Geissler, A. J., Behr, J., Schmid, J., Zehe, A. and Vogel, R. F.

Beer Spoilage Ability of Lactic Acid Bacteria is a Plasmid-Encoded Trait

Beer spoilage ability of lactic acid bacteria (LAB) is known to be a strain-specific trait, while some plasmid-encoded genes were identified in the past 15 years, which are considered to mediate hop tolerance and beer spoilage potential. By comparing up to 114 LAB genomes of brewery and non-brewery isolates, comprising well characterized beer spoiling and non-spoiling strains of the most hazardous LAB species, we investigated the role of plasmids for LAB beer spoilage ability in the context of experimental data. We identified a brewery-specific, plasmid-encoded and highly homologous (between species and strains) shared gene pool. This gene pool encodes for the already known hop tolerance genes *horA* and *horC*, but it is also enriched in genes related to cell envelope metabolism and modification, cation homeostasis, oxidative stress tolerance and pH homeostasis. While most of these genes are suggested to have an auxiliary role for beer spoilage potential, there are others, which are obligatory for LAB growth in beer. These genes include promising diagnostic marker genes for the targeted differentiation of beer-spoiling and non-spoiling LAB.

Descriptors: beer spoilage, lactic acid bacteria, genomics, diagnostic marker genes, plasmidome, genomic plasticity

1 Introduction

Beer is a pleasure for the consumer, a lucrative product for the brewing industry but also a potential ecological niche for bacterial growth. Brewers aim to produce a steady and tasty product, which ties the consumers to their brand. In consequence, any change in quality or sensory appearance of the product, potentially caused by bacterial growth and metabolism, is harmful for the brand and thus for business. Therefore, brewers rely on a reliable quality control for the differentiation of harmful and harmless contaminations in order to prevent a potential economic damage.

Compared to other food producers, brewers are less concerned about the spoilage of their product. This is because beer is a comparatively stable and safe beverage, while the present antibacterial hurdles prevent growth of the vast majority of bacteria, including foodborne pathogens. Besides brewing technological hurdles, bacteria have to cope with several antibacterial properties of beer itself, including the presence of ethanol, hop bitter compounds, a low pH, elevated carbon dioxide levels and a low oxygen content [65, 77]. In addition, beer is a selective nutrient environment for bacterial growth [28, 65, 77]. However, hops or namely the iso-α-acids, which are formed upon heat induced oxidation during wort boiling, are considered as the major antibacterial hurdle for lactic acid bacteria (LAB), being the most important contaminants in the final product [51, 77]. Iso-α-acids have been shown to have two modes of action. On the one hand, they act as protonophores, dissipating the transmembrane proton motive force (pmf) [12, 58], thus basically generating intracellular acid stress, as known for small organic acids such as acetic and lactic acid. On the other hand, it was shown that iso-α-acids participate in cation-dependent (principally shown for Mn^{2+}/Mn^{3+}) transmembrane redox reactions, causing oxidative stress in the cell, while this mode of action has been shown to be of superior significance for the antibacterial effect of hops regarding *Lactobacillus brevis* [13, 56]. Finally, it is important to mention the relation of the pH to the impact of hops on LAB, while the antibacterial properties of iso-α-acids increase dramatically with a decreasing pH (about 50 % with a decrease of 0.2 pH units) [58, 59].

Despite the harsh conditions found in beer, specialized microbes are capable of growing in beer and consequently cause spoilage, while the vast majority of spoilage incidents (up to 90 % from 1980 to 2002) is caused by LAB from the genera *Lactobacillus (L.)* and *Pediococcus (P.)* [5, 6, 65]. LAB species can be classified according to their general hazard potential, which serves as a measure for the significance of a species for brewing microbiology and is based on spoilage statistics, literature and experience of *Hutzler, M.*, et al. [34] and *Back, W.* [6]. Species with a very high hazard potential include *L. backii*, *L. paracollinoides*, *L. collinoides*, *L. brevis*, *L. lindneri* and *P. damnosus*, while the latter three are considered as the major beer spoilers [34, 65]. High hazard potential LAB include *L. acetotolerans*, *L. parabuchneri*, *L. buchneri*, *L. paracasei*, *L. casei*, *L. coryniformis*, *L. perolens*, *L. plantarum*, *L. rossiae*, *P. claussenii* and *P. inopinatus*. Consequently, the majority of LAB species has a low or mostly no hazard potential. While there is obviously some connection to the species level, the actual ability to grow in beer may vary significantly within a species, as beer spoilage ability is a mostly strain specific trait [68]. However, the growth of beer spoiling LAB causes unpleasant sensory effects, including acidification, sedimentation, haze, turbidity, off-flavors, biogenic

Authors

Andreas J. Geissler, Jonas Schmid, Anja Zehe, Rudi F. Vogel, a Technische Universität München, Lehrstuhl für Technische Mikrobiologie, Freising, Germany; Jürgen Behr, Technische Universität München, Bavarian Center for Biomolecular Mass Spectrometry, Freising, Germany; corresponding author: rudi.vogel@wzw.tum.de

amine production and ropiness (slime formation), depending on species and strains [28, 37, 64].

How did these specific LAB adapt to the brewery environment and especially to growth in beer? One general possibility to adapt is to change the phenotype as a response to beer and/or hops. This phenotypic plasticity, reflected by the regulated adaptation to changing environmental conditions, is important for the lifestyle of beer-spoiling LAB in beer [8, 11, 15, 50, 68]. Phenotypic changes were found to affect morphological and metabolic traits, mostly related to hop tolerance, acid tolerance, oxidative stress and cation homeostasis as summarized by *Suzuki, K.* [65], while two examples are given below. A morphological shift was observed for *L. brevis* exposed to hops, resulting in a strong enrichment of higher-molecular-weight lipoteichoic acids (LTAs). LTAs are considered to improve the cell wall barrier function, but also hypothesized to be a reservoir for divalent cations because of their polyanionic properties. LTAs have a potential to bind cations and thus compete for them with hop bitter acids. As hops require Mn^{2+} to exert their full antibacterial properties, the LTA mediated Mn^{2+} homeostasis can be considered as important hop tolerance mechanism [8, 56]. The same species was shown to increase the usage of the arginine deiminase (ADI) pathway as a metabolic response to growth under hop stress [8, 11]. This contributes to an improved energy generation and pH homeostasis (alkalization) by the production of ATP and NH3, respectively [45, 82]. In addition to phenotypic plasticity, bacteria adapt to changing and selective environments by genomic plasticity (adaptive evolution) [18]. Mobile genetic elements, including plasmids and transposons, which are referred to as "the flexible gene pool," can be shared within an environment and contribute to adaptive evolution by horizontal gene transfer (HGT), thus allowing bacterial "evolution in quantum leaps" [18, 22, 30, 72, 79]. The flexible or shared gene pool consists of mobile genetic elements, which might confer a selective advantage. Genomic plasticity, more precisely the uptake of hop resistance genes such as *horA* and *horC* by HGT was already suggested to be responsible for the emergence/origin of beer-spoiling LAB, transforming originally non-spoiling strains into harmful contaminants [65]. Hop tolerance genes were found to be organized in conserved, highly homologous interspecies genetic clusters and have been proposed to encode for multidrug resistance (MDR) transporters, *HorA* as an ABC type MDR transporter and *HorC* as a pmf-dependent MDR transporter. Both are suggested to act as hop efflux transporters. In consequence, a reduced net influx of undissociated and membrane-permeable hop bitter acids is proposed, limiting the antibacterial effect on the cell [54, 65, 66]. The corresponding genes, *horA* and *horC*, are prominent diagnostic marker genes (DMGs) for the species independent identification of beer-spoiling LAB, as the presence of these plasmid-encoded genes has shown a good correlation to beer spoilage ability of various very high hazard LAB species [29, 68].

Based on the first comprehensive genome analyses of brewery LAB, we investigated if beer-spoiling LAB are characterized by a common lifestyle and by shared strategies of adaption to beer. As a consequence, we evaluated, if their adaption to beer results from brewery- and beer-spoilage specific genome properties and genes, which mediate beer-spoilage ability and support the organism to counteract and handle the present antibacterial hurdles. Finally, we evaluated if the respective genes could be potential useful DMGs or even lifestyle genes for the targeted differentiation (quality control) of harmful and harmless brewery contaminations [25]. We sequenced 20 and analyzed up to 114 genomes of LAB strains with very high, high and no hazard potential, brewery isolates and other isolates as well as beer-spoiling and non-spoiling strains. The genomic data and comparisons are discussed in the context of previously published physiological, metabolic and genetic data [10, 25, 28] and with a focus on very high hazard potential LAB (*L. backii*, *L. paracollinoides*, *L. brevis*, *L. lindneri* and *P. damnosus*) and *P. claussenii*, representing the most important beer spoiling LAB species.

2 Material and methods

2.1 Microorganism, media and culture conditions

Supplementary table 1 (the table is added after the article only in PDF format) lists all 94 physiologically characterized strains, alternative identifiers, isolation sources, hazard potential, beer spoilage potential and beer spoilage ability. Strains were cultivated using NBB-Agar, NBB-Bouillon (Döhler, Darmstadt, Germany) and a modified MRS ($mMRS_1$) [55].

$mMRS_1$: 10 g/l peptone, 5 g/l yeast extract, 5 g/l meat extract, 4 g/l K_2HPO_4, 2.6 g/l KH_2PO_4, 3 g/l NH_4Cl, 1 g/l Tween80, 0.5 g/l cysteine-HCl, 10 g/l maltose, 5 g/l glucose, 5 g/l fructose, 0.2 g/l $MgSO_4 \cdot 7H_20$, 0.038 g/l $MnSO_4 \cdot H_2O$

All incubations were carried out at 25 °C under oxygen-reduced conditions. Upon receipt and before storage at –80 °C in 40 vol. % glycerol, all isolates were propagated three times on $mMRS_1$ using single colony transfer, in order to separate possibly mixed cultures. All isolates were checked using Matrix-Assisted-Laser-Desorption-Ionization-Time-Of-Flight Mass Spectrometry, which is a reliable tool for species identification of brewery environment bacteria [42]. The term $mMRS_2$ refers to a MRS variant with the following alterations with respect to $mMRS_1$: No L-cysteine hydrochloride, pH 4.3, 0.16 mg/l $MnSO_4 * H_2O$, 98 mg/l MgSO4 * $7H_2O$ (as found in beer, personal communication). Unless noted otherwise, $mMRS_1$ liquid precultures were used in order to obtain cell suspensions for all kind of experiments. 1.8 ml $mMRS_1$ pH 6.2 was inoculated with a single colony (= biological replicate) and incubated for 4 days at 25 °C before use. These precultures are subsequently referred to as standard precultures [25].

Twelve different beers (Table 1), varying in source, brewing style, pH and hop content were used. All beers were degassed and sterile filtered (0.2 µM, Rapid-Flow Filters, Thermo Scientific, Waltham, USA). Additional analytical data (e.g. ion contents, sugar contents) for the beers of brewery 1 can be found in *Geissler, A. J.* [25].

