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Short communication

Microbial communities in dairy processing environment floor-drains are dominated by product-associated bacteria and yeasts

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ABSTRACT

Floor-drains in food production environments harbour diverse microbes and can be contamination sources. To prevent cross-contamination with spoilage organisms or pathogens, sanitation protocols are regularly applied in food processing facilities. Along with product-associated samples, floor-drain water serves as an important indicator in hygiene monitoring in food processing facilities. However, knowledge about the microbial communities in floor-drains is still low. In this study, the microbial communities in floor-drain water and biofilm samples from an Austrian dairy plant were analysed and revealed that floor-drain microbial communities are dominated by product-associated microbes. DNA was extracted of drain water and drain biofilm samples from three different areas (n = 6) of an Austrian cheese production facility. To characterise the bacterial and eukaryotic communities, 16S rRNA gene pyrosequencing as well as cloning and sequencing of 18S rRNA genes were used. Floor-drain communities were dominated by product-associated bacterial (e.g. *Lactobacillus kefiranofaciens*, *Streptococcus thermophilus*) and eukaryotic phylotypes (e.g. *Debaryomyces hansenii*, *Saccharomyces unisporus*). In addition, putative drain water-derived phylotypes (e.g. *Psychrilyobacter atlanticus, Cobetia marina*) and ciliates were identified.

1. Introduction

Milk and milk products play an important role in human nutrition. Microbial activities are intrinsic to fermented milk products; microbes can be introduced either by raw material (e.g. raw milk), or inoculated intentionally (e.g. as starter or ripening cultures) but also by the production environment. These microbial communities can influence the product quality in a positive or negative way. Milk products harbour a variety of beneficial product-associated microorganisms, but may also harbour food-borne pathogens as a part of the undesired microbiota of milk products (Ferreira, Wiedmann, Teixeira, & Stasiewicz, 2014; Larsen et al., 2014). The main source of microbial contamination in food production can be the food processing plant itself - by contact of the product with processing surfaces and equipment, even when cleaning and disinfection are properly applied (Gibson, Taylor, Hall, & Holah, 1999; Oliver, Jayarao, & Almeida, 2005). Inadequate hygiene measures or improper hygienic design of equipment can foster the formation of biofilms and thereby the formation of potential niches for microbes (Ferreira et al., 2014; Giaouris et al., 2015). When living in biofilms, microorganisms can show increased tolerance towards disinfectants and are involved in either cooperative or competitive interactions within or between species (Giaouris et al., 2015). The importance of biofilms for food safety has been widely recognised (Giaouris et al., 2015; Marchand et al., 2012). In food processing environments, floor-drains (FDs) are one of the niches where formation of biofilms can occur. Due to their open system, FDs are exposed to a wide range of microbes and nutrients and may also serve as a reservoir for food-borne pathogens (Berrang & Frank, 2012; McBain et al., 2003; Zhao, Doyle, & Zhao, 2004; Zhao et al., 2006). However, the knowledge on microbial communities in FDs is still low. So far, few studies have analysed the bacterial and fungal communities in food processing environments and described facility-specific microbiota on cheese and processing surfaces (Bokulich & Mills, 2013; Schirmer, Heir,

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Moretro, Skaar, & Langsrud, 2013). Besides bacteria and fungi, protozoa can survive in FDs or on facility equipment and may influence bacterial communities. In a similar way, few studies are available on the occurrence and diversity of protozoa in food production environments (Vaerewijck, Sabbe, Bare, & Houf, 2008; Bare et al., 2009). Another aspect related to protozoa in FDs is the role of protozoa as potential vectors for food-borne pathogens (Lambrecht et al., 2015; Pushkareva & Ermolaeva, 2010).

The aim of this study was to characterise the composition of microbial communities in FDs using 16S and 18S rRNA gene sequencing technology and to investigate how microbial communities in FDs are influenced by the dairy environment, product- or water-derived communities in an Austrian cheese-production facility.

2. Material and methods

2.1. Facility description

The samples for this study were obtained from a cheese production factory in Austria in 2012. The factory produces a variety of different soft cheeses such as red smear cheese or Camembert and semi-hard cheese products from pasteurised cow, sheep and goat milk.

