Spoilage Potential of Microorganisms and their Detection by real time PCR

Beer spoilage

When it comes to microbial spoilage, beer is rather stable due to the antimicrobial effect of hops, the limited availability of nutrients after fermentation and its low pH. Although the presence of bacteria is ubiquitous in breweries, only a limited number of bacteria and yeast species is capable to survive in beer and spoil it by either producing visible cell growth or metabolites which change its taste.

The typical beer spoiling bacteria are a distinct group of the lactic acid bacteria together with the strict anaerobic species *Megasphaera* and *Pectinatus*. While the latter two only appear in closed systems - mostly seen only in larger breweries which work in an oxygen-free environment - *Lactobacillus* and *Pediococcus* species are always present in each brewery, as they come in with the malt.

Wild yeasts are all those yeasts which are unwanted in the process, independent from their use in brewing. A wild yeast can be a yeast species which may cause superattenuation in finished beer like *Brettanomyces* (*Dekkera*) and *Saccharomyces cerevisiae* var. *diastaticus*. But it can also be a brewing yeast strain which is not intended to be present in a certain batch, as a lager yeast in a top fermenting beer or vice versa, or a *Saccharomyces cerevisiae* var. *diastaticus* yeast in an Ale. Besides, non-*Saccharomyces* yeasts as *Pichia, Candida* or *Zygosaccharomyces* sometimes appear in the brewing environment although not growing in the typical beer styles but most likely in mixed products made with sugar, fruit and spices.

Beer Spoiling Microorganisms

Those bacteria which can not only survive but actively grow in beer and its preliminary process stages are all acid lovers – they can grow at pH values down to pH 3.3 (*Lactobacillus acetotolerans*) and live from the leftovers after brewer's yeast has finished fermentation. Some species are even depending on the presence of yeast: *L. rossiae* for example seems to need the nutrients delivered by dying yeast cells or from yeast metabolism for its proliferation. This species is growing in yeast turbid beers, producing a viscous slime.

Depending on the beer type, different lactic acid bacteria species may cause spoilage - or not.

Although hop resistance is one of the major properties which the beer spoiling bacteria share, the presence of hop resistance genes is not always a safe tool to discriminate between their spoilage and non-spoilage potential. The *hordein* genes which are responsible for hop resistance in bacteria are "jumping genes" which means that these are moving not only between members of the same species, but also from species to species. In laboratory research experiments it was shown that *hordein* genes can be easily transferred into *E. coli* bacteria. Nevertheless, *E. coli* bacteria are not at all regarded as a

beer spoiler, as they have never been growing in beer. Therefore, beer spoilage testing based on the presence of *hordein* genes can give some valuable information, but also could give false positive results.

On the other hand, it is common practice in many breweries to use *Lactobacillus brevis* cultures for preparing the sour mash – this seems to be riskless because that special *L. brevis* culture strain in use is not harboring the *hordein* genes. Nevertheless, these "tame" *L. brevis* cells might easily pick up the *hordein* genes from another beer spoiler cell, and so suddenly turn into a spoiler with a high spoilage potential for all beer styles.

L. brevis is one of the beer spoilers with the highest beer spoilage risk: **Beer spoilage always occurs whenever one of these species is present**: *Lactobacillus brevis, L. lindneri, Pediococcus damnosus, Megasphaera cerevisiae, Pectinatus cerevisiiphilus or P. frisingensis, Brettanomyces* or *Saccharomyces cerevisiae* var. *diastaticus*.

Spoilage by yeasts usually occurs by over fermentation in the finished product. Therefore, the group of beer spoiling yeasts is small – only *Brettanomyces (Dekkera)* and *Saccharomyces cerevisiae* var. *diastaticus* are regarded as typical beer spoilage yeasts. Both can take a few weeks until they start growing, but then can produce immense amounts of CO_2 which might lead filled units to bursting. Off flavors of *Brettanomyces* are the typical "mousiness" or "horse sweat", while growth of *Saccharomyces cerevisiae* var. *diastaticus* often is not even recognized due to off flavor, it only leads to a dry taste, described as a loss of body or mouthfeel, and pressure formation.

The following table 1 gives an overview about the beer spoiling microorganisms, their spoilage potential and occurrence corresponding to beer styles.

The spoilage potential describes the overall risk for a brewery and the beer styles which are produced.