2.2 Evaluation of beer spoilage potential

Beer spoilage potential was assessed as described previously [10, 28]. Strains were tested using a beer spoilage test as described by Suzuki, K., et al. [67], with modifications. 10 ml of lager beer 1 with elevated pH (= $lager_{pH5.0}$, pH 5.0 adjusted with 6 M NaOH)

Table 1 **Beers used for growth experiments. ID-Numbers (1 to 4) refer to different breweries, e.g. wheat beer 1 and pilsner beer 1 were produced by brewery 1. Some basic parameters are given, including the fermentation type, special properties (e.g. organic production), the gravity of the wort used for the respective beer, the alcohol (ethanol) content, the pH and the international bitterness units (IBUs) as a measure for the hop content**

ID	Fermentation type	Special	Gravity (wt. %)	Alcohol (v/v %)	pH	IBU
Wheat beer 1	top fermented	Kristall	12.5	5.5	4.4	14
Wheat beer 2	top fermented		12.7	5.3	4.2	13
Wheat beer 3	top fermented	organic	12.4	5.4	4.4	11
Wheat beer 4	top fermented		12.4	5.4	4.3	12
Lager beer 1	bottom fermented		11.5	5.1	4.3	18
Lager beer 2	bottom fermented		11.7	4.9	4.3	22
Lager beer 3	bottom fermented	organic	12.0	4.8	4.5	21
Lager beer 4	bottom fermented		11.6	4.9	4.6	17
Pilsner beer 1	bottom fermented		11.5	5.1	4.4	29
Pilsner beer 2	bottom fermented		11.9	4.9	4.5	33
Pilsner beer 3	bottom fermented	organic	11.2	4.7	4.5	27
Pilsner beer 4	bottom fermented		11.3	4.9	4.4	28

were inoculated with 2 % of a standard preculture and incubated at 25 °C until visible growth was observed or maximally 14 days, respectively. After visible growth in lager$_{pH5.0}$, the total cell count (hemocytometer) was determined and test beers (wheat, lager, pilsner, 10 ml) were inoculated with approximately 5 x 10^3 cells/ml (final concentration). The inoculated test beers were incubated at 25 °C and examined every second day for visible growth. After 60 days, the OD_{590} and the pH were determined, while the rest of selected samples was frozen at –20 °C for HPLC analysis of bacterial metabolism [28]. The test was performed with three biological replicates; controls contained test beers without inoculation. All strains were characterized with this beer spoilage test and assigned to the respective beer spoilage potential (BSP) group based on the following rules: strong BSP (SB) – growth in pilsner 1, intermediate BSP (IB) – growth in lager beer 1, weak BSP (WB) – growth in wheat beer 1, no BSP (NB) – no growth in test beers. According to Suzuki, K., *Iijima, K.*, *Ozaki, K.* and *Yamashita, H.* [67] strains were additionally assigned to two groups based on the ability to grow in lager beer, which will be referred to as beer-spoiling strains (beer spoilage ability). In case of physiologically variable (instable) strains, tests were performed repeatedly in order to determine the range of beer spoilage potential /ability (e.g. WB-IB) and an average beer spoilage potential. For a selection of strains of each investigated species, we performed the same test with the beers of three different breweries in order to evaluate the transferability (validity for other beers) of test results.

2.3 Genome sequences

Whole genome sequencing strategies and all downstream bioinformatics, up to our publically available genomes were described previously [9, 10, 26, 27]. Additional genomes were obtained from NCBI GenBank [16, 19]. All genomes are listed together with assembly level, accession number and source in supplementary table 2 (the table is added after the article only in PDF format), as well as all accessions of our own genomes. The selection of additional genomes was primarily based on the availability of complete genomes in August 2015 and the following criteria: For the genera *Lactobacillus* and *Pediococcus* we retrieved all genomes with assembly level complete and chromosome, with three exceptions. Genomes with lower assembly levels (*contig, scaffold*) were included for *L. sanfranciscensis* TMW 1.1304, *L. rossiae* DSM 15814 and *L. coryniformis* because of their potential relevance for brewing microbiology or the close relationship to a focused very high hazard potential species, respectively. Finally, the genomes of *Leuconostoc mesenteroides* ATCC8293 and *Lactococcus lactis* IL 1403 were included because of their potential relevance for beer spoiling [34]. Genbank files were converted to fasta files using genbank_to_fasta.py (Lee Bergstrand). All genomes were submitted to RAST (Rapid Annotations using Subsystems Technology) in order to obtain consistently annotated genomes for proper comparison, using default settings: classic RAST, RAST as gene caller, automatically fix errors, backfill gaps [4, 48]. No manual curation was performed. Single annotations were probed using NCBI BLASTp [2, 17] in cases of relevance for specific analyses. Additionally, our own genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline in order to get locus tags and valid accession numbers [3].

2.4 Genome analysis and comparative genomics

2.4.1 Extraction of genomic properties

All bioinformatics analyses were carried out as described previously [10, 25–27]. General genomic properties (GC content, coding density etc.) were extracted using in-house Bash tools, CMG biotools [74] and Psortb for subcellular localization [80]. The number and diversity of insertion elements/transposons was determined with ISfinder [57].

2.4.2 Phylogenetic and phylogenomic analysis

Phylogenetic and phylogenomic trees were constructed based on sequence comparisons of the 16S rDNA and other genetic markers (23S rDNA, *rpoA*, *rpoB* and *recA*), fragmented all-against-all comparison, codon usage, amino acid usage, proteome comparison

and Pancore-analysis. Genetic marker sequences were extracted from uniform RAST annotated genomes, using an in house bash tool. Sequences were aligned with ClustalW and clustered using Splits Tree, applying the UPGMA method [20, 33, 43, 71]. Phylogenomic analyses of chromosomes and plasmidomes were done by fragmented all-against-all alignments using Gegenees with standard settings [1]. Data were exported as nexus files and clustered with Splits Tree, applying the UPGMA method [20, 33]. Marker alignment and phylogenomics based trees were completed with TreeGraph2 [46, 62]. Codon usage and amino acid usage were calculated with CMG-Biotools, followed by heatmap construction and hierarchical clustering (UPGMA) using the R gplots package [74, 78]. Pangenome trees were also calculated with CMG-Biotools, applying standard settings [61, 74].

2.4.3 Pan, core and accessory genomes

Pan, core and accessory genomes on protein level were calculated using CMG-Biotools and BADGE, in each case applying a 50/50 cutoff. Proteins were considered to be in the same family, if 50 % of the alignment was identical and the length of the alignment was more than 50 % of the longest family member sequence [10, 74]. Accessory, strain and group specific genes were also calculated on DNA level using BADGE [10].

2.4.4 Metabolic reconstruction and functional analysis

Functional categorization was performed using the SEED subsystems [4, 48] as well as the cluster of orthologous groups (COG) categories [24, 70], applying the approach of Andreas Leimbach and the corresponding Perl tool cdd2cog. The SEED subsystem analysis allows an assignment of predicted genes to a hierarchical three-level categorization system, ranging from category to subcategory and subsystem. The COG enrichment ends with a classification into 23 categories, with no subdivision. Note that in case of the SEED subsystems a given gene can be assigned to several subsystems. Metabolic capabilities were analyzed using the KEGG mapper [38, 39, 47] and manual BLASTp analysis [2, 17]. The function/annotation of individual genes of interest was evaluated in detail on protein level using the STRING database [60, 69].

2.4.5 Identification of diagnostic marker genes, sequence alignment and primer design

DMGs were identified using BlAst Diagnostic Gene finder (BADGE). Program design, implementation and other information can be found within the corresponding publication and the program manual [10]. If not stated otherwise, BADGE was used with default settings, only changing the minimum DMG occurrence depending on question and number of genomes to be compared. After selection of DMGs for further evaluation (e.g. PCR), the corresponding align.fasta files (containing all identified sequences of a specific DMG) were aligned using Clone Manager 9 (Scientific & Educational Software). After multi-way alignment (exhaustive pairwise alignments), applying the standard linear scoring matrix, a merged consensus sequence was created. Again using Clone Manager 9, DMG specific primer pairs were designed, producing products in a range of 100 to 200 bp. Primers were used for PCR.

2.5 Polymerase chain reaction for the evaluation and validation of predicted diagnostic marker genes

The *Taq* Core Kit 10 (MP Biomedicals, Santa Ana, USA) was used according to the manufacturer. Primers were obtained from MWG Biotech AG (Ebersberg, Germany) and applied with an end concentration of 0.5 µM per reaction. All relevant primers are listed in supplementary table 3 (the table is added after the article only in PDF format), together with their sequence. 1.25 U of *Taq* DNA polymerase was used per reaction. A Mastercycler gradient (Eppendorf AG, Hamburg, Germany) was utilized for thermoprofiles, which were designed according to the "Guidelines for PCR programs" of the *Taq* Core Kit 10. PCR was mostly (after validation of primer functionality) conducted without prior DNA isolation. Therefore, either a single colony was suspended in 200 µl H_2O or, depending on cell density, 0.5 to 2 ml liquid cultures were harvested by centrifugation and suspended in 200 µl H_2O. Finally, 2 µl of these opaque cells suspensions were used as template for PCR. In every case, where PCR was performed without prior DNA isolation, the used cell suspensions were tested for suitability with 16S rDNA PCRs, as controls. Only if these were positive, PCRs with other targets (e.g. *horC*, M05) were accounted as valid. All primers for the amplification of potential DMGs were used at an annealing temperature of 50° C.

PCR products were separated, corresponding to their size, using 2 % gels (agarose in 0.5 x TBE-buffer). Separation was accomplished by applying an electric tension of 100 V in 0.5 x TBE (using an Electrophoresis Power Supply EPS 300, Pharmacia Biotech, Uppsala, Sweden). 6x Loading Dye and different GeneRuler DNA Ladders (Thermo Fisher Scientific, Waltham, USA) were used according to requirements. For visualization, dimidium bromide and an UVT-28 M transilluminator (Herolab, Wiesloch, Germany) were used. Pictures were taken with a CCD camera.

2.6 Statistical quality evaluation of DMGs

The quality of DMGs was tested with Spearman´s rank correlation and Fisher´s exact test, applying Bonferroni-Holm correction [21, 32]. Precision, sensitivity (recall), specifity, accuracy, f-measure, false positive rate and false negative rate as well as the total correct assignments (accuracy) by a specific DMG, were calculated using a confusion matrix and adduced to assess the quality of a given DMG.

3 Results and Discussion

Definitions and terminology for the following sections are given in Box 1. Representatives of all focused LAB species (94 strains) were classified with respect to beer spoilage potential and beer spoilage ability in order to provide a consistent reference data set. Figure 1 (see page 12) shows the basic workflow of the corresponding beer spoilage test as well as the classification scheme. In case of variable strains, tests were performed repeatedly in order to determine the range of beer spoilage potential/ability and an average beer spoilage potential/ability. Supplementary table 1 lists all strains with beer spoilage potential and beer spoilage ability.

For 22 of these characterized strains, most of them isolated from the brewery environment and comprising all focused species, genome sequences were available based on our own sequencing approaches or others [9, 10, 26, 27, 44, 49]. This allowed us to explicitly compare the genomes of beer spoiling and non-spoiling strains, in order to find specific genes, which differentiate these groups and mediate their adaption to beer. These strains were further characterized in detail regarding their metabolism and growth in beer as well as under acid and hop stress [25, 28]. This allowed us to describe the lifestyle of beer-spoiling LAB in beer based on genomic analysis as well as physiological data. The comparison of those genomes to more than 90 other LAB genomes with varying hazard potential, most of them non-brewery isolates, enabled us further to investigate the relation of hazard potential and isolation source to genomic and metabolic properties. More detailed data about all genome analyses and comparisons as well as various physiological data (hop tolerance, ethanol tolerance etc.) can be found in the first author's doctoral thesis [25].

3.1 Chromosomal preconditions and "the core genome of spoilage"

Very high hazard potential LAB species cause the vast majority of all beer spoilage incidents [5, 6, 34, 65]. By comparing the genomes of these species to each other and to those with less or without any relevance for brewing microbiology, we wanted to investigate if hazard potential is defined by the respective species genomes. The first question to be answered was consequently the following: Are the genomes of very high hazard (and high hazard) potential species defined by distinct properties? Does this group of species cluster together based on any parameters and can they be differentiated from those LAB (genomes) without hazard potential, based on these properties? As the chromosome is the actual stable entity of a bacterial genome, we focused on the analysis of chromosomal properties.