2.2. Sampling of floor-drain water and biofilm samples

Three FDs from three different rooms (laboratory, smearing room and ripening room) were selected for this study. The laboratory is used to store and prepare the microorganisms for cheese making processes (starter-, ripening- and smearing cultures). Smearing of fresh cheeses is performed in the smearing room. Afterwards, cheeses are kept for two weeks in the ripening room. Within each room, drain water (DW) and drain water biofilm (DB) samples were taken from the same FD, resulting in six samples in total. From each FD, approximately 500 ml DW was aspirated using 250 ml sterile syringes. DB samples were taken from an approx. 20 × 20 cm area of the FD surface at the same sampling time point as the DW samples using sterile dry sponge sticks (3M, Vienna, Austria). Samples were stored on ice during transport (three hours) to the laboratory and then processed immediately. Briefly, DW samples were centrifuged at 11.000 × *g* for 20 min at 4 °C and the supernatant was discarded. DB samples were homogenised for 7 min in 100 ml sterile 1 × PBS buffer in a stomacher bag using a Stomacher 3500 laboratory blender (Seward, London, United Kingdom). Centrifugation of the biofilm samples was performed at 11.000 × *g* for 20 min at 4 °C. The supernatant was discarded. The pellets of both DW and DB samples were subsequently used for genomic DNA extraction using 250 mg of the pellets using the PowerSoil™ DNA Isolation Kit (MoBio Laboratories, Carlsbad, California, USA) according to the manufacturer's instructions.

2.3. Pyrosequencing of the V1-V2 region of bacterial 16S rRNA genes

PCR amplification of V1-V2 regions of bacterial 16S rRNA genes, library preparation and pyrosequencing were performed at the Center for Medical Research, Core Facility Molecular Biology, Medical University of Graz, Austria, as described recently (Hund, Dzieciol, Schmitz-Esser, & Wittek, 2015).

2.4. Sequence processing and analysis

All pyrosequencing reads (in total 42,914 reads) were analysed using the mothur software, version 1.30.2 (Schloss et al., 2009). For details see Hund et al. (2015). Reads were clustered into operational taxonomic units (OTUs) using a distance limit of 0.03 (97% similarity). An OTU thus harbours all sequences sharing more than 97% 16S rRNA gene sequence similarity to each other. For calculation of species richness and diversity estimators, the data were randomly normalized to the same number of sequences per sample (n = 3177). The 50 most abundant OTUs were analysed for taxonomic affiliation against type strains in the Ribosomal Database Project (RDP) website (Cole et al., 2014).

2.5. Cloning, sequencing and sequence analysis of 18S rRNA genes

To detect protozoa and fungi, 18S rRNA gene PCR was performed using the primers EUK528F (5′-CGGTAATTCCAGCTCC-3′) and U1391R (5′-GGGCGGTGTGTACAARGR-3′) (Edgcomb et al., 2011). For details see (Schornsteiner, Mann, Bereuter, Wagner, & Schmitz-Esser, 2014). One gene library containing approx. 150 clones was created for each sample. Approx. 60 clones per library were randomly chosen and sequenced (Microsynth, Balgach, Switzerland). All sequences (n = 415) were analysed with mothur (Schloss et al., 2009) using only sequences with a minimum length of 750 bp. The remaining sequences (n = 380) were aligned to the SILVA SSUref 119 reference database (Pruesse et al., 2007). Based on this alignment, uncorrected pairwise distances were calculated and used to cluster sequences into OTUs using a distance limit of 0.01 (=99% similarity).

2.6. Accession numbers

Pyrosequencing data and 18S rRNA gene clone sequences were submitted to the European Nucleotide Archive (ENA) with the accession numbers PRJEB11385 (http://www.ebi.ac.uk/ena/data/view/PRJEB11385) and LN897713 – LN898092 (http://www.ebi.ac.uk/ena/data/view/LN897713-LN898092), respectively.

3. Results and discussion

3.1. Bacterial communities in floor-drains

The raw dataset contained 42,914 reads of which 23,515 (54.8%) remained after quality control and were clustered into 680 OTUs. Regardless of the sampling location, species richness and richness estimators were higher in DB than in DW samples (Table S1). In addition, both coverage (Table S1) and rarefaction curves (Fig. S1) indicated that the sequencing approach led to an adequate coverage of the bacterial diversity.

Venn diagrams were generated to evaluate the distribution of OTUs among the different FD samples, revealing largely distinct bacterial communities within each of the analysed FDs (Fig. S2). All DW communities shared only four OTUs (OTUs 3, 11, 16, 27). Similarly, only seven OTUs were common to all DB samples (OTUs 7, 11, 14, 16, 21, 61, 98). Only moderate overlap was found between the DW and DB communities within the same FD. Comparisons between bacterial communities on community level largely confirmed the results from the Venn diagrams revealing moderate similarity between DW and DB communities (Fig. S3). These results strongly suggest the presence of specific indigenous bacterial communities within each FD in certain compartments in this dairy plant.

The sequencing reads were affiliated to eight phyla; the most abundant phylum in all DW and DB samples was *Proteobacteria* (relative abundance 54.0% and 55.1%, respectively) followed by *Firmicutes* (DW: 31.2%; DB: 26.3%). *Bacteroidetes* showed a relative abundance of 4.6% in DW and 12.0% in DB, while *Fusobacteria* had relative abundances of 9.7% (DW) and 4.8% (DB) (Fig. 1). Of note,