- 'High' means that this spoiler can grow fast in mostly every beer style, and a brewery should take measures to get rid of it and find its source immediately. If such a microorganism is constantly in the brewing process, it is a real threat and in the worst case can ruin a brewery.
- 'Low' means that there is a contamination in the process which should be fixed. Such a spoiler it is not an immediate threat to the whole brewery, maybe for a couple of beer styles only - if these styles are produced at all. For those breweries producing hoppy beer styles only, these microorganisms are more regarded as an indicator only.
- 'Medium' is something in between, the spoilage potential and the risk for the brewery is depending on the beer styles which the brewery produces.

The table is listing all microorganisms which have been detected in beer and identified by a reliable method, e.g. DNA sequencing or by species specific PCR analysis.

Other Lactobacillus species that those listed weren't yet proven to grow in beer and/or cause spoilage.

Species	Spoilage potential	Gowth in beer / characteristics	Time until growth starts	Occurrence / comments
Lactobacillus acetotolerans	medium	all	up to 6 months	Grows only anaerobic, often to a low concentration only, causes pH drop
Lactobacillus backii	low	low hops higher pH	up to 4 weeks	stays latent inside the system, often produces no off flavor
Lactobacillus brevis	high	all	growth starting immediately	typical beer spoiler
Lactobacillus casei	medium	low hops	growth starting immediately	Spoilage only in low hopped beer types, wheat beer
Lactobacillus collinoides	low	low hops and low alcohol	growth starting immediately	rarely seen, low acid production often no off flavor
Lactobacillus coryniformis	low	low hops and low alcohol	growth starting immediately	rarely seen, low acid production often no off flavor
Lactobacillus parabuchneri ("frigidus")	low	low hops and low alcohol	growth starting immediately	only rarely seen slime producer
Lactobacillus lindneri	medium	low hops	Up to 4 weeks	cells are often small and might get through filters, low acid production
Lactobacillus perolens	low	low hops, low alcohol and a higher pH	grows in enrichments	spoils only no- or low alcohol beers, rather in beer-fruit mixes
Lactobacillus rossiae	low	low hops, low alcohol and a higher pH	up to 3 months	needs yeast or high nutrient concentration for growth, may cause extreme ropiness
Lactobacillus plantarum	low	low hops, low alcohol and a higher pH	growth starting immediately	often produces only low concentration of lactic acid and minimal off flavor, spoilage by turbidity
Pediococcus damnosus, P. inopinatus	high	all	growth starting immediately	Diacetyl producers, throughout the process, often already in yeast propagations
Megasphaera sp.	high	all	growth starting immediately anaerobic conditions only	dead ends in closed systems, finished product, causes extreme off flavor "rotten eggs", "baby vomit"
Pectinatus sp.	high	all	growth starting immediately anaerobic conditions only	dead ends in closed systems, finished product, causes extreme off flavor, "liquid manure"
Brettanomyces / Dekkera	high	all	up to 3 months	hoses and tanks, often coming from outside – orchards! Off flavors are mousiness /horse sweat
Saccharomyces cerevisiae var. *diastaticus*	high	all	6-8 weeks, might also start immediately depending on temperature	used for Belgian beer styles, sour beers, leads to a dry taste, loss of body and mouthfeel

4e® For everyone Detection Kits

4e® For everyone Detection Kits are available to test for all beer spoiling bacteria and yeasts during all production stages, from pitching yeast down to finished beer, with one single method.

For general routine monitoring with real time PCR, there are screening tests available which cover the detection of different spoilers within a group – you detect them all together in one test.

For the precise identification of a certain spoiler species, additional species-specific tests are available for all beer spoiling bacteria and yeasts.

All 4e® Detection Kits for beer spoiler testing were so solidly developed that they don't pick up any non-spoiling lactic acid bacteria. There won't be false positive results from other lactic bacteria species which are common on malt. Therefore, a positive result from 4e® kits for 'beer spoiling bacteria' doesn't need a second verification like an additional test for *hordein* genes.

4e® For everyone Detection Kit products and the species range which these are detecting are listed in the following table 2:

4e® For everyone Detection Kit	SKU	Species detected within the test
LP Screening Real beer spoiler test	2401-38	Detection of all beer spoiling *Lactobacillus* plus *Pediococcus* species (as in table 1) in one test
LP Identification	2401-37	Single species identification of all the species covered in kit #2401-38
Diverse single species kits	2401-nn	One species only kits are available for all species named in kit #2401-38
Brettanomyces (Dekkera) Screening	2402-20	Common detection of all *Brettanomyces* species in one test
S. cerevisiae var. diastaticus	2402-49	Detection of *Saccharomyces cerevisiae* var. *diastaticus*
Superattenuator yeasts	2402-58	Common detection of all superattenuating yeasts as in kits #2402-20 plus #2402-49

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