Beer-spoiling LAB are not phylogenetically closely related to each other [63, 65]. Accordingly, lactobacilli and pediococci with very high and/or high hazard potential did also not cluster together based on 23S rDNA, *rpoA*, *rpoB* and *recA*. Further, they did not group together based on a phylogenomic analysis of their chromosomes. Neither a proteome based comparison, followed by clustering, nor a pan-genome tree analysis resulted in distinct clusters, correlating to general hazard potential or isolation source. This confirms that these bacteria are not a monophyletic group within the LAB and that their adaption to the niche beer was a rather recent event. Further, LAB with very high and/or high hazard potential could not be related to each other based on genome size, chromosome size, number of proteins, GC content, coding density, codon usage, amino acid usage, proteome similarity, chromosome similarity, functional pattern (SEED, COG) and the proportions of subcellular localizations of proteins (e.g. cell wall, intracellular etc.). To the contrary, these groups include species with completely different genomic preconditions. Exemplarily, they comprise genomes, which belong to the smallest (*L. lindneri*) and to the largest (*L. paracollinoides*) within the lactobacilli, characterized by completely different codon usage and GC contents from 34.3 % (*L. lindneri*) to 47.0 % (*L. paracollinoides*). Regarding general chromosomal properties, very high and/or high hazard potential LAB species cover the whole diversity within the genera *Lactobacillus* and *Pediococcus* [25].

Box 1: The following terms and definitions will be used throughout the results and discussion section and are necessary to follow:

Hazard potential: LAB species are classified according to their general hazard potential for beer. This classification applies to species and was adopted from Hutzler, M., et al. [34]. Species can have a very high or high hazard potential, a positive tendency for beer spoilage and no hazard potential. Species with very high hazard potential also include strains without beer spoilage ability. Definition of a hazard potential serves as a rough measure for the significance of a species for brewing microbiology and is based on spoilage statistics, literature and experience of Hutzler, M., et al. [34] and Back, W. [6]. It is not a measured and clearly defined trait as beer spoilage potential and ability for those strains actually characterized within this study.

Beer spoilage potential: Refers to the ability to grow in beers with increasing antibacterial properties. This property was determined experimentally within this study for all single strains. Thus, it is not a property assigned to a whole species. There are four beer spoilage potential groups, ranging from no beer spoilage potential to strong beer spoilage potential.

Beer spoilage ability: Refers to the ability to grow in (at least) lager beer, representing the average beer regarding antibacterial properties. Includes all strains with intermediate and strong beer spoilage potential. As beer spoilage potential, this property was determined experimentally within this study for all single strains.

Focused species: *L. brevis*, *L. backii*, *L. paracollinoides*, *L. lindneri*, *P. claussenii* and *P. damnosus*. All species regarded to have a very high hazard potential and *P. claussenii*. Focused within this study.

Plasmidome: Totality of plasmids derived from a single strain/genome.

Brewery strain/genome/plasmid/plasmidome: Refers to the isolation source of the corresponding strain, genome etc. Will be used to avoid inconvenient phrases. Consequently, all other will be referred as "other" or "non-brewery" entities.

Diagnostic marker gene (DMG): A gene, capable of differentiating two strains or groups.

Lifestyle: The lifestyle composes from properties, strategies and capabilities of LAB, a species or a strain, which are related to the successful growth in beer and/or can be associated with an enhanced tolerance to beer specific hurdles, thus conferring a potential advantage.

Lifestyle gene: A DMG, where a connection to the lifestyle of a particular bacterium within a given environment is established. E.g. based on physiology or metabolism.

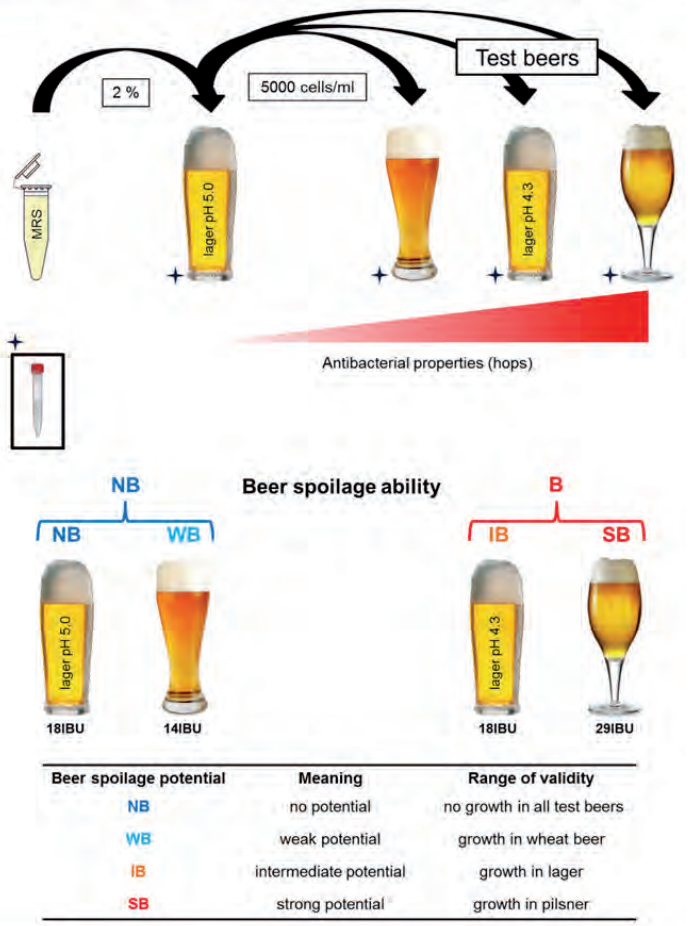

Fig. 1 **Illustration of beer spoilage test - workflow and classification-rules. After cell transfer from mMRS$_1$ to lager beer with an elevated pH of 5.0, cells can adapt to the beer environment at reduced antibacterial properties. Test beers with increasing antibacterial properties, mainly determined by hop content, are inoculated with a low number of cells in order to mimic realistic conditions. Based on their growth behaviour, strains are classified with respect to beer spoilage ability and beer spoilage potential, as shown at the bottom of the figure. IBU = International bitter units (measure for hop content)**

Although LAB with very high and/or high hazard potential share a common chromosomal core genome, we found these sequence based core genomes to be not specific for these groups, as they were covered even to 100 % by other LAB species (e.g. *L. hokkaidonensis*, *L. koreensis*, *L. pentosus* and *L. rhamnosus*) without any documented significance for brewing microbiology. Consequently, the calculated core genomes do not contain any gene, which is specific or unique for the corresponding group, as any included gene was also found within other genomes. Further, these core genomes are characterized by genes almost exclusively related to housekeeping functions, e.g. translation, transcription and central carbon metabolism, making it unlikely that these genes represent those specific traits, which are mandatory for growth in beer [25]. Thus, the chromosomal core genome of very high and/or high hazard potential is not decisive for hazard potential. This is also illustrated by the chromosomally encoded metabolic capabilities of these bacteria. Very high hazard potential LAB include homofermentative and heterofermentative species, some of which are characterized by completely different carbohydrate utilization systems, including metabolically very versatile bacteria (*L. brevis*) and metabolically restricted species (*L. lindneri*), while these differences were also confirmed by HPLC data [25, 28]. *L. paracollinoides* and *L. backii* seem to be prototroph for 19 to 20 amino acids, while *L. brevis* or *L. lindneri* are auxotroph for most amino acids. *L. brevis* encodes for amino acid decarboxylation (glutamate, tyrosine) systems, the ADI and the AGDI system and consequently produces various biogenic amines, which has been confirmed by HPLC analysis [25, 28]. These systems contribute to *L. brevis* energy generation and pH homeostasis. In contrast, *P. damnosus* does neither encode for any of these systems nor did we observe any biogenic amine production. There are various other examples, regarding vitamin biosynthesis, nucleotide metabolism and other metabolic pathways, which emphasize that there is apparently no distinct, shared and chromosomally encoded metabolic foundation or strategy, which is obligatory for LAB to grow in beer [25]. This is in accordance with a previous study, which also came to the conclusion that the "(chromosomal) core genome" of *L. brevis* BSO 464 plays at best a subordinate role for hop tolerance and beer spoilage ability [14]. It is also in tune with our previous metabolic investigations, where we found that the metabolic capabilities measured by HPLC of LAB species with very high hazard potential and *P. claussenii*, regarding carbohydrate, amino acid and organic acid metabolism, did not correlate to beer spoilage potential or beer spoilage ability, but to fermentation type (homofermentative/ heterofermentative) and species. To be exact, this means that we did not find a specific metabolic trait or pathway, related to the abovementioned substance classes, which is obligatory for beer-spoiling LAB or which differentiates beer-spoiling from non-spoiling strains within a species. The corresponding chromosomally encoded metabolic capabilities were almost exclusively found to be conserved within a species [25, 28].

We conclude that very high hazard potential LAB of different species do not share conserved and determinant chromosomal properties; however, these organisms may be characterized by species-specific chromosomal metabolic strategies to support their growth in beer, as discussed later. This also means that very high hazard potential LAB do not form a distinct group based on their chromosomal preconditions and that there are different metabolic strategies to facilitate growth in and adaption to beer. At the same time, we found the abovementioned chromosomal properties to be highly conserved within the individual species, independent of isolation source and beer spoilage potential, which shows that the chromosomal preconditions are also not decisive with respect to beer spoilage potential and ability [25].

3.2 The role of mobile DNA and genomic plasticity

If hazard potential is not defined by chromosomal properties, where are the predicted similarities, which mediate beer spoilage potential? To answer the question if this adaption is mainly a plasmid-encoded, dynamic and therefore unstable trait, we investigated the role of mobile genetic elements for the adaption of brewery isolates to beer and the brewery environment. A first analysis of the available plasmidomes resulted in a significant species-independent correlation for the quantity of plasmids to hazard potential ($\rho = 0.58$), while the correlation to isolation source brewery ($\rho = 0.63$) was even higher. Figure 2 shows the number of plasmids found for the

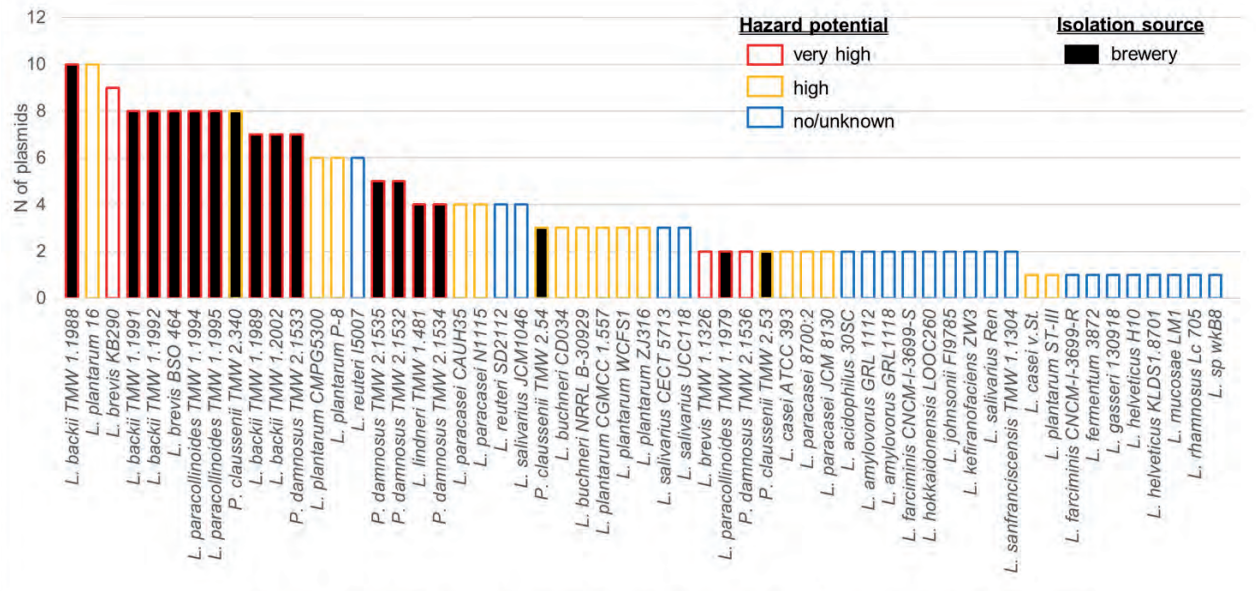

Fig. 2 **Number of plasmids in context of hazard potential and isolation source. The number of plasmids is shown for all complete genomes. Most brewery isolates carry four or more plasmids. *L. casei* v.St. = various strains (7) of *L. casei* containing one plasmid. Strains without plasmids are not included**

included genomes in descending order, labeled according to the respective species hazard potential and the strain´s isolation source. Within individual species, as exemplary shown for *P. damnosus* in figure 3, we further found that the size of the plasmidome not only correlated to the isolation source, but also to the beer spoilage potential [10, 25]. As a consequence, we focused on the analysis of brewery plasmidomes.

