acidification. Mash acidification is especially beneficial if malt of poor quality is being used, because it can compensate for a lower enzyme activity *(140)*. The slightly lower pH enhances the activity of many of the malt enzymes, including limit dextrinase. Simultaneously the activity of the viscosity-relevant $\beta$-glucan solubilases decreases, resulting in a lower mash viscosity and thus yielding a mash with improved lautering performance. Mash acidification can compensate for a lower enzymatic activity. For instance, grist containing up to 20% unmalted barley can be processed without adverse mashing performance *(140)*. Detailed studies have shown that biological acidification of mash and wort results in improved wort and beer characteristics (Table 2) *(139–142)*. Franz and Back *(143)* showed that biological acidification in conjunction with an elevated mashing-in temperature increased flavour stability. Biological acidification results in higher bioavailability of zinc in wort *(144)*, which subsequently results in a better fermentation. Zinc is essential for protein biosynthesis and carbohydrate metabolism of brewer's yeast *(145)*, and plays a role in the production of higher alcohols *(146)*. Through biological acidification, more charged ions are extracted into the wort from the grist. This larger amount and variety of ions interacts with the unaffected amount of chelating compounds present in the wort; thus more zinc remains available in the wort. In order to lower the negative effects resulting from a low pH during wort boiling, such as a lower yield of alpha-acids and a lower splitting rate of the DMS precursor, biological acidification can be performed shortly before the end of wort boiling *(145)*.

**Table 2.** Advantages of biological acidification in the brewing process *(142,147)*

| | |
|---|---|
| *Technological improvements* | |
| Enzymology | Activation of important mash enzymes |
| Nutrients | Improved zinc bioavailability |
| Elimination of proteins | Improved break formation<br>Better hot trub precipitation |
| Redox potential | Lower sensitivity to oxygen, more buffering substances |
| Fermentation | Rapid decrease in pH<br>Higher final attenuation |
| Filtration | Lower wort viscosity, faster lautering<br>Lower beer viscosity, faster filtration |
| *Sensory improvements* | |
| Taste | Fuller and smoother flavour profile |
| Hop bitterness | Smoother bitterness |
| Mouthfeel | Fresh character |
| Foam | Finer bubbles<br>Stable, longer lasting |
| Colloidal stability | Lower risk of protein haze |
| Microbial stability | Lower risk of microbial contamination |

As an alternative to biological acidification, the use of mineral acids or technical lactic acid is commonly employed to lower the pH. The advantages of any form of acidification are the activation of important enzymes during mashing and a lower viscosity of mash, wort and beer *(148)*. However, if the direct addition of mineral acid or lactic acid is either undesired or forbidden, such as under the regulations of the German purity law, the use of biological acidification is the only means to lower the pH of either the mash and/or wort *(139,140)*. To produce biological acidified wort, LAB that have been isolated from malt are typically used to ferment unhopped wort *(149,150)*.The fermented wort is subsequently reintroduced into the brewing process and added either to the mash or to the wort or to both. The requirements for strains selected for modern biological acidification are high hop sensitivity, as thermophilic as possible, homofermentative, no production of diacetyl or biogenic amines and a rapid lactic acid production rate. Most strains used in brewing-related applications belong to the species *Lactobacillus amylolyticus (149)*. *L. amylolyticus* is a Gram-positive, non-spore-forming rod with rounded ends, occurring singly, in pairs or as short chains; it is microaerophilic and catalase-negative, and has a high amylolytic capacity. *L. amylolyticus* is not a beer spoilage bacterium, and can grow up to a temperature of 52 °C, with an optimum between 45 and 48 °C *(149)*. These obligate, homofermentative bacteria produce solely lactic acid from a range of dextrins, and a range of sugars including maltose, sucrose, fructose, galactose, glucose and mannose.

### Berliner Weissbier

An example of a strongly acidified beer is the Berliner Weissbier, which used to be characterized by the fact that the 9 °Plato wort was clarified, but not boiled. This wort was fermented with a mixture containing *Brettanomyces*, *Saccharomyces* and heterofermentative LAB. After the main fermentation, the beer was blended with kräusen and refermented in bottles for up to two years. Napoleon's soldiers used to call the Berliner Weissbier 'The Champagne of the North' ('*Champagne du Nord*') *(151,152)*. As an alternative, the beers were sent to pubs in casks, where the inn-keeper bottled and re-fermented the beer. Later this technique was modified and half of the wort was inoculated with *Lactobacilli* while the rest was fermented using a top fermenting yeast. After some weeks the two fermentations were blended, filtered and re-fermented in bottles using kräusen and top-fermenting yeast *(151)*. Over the last decades, this procedure was cut down to a production using biological acidification and top fermenting yeast, resulting in a sourly refreshing beer with 2.8 ABV and a pH of about 3.2 *(152)*.

