Beer spoilage associated with pediococcus spp

Food & Diet

Introduction

In average lager fermentation gradual decrease of pH and specific gravity is expected while ethanol concentration is increasing. Some flavours are also formed during fermentation as esters and other flavour compounds are formed. They can remain in beer or be gradually removed either due to evaporation or further metabolism of yeast. Deviations from the average pattern of changes can signalise a contamination. Fermenting wort is therefore routinely tested for it’s gravity, pH and also for flavour and odour.

In a sample tested rapid decrease of pH at the end of fermentation was noted. pH reached 3. 7 which was well below expected 4. 1 (Hough 2001). Such significant fall suggested bacterial contamination. Two groups of bacteria may cause lowering of pH. These are Acetic Acid Bacteria and Lactic Acid Bacteria.

pH decrease was noted late in fermentation when no oxygen was available suggesting Lactic Acid Bacteria. Strong diacetyl odour (sweet-buttery), turbidity and time indicated Pediococcus spp., probably Pediococcus damnosus since it is the most common beer-spoiling bacterium in its genus .

The spoilage characteristic associated with *Pediococcus spp* .

Lactic Acid Bacteria are the most common contaminant in fermented wort and beer. They can be responsible for up to 90% of microbial beer spoilage incidents (Taskila et al. 2009). Within them two groups are recognised as the most common contaminants: Lactobacillus and Pediococcus.

The symptoms of bacterial contamination by member of any of those two genera are similar: lowered acidity (giving to the beer sour taste) caused by production of lactic acid by the bacteria and diacetyl (buttery) off-flavour. The latter one is the main reason why pediococcal contamination is so unwanted. According to Whiting (1992) as few as 20, 000 bacterial cells per 1ml can produce diacetyl in concentration of 0. 36mg/L, which is 3 times higher than the taste threshold. Spoilage by Pediococcus is often characterised by ropines, but some of Lactobacilli can also give similar symptoms. Presence of any of those lactic acid bacteria negatively influences yeast performance and health slowing down fermentation (Priest 2006).

Further investigation is required to determine spoilage microorganism, the cause of contamination and methods of removing unwanted bacteria from the system.

Pediococci are spherical, gram positive bacteria that often form tetrads, but also may appear in pairs (Priest 2006). Generally they are catalase negative, but in low glucose medium they can produce pseudocatalase that can also break hydrogen peroxide, which may lead to false catalase test results (Priest 2003). They do not form spores and are nonmotile. The main product of their metabolism is lactic acid (homofermentative bacteria) and thought they are anaerobic they can tolerate presence of oxygen (Priest 2003). The species that can inhabit fermenting wort and beer are hop resistant.

Following species have been isolated from beer:

- *Pediococcus damnosus* (formerly in brewing literature also referred to as P. cerevisiae), which is found in beer, late fermentation and brewing yeast (also wine) and is thought to be responsible for 90% of all spoilage incidents caused by Pediococci (Priest 2003, Whiting et al. 1992,).
- *Pediococcus inopinatus found in beer, brewing yeast, vegetables, wine, milk (Priest 2003).*
- *Pediococcus dextrinicus (Priest 2003).*
- *Pediococcus pentosaceus (Priest 2003).*
- *Pediococcus claussenii (Priest 2006).*

Isolation and confirmation of the presence of Pediococcus spp.

To detect presence of Pediococcus species in wort it should be first filtrated (volume – 100ml). A 0. 2 – 0. 45 microns membrane filter is recommended. (Lewis and Bamforth 2006).

Colonies should be incubated then on suitable medium (e. g. MRS[1]with Actidione to suppress yeast growth, Raka-Ray or NBB medium) for 5 days in temperature 25oC in anaerobic conditions (Lewis and Bamforth 2006, Briggs et al. 2004).

Grown bacteria can be Gram-stained and examined under microscope. Gram-positive cocci organised in tetrads suggest Pediococcus spp. although that should be checked by further tests:

Catalase test should be negative – no bubbles formed after dripping 3% H2O2 solution onto colony. Colonies growing have sour odour.

No gas should be produced from glucose using Gibson and Abd-el-Malek method (Priest 2003, p. 211).

An improved methodology for the recovery of Pediococcus spp.

Traditional methods of identifying bacteria are very time consuming. The results are too slow for commercial requirements and may result in dispatching a product that does not meet health and safety criteria. Therefore rapid testing methods were developed to identify spoilage microorganisms. There are used in diagnostic tests, often designed specifically for a given industry, such as LightCycler foodproof Beer Screening Kit.

The LightCycler foodproof Beer Screening Kit is based on polymerase chain reaction (PCR). In order to carry on the test sample has to be filtrated and inoculated into enriching broth as the count of about 1000 cells/ml is required to increase reliability of the result. The sample is then centrifuged, cells are lysed and DNA extracted, amplified and identified.

The test can detect 24 most common species of beer spoilage bacteria of genera Lactobacillus, Pediococcus, Pectinatus and Megasphaera and identify Pediococcus damnosus as well as P. inopinatus, Lactobacillus brevis, L. lindneri and Megasphaera cerevisiae (Biotecon 2009).

The method is very quick in compare to traditional methods (2 days versus up to 14) but is much more expensive. It requires three more kits and specialist equipment to carry the test and read the results, therefore can not be applied in small breweries.

Solution

Pediococcus damnosus was found in the fermenting wort and in beer. Special cleaning regime was employed with use of antibiotics to remove contamination as well as acid wash was implemented to pitching yeast.

References

- BIOTECON. 2009. http://bc-diagnostics. de/? cid= 1201264657〈= 1&name= foodproof+Beer+Screening+Kit %2C+Hybridization+Probes+(LC+1. x)+
- http://www. bc-diagnostics. com/public/DB_Data/files/Downloads/BeerScreening. pdf leaflet. Biotecon Diagnostic GmbH. Potsdam.
- Briggs, D. E., Boulton, C. A., Brookes, P. A., Stevens, R. (2004). Microbiology. In Brewing. Science and Practice. pp 606 – 649. Woodhead Publishing Limited and CRC Press, LTD. Cambridge.
- Hough, J. S., (2001). The Biotechnology of Malting and Brewing. Cambridge University Press. Melbourne.
- Lewis, M. J., Bamforth, C. W., (2006). Microbiology. In Essays in Brewing Science. pp. 58 – 68. Springer Science+Business Media LLC. New York.
- Priest, F. G., (2003). Gram-positive Brewery Bacteria. In Brewing Microbiology. 3rd ed. (ed. by Priest, F. G. and Campbell, I.). pp 181 – 218. Kluwer Academic/Plenum Publishers. New York.
- Priest, F. G., (2006). Microbiology and Microbial Control in the Brewery. In Handbook of brewing. 2nd ed. (ed. by Priest, F. G. and Stewart, G. H.). pp 607 – 628. Tylor and Francis. Boca Roca.

- Taskila, S., Nebauer, P., Tuomola, M., Breinstein, A., Kronlöf, J., Hillukkala, T. (2009). Improved Enrichment Cultivation of Beer Spoiling Lactic Acid Bacteria by Continuos Glucose Addition. In Journal of Institute of Brewing. Vol. 15. No 3. 177-182.
- Whiting, M., Crichlow, M., Ingledew, W. M., Ziola, B., (1992). Detection of Pediococcus spp. In Brewing Yeast by a Rapid Immunoassay. In Applied and Environmental Microbiology. Vol. 58. No 2. 713-716