In general, brewery isolates where found to have up to ten plasmids, ranging in size from less than 5 kbp to 144 kbp. The respective plasmidomes represent up to 10.6 % of their genomes. Within the genera *Lactobacillus* and *Pediococcus*, we could only find a higher ratio of plasmidome to genome size for strains of *Lactobacillus salivarius*, while *L. salivarius* plasmids are considered to contribute to niche adaption, in this case the mammalian gastrointestinal tract [10, 53]. In total, we found 105 brewery plasmids encoding for 3,381 genes (RAST), while 19 pairs of nearly identical plasmids (95 % identity, 90 % coverage) were found, ending up with 86 individual plasmids. With three exceptions, all plasmid pairs were found within a single species. Brewery plasmids comprise GC contents from 34.6 to 46.6 % and consequently do not show a consistent codon or amino acid usage. Nevertheless, based on codon usage and a fragmented all-vs-all alignment of their plasmidomes, (almost all, codon usage) all brewery isolates cluster together, as illustrated by figure 4 (see page 14). In addition, the respective cluster includes not only all brewery isolates, but also all beer spoiling strains with very high hazard potential. In contrast, non-brewery and non-spoiling isolates of the same species are found outside of the respective cluster. This strongly indicates that beer spoilage ability is more related to isolation source and plasmidome, than to species and chromosome [25].

Where does the similarity of these brewery plasmidomes come from, as it cannot be solely explained by those 19 nearly identical plasmids, shared by only a few strains analyzed? We investigated the distribution and similarity of brewery plasmidome encoded genes, their occurrence in non-brewery isolates and if they are part of conserved genetic clusters (compare to *horA*, *horC* clusters). This was also done to differentiate strain specific genes from niche (brewery, beer) specific genes, in order to extract the part of the brewery plasmidomes, which might be of high relevance for their adaption to the niche. Therefore, we used BADGE, looking specifically for highly similar, homologous, shared DNA. Threshold values for homologous genes were set to 90 % sequence identity and 90 % subject-to-query/query-to-subject coverage. In order to compare the degree of shared plasmidome information within a species and between species, a shared gene pool was defined for each species and each pair of species. A gene was considered part of this pool, if it occurred twice within a species, or at least twice within a pair of species (at least one strain of each species). The analysis revealed a shared gene pool of highly homologous (mostly 99 to 100 %, see Figure 5, see page 15), mostly brewery-specific genes, in various cases organized as genetic clusters and flanked or associated with transposases and mobile element proteins. Of 3,381 brewery plasmid-encoded genes, 589 were found to be present in at least

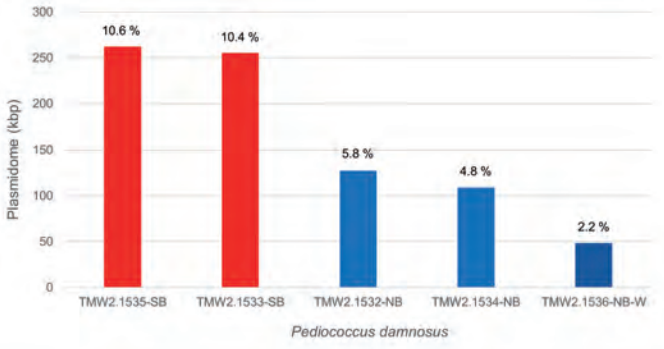

Fig. 3 **Cumulative size of the plasmidomes of *P. damnosus* genomes. The colour code illustrates the beer spoilage potential (SB = strong beer spoilage potential, NB = no beer spoilage potential) and the isolation source in case of the winery isolate (W). The proportions (%) on top of the bars are the calculated proportions of the plasmidome to the whole genome**

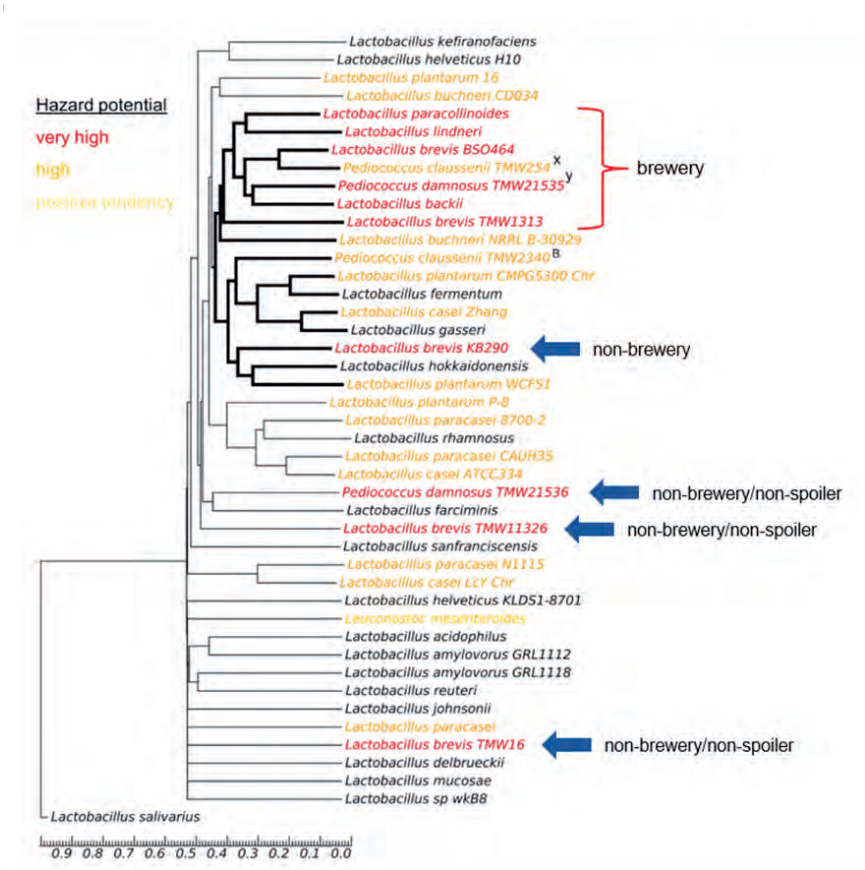

Fig. 4 Similarity tree of lactic acid bacteria plasmidomes, based on a fragmented all-against-all comparison (Gegenees). If all strains of a species clustered together, they were condensed to the respective species. Elsewhere strain designations were kept for all or for representative strains (see x and y). The bold subtree contains all brewery isolates, while the included upper subordinate subtree contains all brewery isolates, with the exception of *P. claussenii* TMW 2.340-IB. *L. buchneri* NRRL B30929 was isolated from an ethanol production plant. Note that all non-brewery strains of the very high hazard potential species (*L. brevis* KB 290, TMW 1.1326-NB and TMW 1.6-WB, *P. damnosus* TMW 2.1536-NB) do cluster elsewhere based on their plasmidome sequences. x = represents TMW 2.54-SB and TMW 2.53-SB, y = all brewery isolates of *P. damnosus*

two brewery plasmidomes. Regarding these 589 genes, we found 543 of them to be shared by those 19 (nearly) identical plasmid pairs mentioned above, while only 25 % of these genes were found exclusively within these plasmids. The other 75 % are encoded in at least one more brewery plasmidome, showing that the shared gene content of brewery plasmidomes and the similarity of brewery plasmidomes (see Figure 4) are not only based on the presence of identical plasmids. It also indicates that a distinct proportion of brewery DNA might be transferred transposon-mediated, as suggested by Suzuki, K. [65]. This is supported by the fact that we found various shared conserved clusters to be encoded on otherwise different plasmids [25]. Figure 6 illustrates that the shared gene pool within a species is always the largest. While 41 % of all shared genes are only present within a single species, the rest was found in at least two species, including 17 genes with occurrence in all six focused species. About one quarter (27 %, 160 genes) of all shared genes was also found within the analyzed non-brewery plasmids, thus being not brewery-specific. Note that these other plasmids include those of non-brewery *L. brevis* and *P. damnosus* strains, as well as 164 additional plasmids comprising 19 other LAB species. These encode in total for more than 5,400 genes. Within brewery isolates and individual species, beer-spoiling strains were further found to share more genes with each other as with non-spoiling strains, while several beer-spoiling strains of different species share even more genes with each other as with non-spoiling strains of the same species (see Figure 7, see page 16). For example, both beer-spoiling strains of *P. damnosus* share 84 +/– 10 genes with the beer-spoiling *L. backii* strains, 61 +/– 5 with the non-spoiling brewery isolates of *P. damnosus* and only 18 +/– 2 with winery isolate *P. damnosus* TMW 2.1536NB. Thus, the number of shared homologous genes is apparently related to niche adaption and beer spoilage potential, which is also illustrated by figure 8 (see page 17) [25].

Various shared genes within the pool were found to be partially or exclusively encoded within contiguous pieces of DNA, while figure 9 (see page 17) shows the distribution of the identified genetic clusters. *HorA* and *horC*, as well as their corresponding genetic clusters, have the highest prevalence. As they are considered to confer active hop tolerance, their high prevalence makes sense if we consider hop efflux transporters as a first defense line against hops [75]. In case of *horC*, we found two general cluster variants, while the truncated variant consists of *horC* and *horB* only. Further, we identified 16 additional clusters, some of them found within various species and others restricted to single species or groups [25]. Beer-spoiling *L. backii* and *P. damnosus* strains were found to encode for a complete plasmid-encoded fatty acid biosynthesis (FAS) cluster. Both species lack a chromosomal encoded FAS, while we could clearly demonstrate in a previous study that only strains harboring the FAS cluster are able to grow in beer [10], which is a beverage characterized by a very low content of long chained fatty acids (C12 to C18) [51]. Interestingly, all brewery plasmidomes of *P. damnosus*, as well as four out of five plasmidomes of *L. backii* carry one of two identified clusters (clusters 8/15, see Figure 9) containing genes for the uptake and utilization of biotin. Biotin is required as a covalently attached cofactor of the FAS-initiating enzyme acetyl-CoA carboxylase [81]. Cluster 8 in addition encodes for a cyclopropane-fatty-acyl-phospholipid synthase and a fatty acid binding protein of the DegV family. Both clusters were not found within the other core-species plasmidomes [10, 25]. As the other species encode a complete chromosomal FAS, there is no need for an additional plasmid-encoded FAS cluster. This indicates that brewery LAB do not just randomly take up mobile DNA or plasmids, but specifically take what they need from the brewery-specific flexible and shared gene pool [25].

