Use of Matrix-assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for the identification of beer spoilage bacteria.

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Beer is a beverage with good microbiological stability because it contains almost no oxygen and nutrients for bacterial growth. In addition, low pH, high CO_2-content and the presence of ethanol and antibacterial hop compounds ensure microbial stability. Nevertheless, beer spoilage induced by bacteria is a common problem in the brewing industry and these spoilage bacteria typically cause visible turbidity, acidity and off-flavours. Currently, these bacteria are detected with culture-dependent methods using selective media or with faster identification methods such as DNA-typing, ribotyping and other PCR-based techniques. These approaches are notoriously laborious, expensive, time-consuming and moreover, lack specificity and sensitivity. The present study aims to develop a quick, specific and inexpensive method to detect and identify beer spoilage bacteria in the brewing industry. To achieve this, an extensive database comprising MALDI-TOF MS-profiles of more than 260 established and accurately identified contaminants and beer spoilage strains was built. In addition to these strains, strains of the same species originating from other niches, besides spoiled beer, were also included in order to encompass the phenotypic diversity of the spoilage species. Among others, strains of *Lactobacillus brevis* (29), *Lb. lindneri* (3), "*Lb. brevisimilis*" (1), *Lb. buchneri* (5), *Lb. coryniformis* (1), *Lb. plantarum* (8), *Lb. parabuchneri* (15), *Lb. paracollinoides* (2), *Lb. perolens* (10), *Pediococcus damnosus* (9) , *P. inopinatus* (10), *Pectinatus cerevisiiphilus* (1), *P. frisingensis* (2), *Selenomonas lacticifex* (1), *Megasphaera cerevisiae* (2), and *Zymophilus raffinosivorans* (2) were included in the database. The resulting set of profiles (± 6500 good quality profiles) allowed the assignment of reproducible species-specific biomarker peaks for all spoilage species. All strains were not only cultured at species-specific conditions (type medium, growth temperature, oxygen requirements, growth time), but also on selective and non-selective media. Different media are used to enable the exclusion of medium-associated peaks from the species-specific biomarker peaks. Consequently, identification of novel beer spoilage isolates can be easily and rapidly performed. Nevertheless, the final aim of this research is to detect and identify these bacteria in a spoiled sample with minimal time-consuming culture steps.

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