### Lambic beer

Lambic beers and the newer American Coolship Ales are also examples of a beer style that depends on the activity of bacteria for part of its sensory characteristics. Lambics are produced using malted barley and wheat, where the percentage of wheat has to be at least 30% of the grist bill. Hopping is done with a high dose of aged hop cones, which are not used for aroma or bitterness, but solely for their anti-microbial properties, preventing the growth of any pathogenic Gram-positive bacteria in the spontaneous fermenting wort. Because of the microflora required for Lambic fermentation, and the limited control of the inoculation practice, this kind of beer can only be produced during the cold season. Wort boiling is quite intensive, resulting in a total evaporation of about 30%. After boiling, cooling takes place overnight in an open shallow vessel known as a coolship, and during this period the wort picks up a variety of microorganisms from the air that is blown over the wort *(153,154)*. This inoculated/infected wort is run into large casks and stored at a temperature between 0 and 25 °C without any deliberate inoculation with yeasts *(155)*. It takes around 4–8 months to decrease the wort density from 12 to around 3 °P. After the main attenuation of about 80% is reached, the wort is further fermented for up to two years, resulting in a wort of 1 °P or less *(156)*. The microbial population present in Lambic beers after one year of spontaneous fermentation consists mainly of *Brettanomyces* yeasts, LAB and acetic bacteria. It has been found that *Brettanomyces* is the main organism responsible for superattenuation, although this was less pronounced when *Pediococcus* was absent *(153)*. It was found that *Brettanomyces*, but not *Saccharomyces*, survive well under the conditions found in a one-year-old Lambic *(156)*. During Lambic fermentation multiple phases can be differentiated. In the enterobacterial phase, *E. cloacae* and *Klebsiella aerogenes* are most frequently recognized *(154,155,157)*. The early yeasts during a Lambic fermentation are maltose non-fermenting yeasts such as *Kloeckera apiculata*, *Saccharomyces globosus* and *Saccharomyces dairensis*. These organisms disappear when the pH is lowered and glucose in wort becomes depleted. The main ethanolic fermentation happens in the *Saccharomyces* phase, the main population being *Saccharomyces cerevisiae*, *Saccharomyces bayanus*, *Saccharomyces uvarum* and *Saccharomyces inusitatus*. After about 4 months, LAB, mainly belonging to the genus *Pediococcus*, increase *(153)*. Owing to sugar exhaustion *Saccharomyces* species disappear and superattenuating *Brettanomyces* yeasts increase. In the ripening phase after around 10 months, LAB and *Brettanomyces* decrease as the final wort attenuation is reached *(155)*. The final phase of Lambic fermentation is the bottle refermentation, resulting in Gueuze *(155,158)*. During bottle refermentation, species

such as *Candida*, *Torulopsis*, *Hansenula*, *Pichia* and *Cryptococcus* are present at low numbers, with the predominant organisms being *Brettanomyces* and LAB *(159)*. Lambic fermentation is probably the most important natural growth medium for *Brettanomyces* yeasts. Almost all known *Brettanomyces* species can be found during Lambic fermentations *(160)*.

### Acidulated malt

An alternative to biological acidified wort is the use of acid malt. Acid malt is malt which is inoculated with LAB during the germination step. The LAB will metabolize the sugars that are present on the surface of the kernels into lactic acid. The lactic acid will be retained on the surface of the kernels during germination, kilning and subsequent handling. Such malts may be added at rates from 3 to 5% of the grist, resulting in a pH reduction of about 0.15–0.25 units *(140)*. The pH of a typical acid malt based mash is 5.6, while the resulting wort has a pH of 5.2, as compared with 5.9 and 5.5 for non-acid malt based mash and wort, respectively *(150)*.

The use of LAB as starter cultures during the malting process focuses on two problems. On the one hand rootlet growth of the germinating kernels can be suppressed and thus malting losses be reduced. On the other hand LAB show a certain antifungal and antimicrobial activity, inhibiting the growth of fungi involved in gushing and health relevant mycotoxin producing microbes *(84,161,162)*. Reported beneficial effects on the malting process include a lower viscosity and $\beta$-glucan content of wort, increased malt yield and a pronounced improvement of mash and wort filterability. Furthermore, the growth of potential gushing inducing *Fusarium* species, as well as the growth of mycotoxin producing fungi, is suppressed *(84)*. LAB starter cultures have positively influenced the malting process by actively contributing to barley germination and/or malt modification *(85,163)*. Microbial starter cultures have been applied by spraying LAB starter cultures during the germination step of the malting process. This results in several beneficial effects on the quality of malt, such as a decreased proportion of kernels contaminated with *Fusarium*, decreased water sensitivity, increase in falling number, improved extract, improved free amino nitrogen, better malt modification, improved wort filterability, an increase in $\alpha$-amylase activity and a decreased tendency to gushing *(164–166)*. Malting losses can be reduced by 50% when malt is treated with *Lactobacillus plantarum (164)*. *L. plantarum* performed significantly better than chemical rootlet inhibitors such as potassium bromated, which were once employed by the industry *(164,165)*.