What other functions and properties are encoded by brewery plasmidomes, the identified clusters and other shared genes? Functional analysis of brewery plasmidomes revealed an enrichment of certain functional categories, while the vast majority of all plasmid-encoded/

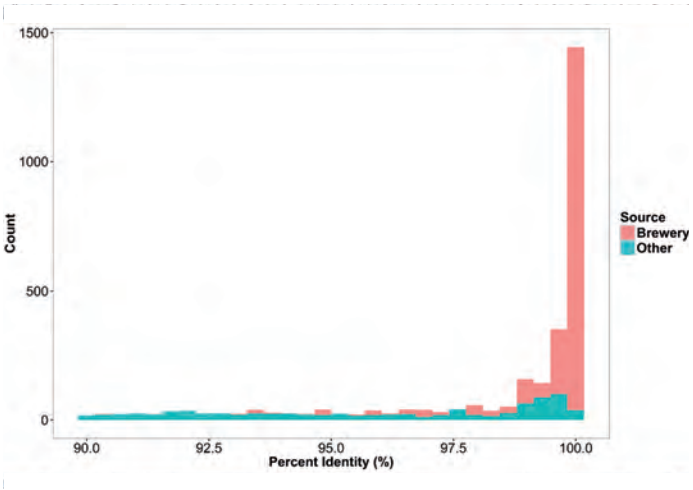

Fig. 5 **Sequence similarity (homology) within brewery plasmid shared gene pool. The histogram shows the total count of BLAST hits versus sequence similarity (percent identity) of these matches. We found 589 genes shared by at least two brewery plasmidomes. The BADGE settings for the identification of these 589 genes were set to 90 % sequence identity and 90 % subject-to-query/query-to-subject coverage. All 589 genes were blasted against all brewery plasmids and against all non-brewery plasmids. The red histogram shows the similarity of these 589 shared genes within the brewery plasmidomes, from minimum 90 % (Identity threshold) to 100 %. The blue histogram shows the sequence similarities of those 160 genes, which were also identified in non-brewery plasmids, but only those similarities to non-brewery plasmid genes**

shared genes encodes for either hypothetical proteins, or products with a non-clearly defined or non-informative biological function. Figure 10 (see page 18) highlights the most abundant genes and functions found on these brewery plasmids in a wordcloud. Not all of these genes and functions are actually brewery-specific, while many of them are still interesting, as they are noticeable abundant. However, while most of the functions, for example FAS, are not at all specific for the brewery environment, many genes and clusters are specific for the brewery environment on DNA sequence level. Thus they are perfect potential DMGs for molecular quality control. Based on functional analysis (SEED, COG, data not shown, see [25]) and the analysis of all shared genes and clusters, we further found some global biological functions or categories to be enriched within brewery and beer-spoiling plasmidomes. These biological functions include genes for: metabolic traits related to alternative substrates and pH homeostasis, cell envelope metabolism and modification, ion homeostasis and oxidative stress tolerance. This is quite interesting, as all these traits can be in general connected to beer-typical hurdles (especially hops), thus potentially representing an advantage for LAB adaption to beer [25].

Metabolic traits: The ability to produce the unwanted off-flavor diacetyl (indirectly) from pyruvate is a plasmid-encoded trait in case of *P. damnosus*, while the corresponding α-acetolactate operon (Cluster 1, see Figure 9) was only found in brewery genomes. We suggest that this metabolic capability is not only troubling brewers, but also represents a metabolic advantage for growth in beer [10, 25]. The production of non-acidic end products in an intrinsically acidic environment is an advantage, especially if we consider the increase of hop´s antibacterial properties with a decreasing pH [58, 59]. A conserved cluster for a complete agmatine deiminase pathway (AGDI, Cluster 12 see Figure 9) was found, encoded by the plasmidomes of two *L. paracollinoides* strains and *L. backii* TMW 1.1991-SB. The AGDI pathway allows the production of 2 ATP and 2 NH3 from agmatine, resulting in the biogenic amine putrescine, while agmatine is often found in beer, also as a potential metabolic

	L. backii	*P. damnosus*	*L. paracollinoides*	*P. claussenii*	*L. brevis*	*L. lindneri*	Other plasmids
L. backii	379	177	116	103	97	24	54
P. damnosus	177	300	87	83	72	25	110
L. paracollinoides	116	87	249	91	88	27	120
P. claussenii	103	83	91	183	70	21	98
L. brevis	97	72	88	70	165	23	97
L. lindneri	24	25	27	21	23	36	23

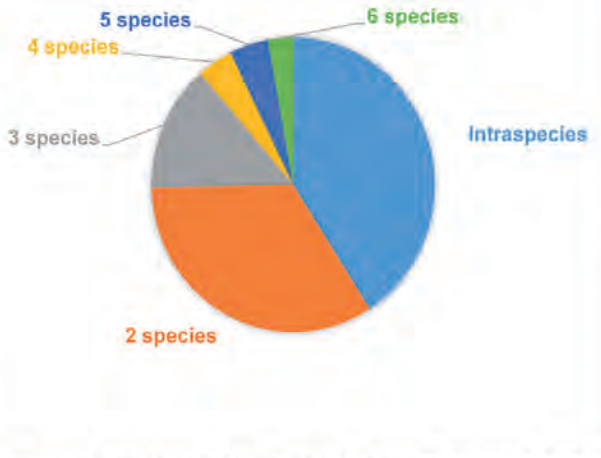

Fig. 6 **The shared gene pool of brewery plasmidomes on species level. Only species were considered, where evident brewery genomes were available. The table on the left side shows the number of shared plasmid-encoded genes between species. Species were summarized, omitting non-brewery strains, which were added to all other non-brewery plasmids. Only those 589 genes are considered, which were found to be encoded by at least two different brewery strains/plasmidomes. A gene was further considered part of the gene pool, if it occurred at least twice within a species, or at least twice within a pair of species. Note that a gene being part of a gene pool does not have to be absent within another species or the other plasmids. This figure is not about exclusively shared genes. A colour code from green (high number of shared genes) to red (low number of shared genes) was applied to each row of the table independently in order to illustrate similarities between distinct species. The pie chart on the right side shows the proportions of shared genes within and between the six species. In detail: Intraspecies: 41 %; two species: 34 %; three species: 14 %; four species: 4 %; five species: 4 %; six species: 3 %**

	L. backii TMW 1.1988-SB	*L. backii* TMW 1.1991-SB	*L. backii* TMW 1.1992-SB	*L. backii* TMW 1.1989-SB	*L. backii* TMW 1.12002-IB	*P. damnosus* TMW 2.1533-SB	*P. damnosus* TMW 2.1535-SB	*P. damnosus* TMW 2.1532-NB	*P. damnosus* TMW 2.1534-NB	*P. damnosus* TMW 2.1536-NB	*P. claussenii* TMW 2.53-SB	*P. claussenii* TMW 2.54-SB	*P. claussenii* TMW 2.340-IB	*L. paracollinoides* TMW 1.1994-SB	*L. paracollinoides* TMW 1.1995-SB	*L. paracollinoides* TMW 1.1997-NB	*L. brevis* BSO464	*L. brevis* TMW 1.313-SB	*L. brevis* KB290	*L. brevis* TMW 1.1326-NB	*L. lindneri* TMW 1.481-IB
L. backii TMW 1.1988-SB		121	118	77	78	88	91	35	36	27	27	33	29	42	48	22	53	13	37	11	17
L. backii TMW 1.1991-SB	121		130	70	73	104	85	42	41	27	34	45	44	55	61	34	66	16	51	13	21
L. backii TMW 1.1992-SB	118	130		77	79	85	86	36	36	25	33	40	49	49	61	26	54	15	48	13	16
L. backii TMW 1.1989-SB	77	70	77		159	67	82	34	35	15	26	29	29	30	46	19	55	16	36	11	14
L. backii TMW 1.2002-IB	78	73	79	159		68	82	34	35	15	25	29	29	31	47	20	58	16	37	11	16
P. damnosus TMW 2.1533-SB	88	104	85	67	68		152	66	56	20	29	37	43	48	54	23	55	13	49	12	23
P. damnosus TMW 2.1535-SB	91	85	86	82	82	152		65	56	16	29	37	24	43	49	21	53	14	35	10	19
P. damnosus TMW 2.1532-NB	35	42	36	34	34	66	65		104	10	12	21	24	25	30	13	28	7	19	8	15
P. damnosus TMW 2.1534-NB	36	41	36	35	35	56	56	104		10	14	23	24	25	32	13	30	6	18	8	15
P. damnosus TMW 2.1536-NB	27	27	25	15	15	20	16	10	10		8	10	11	14	16	12	17	5	35	5	9
P. claussenii TMW 2.53-SB	27	34	33	26	25	29	29	12	14	8		74	4	10	36	9	28	6	10	9	5
P. claussenii TMW 2.54-SB	33	45	40	29	29	37	37	21	23	10	74		36	19	42	16	47	12	16	10	10
P. claussenii TMW 2.340-IB	29	44	49	29	29	43	24	24	24	11	4	36		35	45	24	41	14	37	9	18
L. paracollinoides TMW 1.1994-SB	42	55	49	30	31	48	43	25	25	14	10	19	35		73	45	44	11	35	10	24
L. paracollinoides TMW 1.1995-SB	48	61	61	46	47	54	49	30	32	16	36	42	45	73		65	64	19	39	14	19
L. paracollinoides TMW 1.1997-NB	22	34	26	19	20	23	21	13	13	12	9	16	24	45	65		33	10	27	8	12
L. brevis BSO464	53	66	54	55	58	55	53	28	30	17	28	47	41	44	64	33		28	49	15	22
L. brevis TMW 1.313-SB	13	16	15	16	16	13	14	7	6	5	6	12	14	11	19	10	28		15	3	4
L. brevis KB290	37	51	48	36	37	49	35	19	18	35	10	16	37	35	39	27	49	15		15	13
L. brevis TMW 1.1326-NB	11	13	13	11	11	12	10	8	8	5	9	10	9	10	14	8	15	3	15		6
L. lindneri TMW 1.481-IB	17	21	16	14	16	23	19	15	15	9	5	10	18	24	19	12	22	4	13	6	

Fig. 7 The shared gene pool of brewery plasmidomes on strain level. Only strains of those species were considered, where evident brewery genomes were available. Brewery plasmidomes are coloured yellow. A colour code from green (high number of shared genes) to red (low number of shared genes) was applied to each row of the table independently. This way one can visually assess those strains which share the most genes to a defined / selected strain (row) and which strains share least genes. The red box highlights the fact that both beer-spoiling strains of *P. damnosus* share more genes with the beer-spoiling *L. backii* strains, compared to non-spoiling strains of the same species. Beer spoilage potential (BSP) is shown, where available: SB = strong BSP, IB = intermediate BSP, WB = weak BSP, NB = no BSP

product of yeast metabolism [23, 37]. Agmatine consumption as well as putrescine production were confirmed by HPLC analysis [25] for *L. backii* TMW 1.1991-SB and *L. paracollinoides* TMW 1.1994-SB, while this was not detected for the other strains of both species. *L. paracollinoides* TMW 1.1979-NB, also positive for cluster 12, did not show beer spoilage ability. This indicates that the AGDI pathway has rather an auxiliary function, and, all alone, just like the acetoin pathway, is not sufficient for successful growth in beer [25]. Previous studies already indicated that some plasmids or plasmid-encoded genes are obligatory for beer spoilage ability [14, 68], while others have an auxiliary function [14, 50]. Nevertheless, ATP generation, alkalization of the intracellular space and a no-cost substrate/product antiport make it a very attractive system for LAB growing at a low pH [25, 45, 82].

