

## EXPLORATION OF MATRIX ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY AS A FAST IDENTIFICATION TOOL FOR BEER SPOILAGE BACTERIA

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Beer is a beverage with good microbiological stability because it contains almost no oxygen and nutrients for the growth of many bacteria. In addition, low pH, high CO<sub>2</sub>-content and the presence of ethanol and antibacterial hop compounds ensure microbial stability. Beer spoilage bacteria are nevertheless a common problem in the brewing industry and typically cause visible turbidity, acidity and off-flavours and are worldwide a source of concern for the brewing industry<sup>1,2</sup>. In modern breweries the hop-resistant lactic acid bacteria *Lactobacillus brevis*, *Lb. lindneri*, *Lb. brevisimilis*, *Lb. coryneformis*, *Lb. plantarum*, *Lb. malefermentans*, *Lb. parabuchneri*, *Pediococcus damnosus*, *P. inopinatus* and *P. dextrinicus* are generally regarded as the most hazardous beer spoilage bacteria. Due to improved process technology the importance of aerobic bacteria has decreased, yet the appearance of strictly anaerobic Gram negative bacteria<sup>3</sup>, such as *Pectinatus cerevisiiphilus*, *P. frisingensis*, *Selenomonas lacticifex*, *Megasphaera cerevisiae* and *Zymomonas mobilis* has increased. Currently, spoilage bacteria are detected with culture-dependent methods using selective media or faster identification methods such as DNA-typing, ribotyping and PCR-based techniques. These approaches are notoriously laborious, expensive, time-consuming and moreover, they lack specificity and sensitivity. This research aims to develop a quick, specific and inexpensive method to detect and identify contaminants and spoilage bacteria in food products, more particularly in the brewing industry. To achieve this an extensive database comprising matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)<sup>4,5</sup> profiles of well-established and correctly identified beer spoilage bacteria and contaminants is build. In addition, strains originating from other niches will also be included in order to encompass the phenotypic diversity of all spoilage species. The resulting set of profiles will allow to assign species-specific biomarker peaks, specific and reproducible for a given spoilage species (or even strain) which will allow identification of an unknown beer spoilage organism. At this moment the database contains the following genera (number of species between brackets): *Acetobacter* (7), *Chryseobacterium* (4), *Enterobacter* (2), *Gluconacetobacter* (5), *Lactobacillus* (19), *Obesumbacterium* (1), *Pectinatus* (1), *Pediococcus* (4) and *Zymomonas* (1). In order to complete the database spectra of every known deposited beer spoilage strain and other strains of the same species are generated and added to the database.

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<sup>1</sup> Sakamoto, K., Konings, W. N., 2003. Beer spoilage bacteria and hop resistance. *Int. Journal of Food Microbiology* 89, 105-124.

<sup>2</sup> Jespersen, L., Jakobson, M., 1996. Specific spoilage organisms in breweries and laboratory media for their detection. *Int. Journal of Food Microbiology* 33, 139-155.

<sup>3</sup> Juvonen, R., Koivula, T., Haikara, A., 2008. Group-specific PCR-RFLP and real-time PCR methods for detection and tentative discrimination of strictly anaerobic beer-spoilage bacteria of the class *Clostridia*. *Int. Journal of Food Microbiology* 125, 162-169.

<sup>4</sup> Fox, A., 2006. Mass spectrometry for species of strain identification after culture or without culture: past, present and future. *Journal of Clinical Microbiology* 44, 8, 2677-2680.

<sup>5</sup> Sauer, S., Kliem, M., 2010. Mass spectrometry tools for the classification and identification of bacteria. *Nature Reviews Microbiology* 8, 74-82.