A variety of microbes are carried on the raw materials used in beer brewing, rendering the process susceptible to contamination and often resulting in spoilage or inferior quality of the finished product. The application of antimicrobial-producing LAB at various points in the malting and brewing process could help to negate this problem, providing an added hurdle for spoilage organisms to overcome and leading to the production of a higher quality beer. The bioprotective potential of LAB and their application might be of interest for the brewing industry. Antifungal-producing LAB may reduce the need for chemicals such as fungicides, which are undesirable from a consumer viewpoint. Fungicides also can be inefficient for eliminating fungal growth and do not consistently reduce mycotoxin levels in *Fusarium*-infected cereals *(167)*. An example of applying LAB in malting is the development of LAB starter cultures for use as inoculants during the malting process in order to improve the quality of the malt *(84,163,168,169)*. A number of *L. plantarum* strains have shown a fungistatic effect against different plant pathogenic, toxigenic, and gushing-active *Fusarium* fungi *(170–173)*. The antifungal activity of LAB is poorly characterized, but organic acids, as yet uncharacterized proteinaceous compounds, and cyclic dipeptides have been implemented as inhibitory with regards to some fungi *(164,172,174)*. Certain LAB produce antibacterial substances that restrict the growth of various potential harmful Gram-negative and Gram-positive bacteria *(134,168–171,175–178)*. This antibacterial activity is active across a wide pH range, and relatively insensitive to heat treatment. The secreted compounds are sensitive to treatment with proteolytic enzymes and therefore proteinaceous in nature, which implies that they are bacteriocin-like inhibitory substances. Bacteriocins share a common inhibitory mechanism – the depletion of the proton motive force across the plasma membrane *(179)*. Bacteriocins produced by bacteria comprise a heterogeneous group of physicochemically diverse ribosomally synthesized peptides, with a varied antimicrobial activity spectrum against a range of Gram-positive bacteria *(168,180)*.

Lactic acid, in addition to its antimicrobial property owing to the lowering of the pH *(175,180)*, also functions as a permeabilizer of the Gram-negative bacterial outer membrane and may act as a potentiator of the effects of other antimicrobial substances *(181,182)*. The employment of starter cultures in malting is a relatively new process that controls indigenous microbial growth and is both technically and economically feasible. The utilization of LAB as starter cultures in malting reduces fungal contamination, lowers the aerobic bacterial flora and leads to a higher malt quality regardless of the natural variation of the microflora of the barley *(84,133,183)*.

### Novel, malt-based beverages

In recent years a number of novel, innovative malt-based beverages have been launched. Consumer awareness of the negative impact of poor nutrition has grown in recent years. Consumers are looking for new products with natural ingredients and beneficial health attributes *(184–186)*. The intent of novel malt-based beverages is to produce new beverages employing the facilities and the knowledge already existing in breweries *(187–189)*. Brewers can take advantage of the biotechnical knowledge they have gained over the centuries and the technical know-how of their existing brewing facilities *(185,186)*. Novel malt-based beverages are usually artificially carbonated since the microorganisms selected to facilitate the fermentation do not produce a sufficient amount of carbon dioxide, if any is produced at all. With alternatively fermented substrates, a great variety of potential drinks can be produced by adding fruit juices, flavour or functional ingredients *(186,188)*. In one example of a LAB facilitated, malt-based fermented beverage, unhopped beer wort is used as the substrate with *L. amylolyticus* as the fermentative organism at a fermentation temperature of 48 °C. The fermentation rapidly commences and stops after about 42 h with a pH of about 2.9. The pH is the limiting factor; after reaching the low pH there is neither an increase in lactate production nor an increase in cell count *(184–186,189)*. Another possible application of LAB is their use during the production of non-alcoholic beers *(190)*, in particular, beverages produced

by the method of stopped fermentation, that is, stopped by either filtration or flash pasteurization, whereby a LAB fermentation provides a fresh character and an improved drinkability compared with a yeast-driven fermentation *(142)*.

## Concluding remarks

Whereas bacteria in beer are of concern with regards to potential negative impacts on a range of quality characteristics (the Bad), they very rarely pose a concern with regards to food safety (the Ugly). The intrinsic and extrinsic antibacterial hurdles associated with beer and its production make it an inherently safe beverage to consume. Nevertheless, bacteria have been proven to play a significant beneficial role (the Good) with regards to the production of malt, specific beer styles and the production of alternative malt-derived beverages. Hence, not all bacterial involvement in the production of beer can be viewed through a single eyeglass, or measured by a single yard stick. While often scorned and unwanted in the beer production process, the presence of bacteria can sometimes underscore positive beer attributes.

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