Cell envelope metabolism and modification: Several shared genes and enriched functions, especially associated with the biosynthesis and modification of teichoic/lipoteichoic acids and peptidoglycan metabolism were identified, while most of these genes were found on distinct genetic clusters such as cluster 2, the *horA* and the *horC* clusters. Suzuki, K., Iijima, K., *Sakamoto, K.*, *Sami, M.* and *Yamashita, H.* [68] already stated that the highly conserved regions flanking *horA* and *horC* might potentially be involved in cell envelope metabolism, while a general relevance of the cell wall and LTAs for hop resistance was already detailed in the introduction. However, while a modification of LTAs has been identified as a specific response of *L. brevis* to acid and hop stress [8], this is hardly a part of *L. lindneri´s* lifestyle in beer, as this species was found to lack cell wall teichoic acids [6, 7]. This lack of teichoic acids is reflected by an apparent lack of genes associated with this trait. We further identified shared genes, which are potentially involved in peptidoglycan recycling and peptidoglycan biosynthesis. Two different N-acetylmuramyl-L-alanine amidases (EC 3.5.1.28) were found, one variant on cluster 2 (see Figure 9), which is encoded by all brewery *P. damnosus* and *L. brevis* BSO 464, and another variant encoded by *L. brevis* TMW 1.313-SB and *L. paracollinoides* TMW 1.1995-SB. These autolysins separate the glycan strand from the peptide, allowing the further degradation/recycling of the stem peptides and the remaining glucans [76]. Recent comparative proteomic research with *L. brevis* TMW 1.465-SB indicated an upregulation of proteins involved in peptidoglycan metabolism under acid and hop stress [11]. However, the actual role of N-acetylmuramyl-L-alanine amidases for growth in beer or hop tolerance remains elusive [25].

Ion transport and homeostasis: With the exception of *L. lindneri*, we found all focused species to harbor plasmid-encoded genes and clusters related to cation transport and homeostasis. Most of these genes were found to be linked to copper homeostasis, namely to the ATP-dependent export of copper ions (CopB). We further identified a brewery-specific DNA sequence encoding a magnesium transporter (CorA). It is located on a distinct cluster together with the already published lifestyle gene *hitA*, which is a potential hop inducible Mn^{2+}/H^{+} symporter [31], and a transcriptional regulator of the TetR family. We found the *hitA* cluster encoded by brewery *L. brevis*, *P. damnosus*, and, for the first time, encoded by *L. backii* and *L. paracollinoides* strains. CorA is considered to be a major ubiquitous Mg^{2+} uptake system, which is involved in Mg^{2+} homeostasis in all kind of bacteria [41]. Interestingly, *Preissler, P.* [52] observed Mg^{2+} uptake and a concomitant release of Mn^{2+} by *hitA*-positive *L. brevis* TMW 1.313-SB grown in pilsner beer. As Mn^{2+} and Mg^{2+} are suggested to be interchangeable with respect to some physiological functions [36], the observed ion homeostasis could be a specific strategy to reduce the antibacterial effects of hops, as additionally the antibacterial properties of hops were found to be less pronounced (compared to Mn^{2+}) [12] or even reduced in the presence of Mg^{2+} [52, 59]. The association of *hitA* and *corA* encoded on a shared cluster, potentially simultaneously regulated, was previously unknown, but indicates that not only manganese

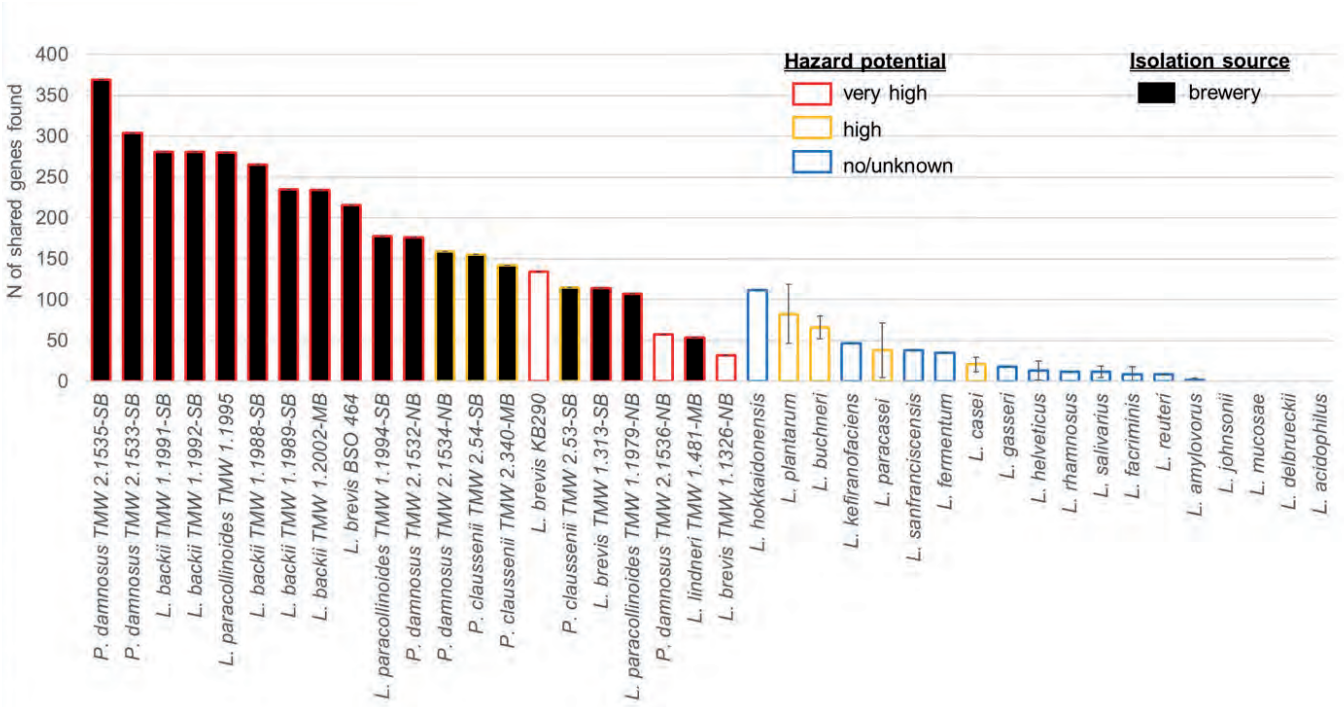

Fig. 8 The relation of hazard potential, isolations source and beer spoilage potential to the shared gene pool of brewery plasmidomes. The number of genes, shared with ("taken from") the shared gene pool is shown for single strains of all relevant species. Species without brewery isolates were summarized, represented by an average value and standard deviation. Only those 589 genes are considered, which are encoded by at least two different brewery plasmidomes. The hazard potential and the isolation source are indicated. Beer spoilage potential (BSP) is shown, where available: strong BSP (SB), intermediate BSP (IB), weak BSP (WB), no BSP (NB)

Cluster	DMG	Function	brewery specific DNA	*L. backii* TMW 1.1988-SB	*L. backii* TMW 1.1991-SB	*L. backii* TMW 1.1992-SB	*L. backii* TMW 1.1989-SB	*L. backii* TMW 1.2002-IB	*P. damnosus* TMW 2.1533-SB	*P. damnosus* TMW 2.1535-SB	*P. damnosus* TMW 2.1532-NB	*P. damnosus* TMW 2.1534-NB	*P. claussenii* TMW 2.53-SB	*P. claussenii* TMW 2.54-SB	*P. claussenii* TMW 2.340-IB	*L. brevis* BSO464	*L. brevis* TMW 1.313-SB	*L. paracollinoides* TMW 1.1994-SB	*L. paracollinoides* TMW 1.1995-SB	*L. paracollinoides* TMW 1.1997-NB	*L. lindneri* TMW 1.481-IB	*P. damnosus* TMW 2.1536-NB	*L. brevis* KB290	*L. brevis* TMW 1.1326-NB	*L. brevis* TMW 1.6-WB
FAS-Cluster	M05 (*fabZ*)	Type II fatty acid biosynthesis	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
horA-Cluster	*horA* / ORF5	Hop export / cell envelope modification	-	+	+	-	-	-	+	+	+	+	v	+	+	+	-	+	-	-	+	-	-	-	-
horC-Cluster-1	*horB* / *horC*	Hop export / cell envelope modification	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	-	+	-	-	-	-	-	-
horC-Cluster-2	*horB* / *horC*	Truncated version - *horB* / *horC* only	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
hitA-Cluster	*hitA*	Mn^{2+} / Mg^{2+} transport	+	-	-	-	-	v	+	+	-	-	-	-	-	+	+	-	v	-	-	-	-	-	-
Cluster 1		Acetoin / butanediol metabolism	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-
Cluster 2		Peptidoglycan recycling / metal ABC transport	+	-	-	-	-	-	+	+	+	+	-	-	-	p	-	-	-	-	-	-	-	-	-
Cluster 3		Sugar utilization / DNA modification	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Cluster 4	M09	Maltose utilization	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cluster 5	M28	Unknown	+	+	+	+	+	+	+	+	-	-	+	+	-	+	-	p	p	-	-	-	-	-	-
Cluster 6	M03	Metal ABC transport	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cluster 7		Unknown	+	+	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cluster 8		Biotin utilization / cyclopropane FA synthesis	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Cluster 9		Conjugation	-	-	+	+	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-
Cluster 10	M42	Various (Lipase / NADH peroxidase)	+	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-
Cluster 11		Unknown (Enolase)	+	+	+	+	-	-	+	+	-	-	+	+	-	+	-	-	+	-	-	-	-	-	-
Cluster 12		Agmatine deiminase pathway	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-
Cluster 13		Sugar transporter	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-
Cluster 14	M22 / M23	Maltose utilization	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Cluster 15		Biotin utilization	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Fig. 9 Distribution of genetic clusters within brewery plasmidomes. Brewery plasmidomes are coloured yellow. For comparison, non-brewery plasmidomes of the same focused species are shown. Brewery-specific DNA: + = no high identity hits (minimum 50 % query/subject coverage, 75 % identity) in nr/nt BLAST database with exception of brewery isolates. Beer spoilage potential (BSP) is shown, where available: SB = strong BSP, IB = intermediate BSP, WB = weak BSP, NB = no BSP. + = presence and – = absence of respective cluster. v = presence indicated in vitro (PCR), p = partially present

but also magnesium homeostasis could be important for the growth of beer-spoiling LAB in beer. Altogether, plasmid-encoded and shared genes were found, associated with ion homeostasis and the transport of metals (additionally for cobalt/zinc/cadmium and potassium). As these metals partially have antibacterial properties themselves [73] and considering the knowledge about the evident

