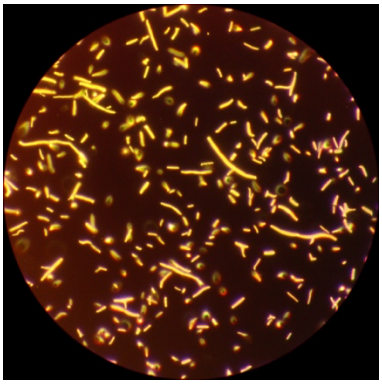
APPLICATION NOTE

Detection of Anaerobic Beer Spoilers

Why is the detection of anaerobic beer spoilers so hard?

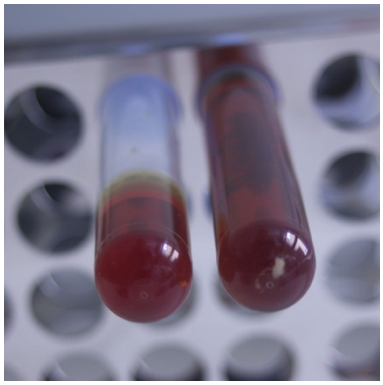
Anaerobic beer spoilers only became an issue since the technology of beer production was improved following concern of avoiding entry of oxygen into the production process. The first anaerobic spoiling Pectinatus strains were isolated in 1978. Megasphaera was first detected in beer and described as a new species in 1986.



The Problem

Besides the spoilt beer, the problem for the German brewery at that time was that *L. acetotolerans* was not even yet on the list of beer spoilers. First these bacteria had to be identified to get an idea what this is all about.

In our lab we were able to isolate a slowly anaerobic growing *Lactobacillus* species from a contaminated bottle which we identified by DNA sequencing as *Lactobacillus acetotolerans*. This bacterium was very sensitive to oxygen; besides, *L. acetotolerans* is resistant to higher alcohol concentrations. The strain description of *Lactobacillus acetotolerans* gives the following information: Facultative anaerobic, growth is generally observed at pH 3.3 to 6.6 and at 23 to 40 °C, no growth at 15 °C. The bacterium is resistant to 4 to 5% and 9 to 11% of acetic acid at pH 3.5 and 5.0, respectively.



The Solution

In order to detect *L. acetotolerans*, the routine sampling and enrichment methods in the brewery had to be changed as this species is not only slow growing but also picky concerning nutrients.

By applying a close to anaerobic liquid enrichment in FastOrange® B bouillon followed by a *L. aetotolerans* species specific PCR analysis, the brewery was able to constrict the most probable contamination source: It was the area where the tanker trucks were loading the beer – rinse water from one of the empty trucks gave a positive result for *L. acetotolerans*.

The final conclusion was that the trucks were insufficiently cleaned and *L. acetotolerans* was already on board when the beer was filled in. This constellation was probably evident throughout the year, but seemed to bear a high spoiler potential during the warmer months when even short times on the truck could lead to a concentration of lactic acid bacteria sufficient for spoiling the beer.

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Interestingly, there is not much literature available about *Lactobacillus acetotolerans*. There are publications which proof *L. acetotolerans* as one of the dominant lactic acid bacteria species in traditional fermentations, e.g. liquor from fermented grains (Luzhou-flavor liquor, more than 50% alcohol) as well as in the natural fermentations of sourdough (Mantou) and Sichuan pickles, all in China, in a Bamboo shoot fermentation in north-east India, in a traditionally fermented fish and rice dish (Narezushi) in Japan, and as a contaminant of yeast used for the production of in a Brazilian liquor (Cachaça).

The species *Lactobacillus acetotolerans* usually only is identified as part of the microflora of quite different fermentation processes if the analysis is based on cultivation independent methods. It seems that the detection of *L. acetotolerans* is mostly failing in those common analyses methods which need bacterial proliferation before the bacteria from a sample are identified.

This observation supports our finding that the sampling and sample processing are the major challenges for the detection of *L. acetotolerans*.

To detect *Lactobacillus acetotolerans*, it essential to work in a low oxygen atmosphere from the beginning, which includes the sampling itself. The easiest way to analyze liquids for anaerobes is by direct sampling into an incubation bottle or tube prefilled with liquid enrichment medium, filling the vessel up to the brim with liquid sample. Besides, this *Lactobacillus* species is very slow in growing. Often it takes 2-3 weeks until *L. acetotolerans* becomes visible in enrichments, and then often it forms only a small flake of haze in the bottom of the tube (refer to picture, right tube: *L. acetotolerans* in FastOrange B® Tube after 10 days incubation).

When using swab samples, always keep in mind that a dry cotton or sponge is full of air which means it is full of oxygen. Therefore, it is absolutely essential to use wet swabs only for anaerobic sampling, and to immerse the swab immediately into a liquid culture medium.

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