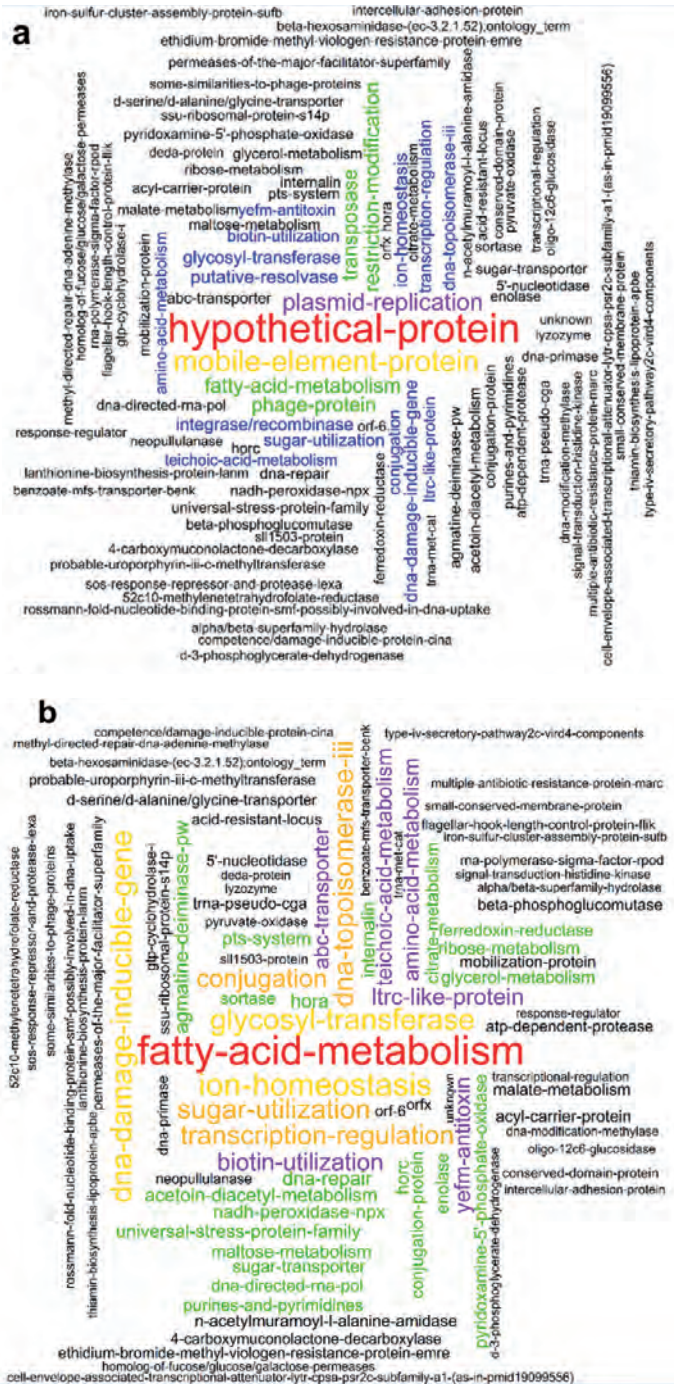

Fig. 10 Wordcloud of brewery plasmidome-encoded genes and global functions - illustration of gene and function/trait frequencies. Genes with a distinct association to a global function, e.g. conjugation or fatty acid metabolism, were summarized. In contrast to the SEED subsystem analysis or a comparable analysis, all genes/functions are depicted. The upper panel (a) shows all brewery genes/functions, which occur more than two times in the total brewery plasmidome. The lower panel (b) shows the same but without genes/functions lacking an informative biological function (e.g. hypothetical protein, mobile element protein). Thus, the lower panel has a different scaling (color/font size)

linkage of hop`s antibacterial properties to the content of various cations [12, 59], the presence of such a high number of ion transport related genes indicates that active ion homeostasis, not only in case of manganese, is an important biological process for LAB growing in beer and/or the brewery environment and thus part of their lifestyle in beer [25].

Oxidative stress: A NADH peroxidase (M42, also part of Cluster 10, see Figure 9) was encoded in 11 beer-spoiling plasmidomes, but the DNA-sequence itself is not specific for the brewery environment and was also found in other LAB genomes. PCR screening of all 94 strains revealed a species independent enrichment of M42 in beer-spoiling strains (Fisher's p = 0.037 for all focused species), which is especially prominent for *P. damnosus*. NADH-peroxidases (*npx*, EC 1.11.1.1) catalyze the reduction of H_2O_2 to H_2O using NADH as electron donor, thus counteracting oxidative stress but also offering the possibility to recycle NAD [40]. Further, we found a ferredoxin reductase (Fdx) and a ferritin-like antioxidant protein (Dps), again encoded by several brewery plasmidomes, but also by non-brewery isolates. Both are considered part of the cellular defense against oxidative stress [35, 40]. An auxiliary role of these genes for growth in beer is conceivable, considering the fact that only small amounts of oxygen in beer, together with Fe^{2+} or Cu^{+}, can cause the formation of H_2O_2 and other reactive oxygen species [51]. Further, oxidative (redox) stress was previously identified as a major antibacterial property of hops [13, 56], although it remains elusive how this could participate in the generation of H_2O_2 or any other ROS. A potential significance of Npx and Fdx for growth in beer is also supported by the findings of *Bergsveinson, J.*, *Baecker, N.*, *Pittet, V.* and *Ziola, B.* [14]. They demonstrated that the loss of pLb464-8, which carries both abovementioned genes, had a significant negative effect on *L. brevis* BSO 464 growth behavior in beer, while its relevance for hop tolerance was not clear. They suggest that pLb464-8 might be important to cope with other stresses found in beer [14]. This could be oxidative stress, although pLb4648 encodes for 45 other potential genes with different functions/annotations. The same research group could also demonstrate that several oxidative-stress-related genes of *P. claussenii* ATCC BAA-344 (TMW 2.340-IB), including the plasmid-encoded dps gene, were significantly upregulated upon growth in beer [50], while a general significance of oxidative stress tolerance for *L. brevis* hop tolerance was indicated by various other studies [8, 11, 13, 25, 75].

Further, shared genes were found related to the uptake of unknown substrates, growth at cold temperatures (*ltrA*), DNA restriction/modification, DNA repair, general stress tolerance and others. It is important to get a deeper knowledge of the relevance of shared genes and clusters, in order to "discard" those that have no relevance for beer spoilage. We think that the approach performed by Bergsveinson, J., Baecker, N., Pittet, V. and Ziola, B. [14], including the generation of plasmid-cured variants and the consequent characterization of their beer spoilage potential with respect to some key parameters, will help to better understand the role of brewery plasmids and clusters [25].

In conclusion, the comparative analysis of brewery plasmidomes emphasizes the known relevance of cation homeostasis, oxidative stress tolerance and cell envelope metabolism for hop tolerance and beer spoilage potential. Further, we suggest that plasmid-encoded metabolic traits, which allow the reduction of the acid load, e.g. by producing non-acidic end products such as diacetyl or butanediol, contribute to the acid tolerance of beer-spoiling LAB. These metabolic strategies are suggested to be part of their lifestyle in the

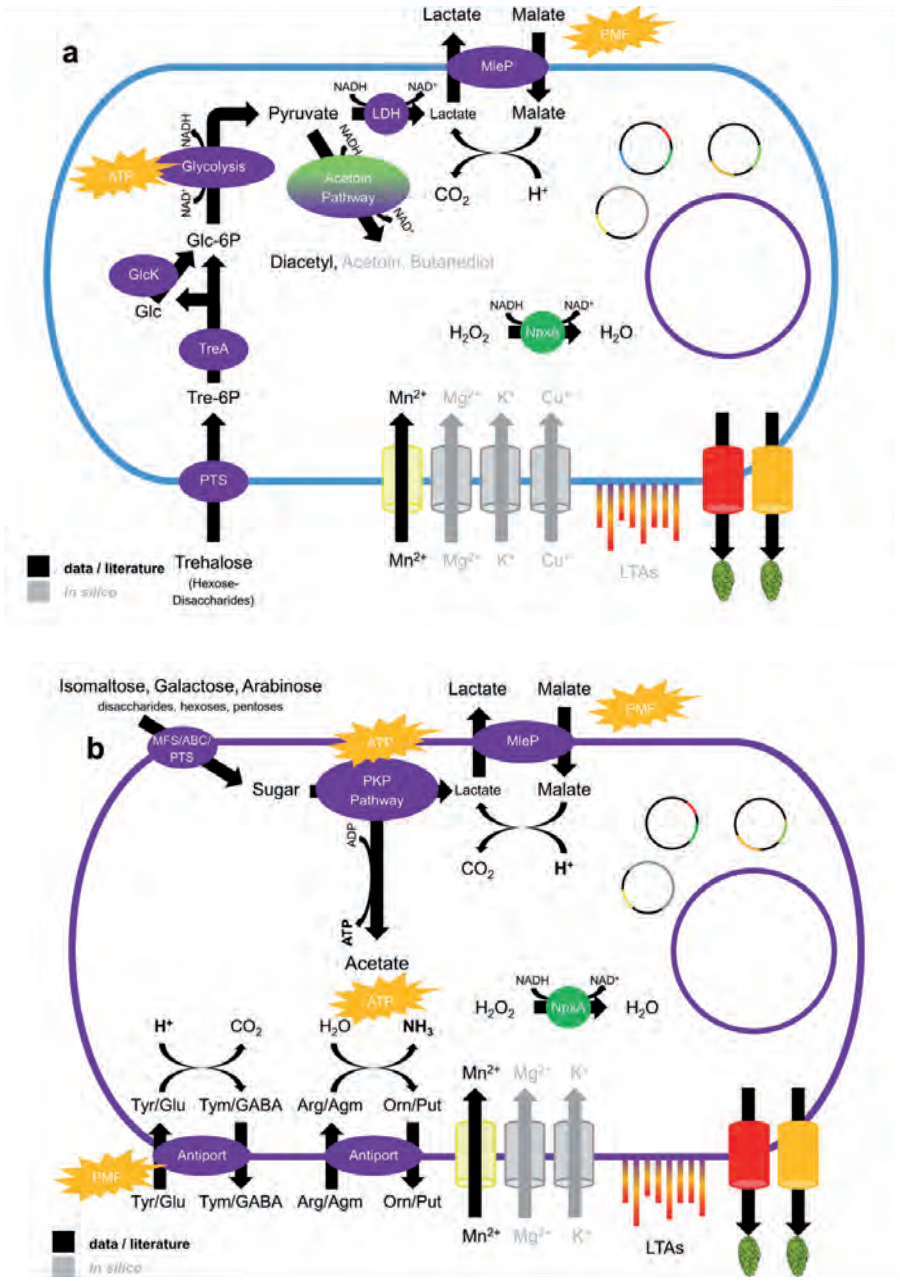

Fig. 11 **The lifestyle of *P. damnosus* and *L. brevis* in beer – metabolic traits and strategies, which are proposed to contribute to their adaption to beer and especially to hops and the low pH. The colour code of the included features illustrates the genetic origin of the included traits (chromosomal = purple / plasmid-encoded = other colours). The font colour corresponds to the evidence level: black = data / literature, genomic prediction confirmed by metabolic data, genetic correlation data and other experimental data, or supported by published literature; grey = *in silico* prediction only. Note that this is only a simplified scheme and that not all shown plasmid-encoded traits are present in all beer-spoiling strains of the respective species, but part of the corresponding species flexible gene pool. Panel (a) shows selected features for *P. damnosus*. The ability to build an intact membrane in the low-fat environment beer relies on the presence of a plasmid-encoded fatty acid biosynthesis cluster (blue), while only strains carrying that cluster have beer spoilage ability. At least one hop tolerance gene was found for all beer-spoiling strains (red, yellow). Further, *P. damnosus* is characterized by various plasmid-encoded genes and clusters related to cation homeostasis, cell envelope metabolism and modification and oxidative stress tolerance. Trehalose and gentiobiose are the preferred substrates of this homofermentative species. Sugars are taken up using phosphotransferase systems (PTS), while the resulting C6 sugars are catabolized to lactate only under laboratory conditions. In beer and under hop stress, the production of lactate is reduced markedly, while diacetyl, acetoin and butanediol (plasmid encoded trait) are produced. This way *P. damnosus* produces non-acidic end products, which is a clear advantage considering the dependence of hops antibacterial action on a low pH, while remaining redox balance. Panel (b) shows selected features for *L. brevis*. Note that the majority of all plasmid-encoded features were also found in the plasmidomes of *L. brevis*, while the chromosomal metabolic strategies are, with the exception of malolactic fermentation, others. Especially the intensive usage of amino acids and agmatine for energy generation and active pH homeostasis differentiates this heterofermentative bacterium from *P. damnosus*. ATP = adenosine triphosphate, ADP = adenosine diphosphate, PMF = proton motive force, Tyr = tyrosine, Tym = tyramine, Glu = glutamate, GABA = γ-aminobutyric acid, Arg = arginine, Agm = agmatine, Orn = Ornithine, Put = putrescine, MFS = major facilitator superfamily, ABC = ATP-binding cassette, PKP = phosphoketolase pathway, LTAs = lipoteichoic acids**

low pH environment beer. The abundance of plasmid-encoded, partially brewery-specific genes, encoding for various functions, which might confer an advantage in the brewery environment or can be linked (in theory) to an improved growth in beer, suggests that these plasmids are the foundation for LAB beer spoilage ability and consequently important for their lifestyle in beer [25].

3.3 The lifestyle of beer spoiling lactic acid bacteria

So how does the lifestyle of beer spoiling LAB look like? We suggest that it is a combination of species-specific and chromosomally encoded metabolic traits which are complemented by plasmid-encoded traits and strategies, which enable LAB to overcome the beer-specific antibacterial hurdles and to grow in beer. The chromosomal traits are conserved within a species and thus not decisive with respect to beer spoilage potential, while the brewery shared gene pool encodes for properties, which are essentially for growth in beer and thus convert harmless contaminants into beer-spoiling strains. Figure 11 shows a selection of identified traits and metabolic strategies of beer-spoiling strains of *L. brevis* and *P. damnosus*, being the most important beer-spoiling LAB species. While they clearly differ regarding their chromosomally encoded

metabolic strategies, they share most of their plasmid-encoded traits, which emphasizes their decisive role for beer spoilage potential. Although chromosomally encoded metabolic traits are not sufficient for beer spoilage, they are relevant for growth in beer. Hops are considered to increase the energy demand by LAB. This is firstly because hops act as pH dependent proton ionophores [12, 58], thus reducing the pmf and consequently the pmf dependent uptake of substrates [64]. Secondly, hop tolerance itself is considered energy-intensive, especially as the active hop transporters *HorC* and *HorA* rely on energy in terms of ATP and pmf [54, 65, 66, 75]. We were able to actually measure this increased energy demand under hop stress, while we observed a distinct metabolic response of beer spoiling strains of all focused species to an exposure of hops, compared to acid stress only. This response resulted in an increased substrate and energy demand, despite decreasing cell density and a reduced broth acidification and specifically a reduced lactate production [25]. Thus, LAB need to have an efficient energy generating metabolism and active pH homeostasis mechanism in order to grow under hop stress and thus in beer. This is achieved by mostly species-specific combinations of chromosomally encoded metabolic strategies, including the formation of non-acidic end products (acetoin, diacetly, butanediol), a shift to acetate production, which is a weaker acid than lactate, citrate fermentation, malolactic fermentation and the utilization of free amino acids by amino acid decarboxylation and deiminase pathways. However, none of these traits are used by all beer spoiling organisms and are thus non-essential [25, 28]. In contrast, hop tolerance genes such as *horA* and *horC*, as well as the FAS cluster in case of *P. damnosus* and *L. backii*, are essential for beer spoilage ability [10, 25, 29, 68]. Altogether, this illustrates that any molecular approach (e.g. PCR) to differentiate beer-spoiling and non-spoiling strains has to rely on the brewery-specific mobile and shared genetic pool encoding additional traits beyond *horA* and *horC*, which finally enable and facilitate LAB growth in beer [25].

3.4 Mobile DNA as target for quality control

As initially stated, brewers need to perform quality control in order to provide consumers with a stable product. Thus, detection and identification of harmful contamination with beer-spoiling LAB has to be highly selective, fast, robust and cheap. The usage of detection media such as NBB allows the detection and enrichment of contaminations, but does not provide taxonomic identification or information on the beer spoilage potential of an isolate. Challenge tests are still considered the gold standard for the prediction of beer spoilage ability and thus for the product hazard of a given contaminant. However, these tests are quite tedious and simply take too long. Consequently, rapid and reliable alternatives are desired [65, 68]. For the prediction of novel DMGs from genome sequences, we designed and programmed the BlAst Diagnostic Gene findEr (BADGE), a simple and fast bioinformatics tool which is addressed in detail within a previous publication [10]. We successfully used BADGE to predict DMGs, which were present in beer-spoiling genomes but not in non-spoiling genomes, while the respective genes were almost exclusively part of the brewery shared gene pool. Employing a PCR assay, we evaluated 45 predicted DMGs and consequently validated the significance of some novel and promising DMGs. PCR was performed without prior DNA extraction using single colonies from NBB plates. This allows the prediction of beer spoilage ability within a few hours after detection. It is also possible to further reduce the detection time by analyzing (PCR) beer without a previous cultivation-based preenrichment (e.g. QuickGEN, Gen-ial, Troisdorf, Germany) [25].

Because of a high degree of instability (varying beer spoilage ability, genetic instability), low transferability (varying beer spoilage ability in different beer systems), a low number of non-spoiling strains and/or a lack of significance (for details, rationale see [25]) we suggest that a species identification is sufficient for *L. backii*, *L. lindneri*, *L. paracollinoides* and *P. claussenii*, although we found DMGs to differentiate beer spoiling strains from non-spoilers for 3 out of 4 of these species [25]. However, species identification can be achieved using species-specific PCR (e.g. First-Beer Differentiation PCR Kit, Gen-ial, Troisdorf, Germany) or other non-DNA-based approaches, such as MALDI-TOF MS. A detection of these species should be considered as product hazard even in the absence of intraspecies differentiation of beer spoiling strains. Differentiation of beer-spoiling strains is necessary for *L. brevis* and *P. damnosus* because of a distinct strain-specific beer spoilage ability and a high relevance of both species for beer spoilage. The lifestyle genes *fabZ* (FAS cluster), *horA* and *horC* are used in case of *P. damnosus*, while the presence of fabZ and one of the "hor"-genes together is counted as positive result. Applying this system, we were able to differentiate 20 strains into beer-spoiling and non-spoiling strains 100 % correctly. *L. brevis* is differentiated using *horC* and M37, while the presence of either is counted as positive result. Almost all (18 of 20) strains were correctly identified as beer-spoiling or non-spoiling strains using *horC* and M37. Altogether, despite the fact that we did not differentiate on a strain-level within four of six focused species, this approach led to a detection of 99 % of all beer-spoiling strains with a false positive rate of 30 %. This way we end up with a specifity (70 %) of the identification system, where a strain differentiation actually pays off for quality control, as it is possible to differentiate the majority of harmful and harmless contaminations with very high hazard potential LAB and *P. claussenii*. For comparison, the often suggested combination of *horA* and *horC* for the species-independent identification of beer-spoiling LAB [68] identified only 95 % of all beer-spoiling strains in this study, in addition characterized by a false positive rate of 58 %. The necessity of the FAS cluster for beer spoilage ability of *P. damnosus* and *L. backii* shows that there are species-specific lifestyle genes (clusters), which are obligatory for growth in beer. A species-independent approach might thus not be the right choice, as we still do not know the relevance of the majority of all genes encoded in the brewery shared gene pool. A selection of further DMGs with significant correlation to beer spoilage ability and potential, as well as suggestions for distinct quality control approaches, species-specific and species-independent, is described by Geissler [25].

4 Conclusion

Beer-spoiling LAB differ with respect to their species-specific metabolic strategies that support bacterial growth in beer. These chromosomally encoded traits are species-specific and consequently not suited as diagnostic marker genes (DMGs). In contrast, beer

spoilage ability is a plasmid-encoded trait. We found that strains of different species share a mostly brewery-specific, dynamic mobile genetic pool, encoding additional traits beyond *horA* and *horC*, which finally enable and facilitate LAB growth in beer. Thus, genomic plasticity is the key to beer spoilage ability. The respective additional genes contribute, amongst others, to cation homeostasis, oxidative stress tolerance and cell envelope modification and metabolism. These were already suggested to be relevant for beer spoilage potential and hop tolerance, while their significance so far has only been indicated in terms of phenotypic plasticity [65]. Further, the ability to produce long-chain fatty acids was found to be essential for LAB growth in the low-fat environment beer. This and other shared plasmid encoded traits comprise DMGs for the enhanced molecular biological detection of beer-spoiling LAB in quality control.

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Supplementary Table 1: Beer spoilage test results - Classification of strains into beer spoilage ability (BSA) and beer spoilage potential (BSP) groups inlcuding spoilage related data (turbidity / pH after 60 days, first visible growth assessment = growth (d)). Hazard potential, alternative strain IDs and the source of each strain are listed. BSA and BSP were determined applying the beer spoilage test. Classification based on beer spoilage test: strong BSP (SB) - growth in pilsner 1, middle BSP (MB) - growth in lager beer 1, weak BSP (WB) - growth in wheat beer 1, no BSP (NB) - no growth in test beers; BSA (B/NB) defined by growth in lager beer 1. BSP and BSA are once listed as range to illustrate the variability found for some strains. Additionally, the average BSP/BSA is shown. The average BSP/BSA were defined (calculated) applying the following rules: Simple median point: e.g. 5 x SB, 1 x WB → SB, average category: 3 x WB, 3 x SB → MB, "safe side": 3 x MB, 3 x SB → SB. Classification of a selection of tested strains is also shown for test beers of three other breweries (transferability test).

Species	Strain					Beer spoilage test with beers of brewery 1																Beer spoilage test with beers of brewery 2				Beer spoilage test with beers of brewery 3				Beer spoilage test with beers of brewery 4			
						Spoilage Potential - with Variability		Spoilage Potential		Lager beer pH 5.0 - Lager beer, adjusted to pH 5.0 with 6 M NaOH			Kristall Wheat beer pH 4.4, 14 IBU, gravity 12.5 wt. %, alcohol 5.5 vol. %			Lager beer pH 4.3, 18 IBU, gravity 11.5 wt. %; alcohol 5.1 vol. %			Pilsner beer pH 4.4, 29 IBU, gravity 11.5 wt. %, alcohol 5.1 vol. %			Spoilage Potential		Spoilage Potential - with Variability		Spoilage Potential		Spoilage Potential - with Variability		Spoilage Potential		Spoilage Potential - with Variability	
	original ID	TMW	alternative ID	source	brewery isolate	beer spoilage ability	spoilage potential groups	beer spoilage ability	spoilage potential groups	growth (d)	OD590 (60d)	pH (60d)	growth (d)	OD590 (60d)	pH (60d)	growth (d)	OD590 (60d)	pH (60d)	growth (d)	OD590 (60d)	pH (60d)	beer spoilage ability	spoilage potential groups	beer spoilage ability	spoilage potential groups	beer spoilage ability	spoilage potential groups	beer spoilage ability	spoilage potential groups	beer spoilage ability	spoilage potential groups	beer spoilage ability	spoilage potential groups
Lactobacillus brevis very high hazard potential	TMW 1.6	TMW 1.6	DSM 20054[T]	faeces	no	NB	WB	NB	WB	4 +/- 1	0.2 +/- 0.11	4.51 +/- 0.06	11 +/- 4	0.12 +/- 0.08	4.37 +/- 0.08	no - 60	0 +/- 0	4.38 +/- 0.08	no - 60	0 +/- 0	4.34 +/- 0.04												
	TMW 1.1326	TMW 1.1326	ATCC 367	silage	no	NB	NB	NB	NB	3 +/- 0	0.1 +/- 0.02	4.81 +/- 0.02	no - 60	0.01 +/- 0	4.51 +/- 0	no - 60	0 +/- 0	4.47 +/- 0.01	no - 60	0 +/- 0	4.42 +/- 0.01	NB	NB	NB	NB	NB	NB	NB	**NB**-WB	NB	NB	**NB**-B	**NB**-MB
	TMW 1.100	TMW 1.100		sourdough	no	NB	WB	NB	WB	1 +/- 0	0.36 +/- 0.05	3.97 +/- 0.03	14 +/- 4	0.07 +/- 0.02	4.01 +/- 0.02	no - 60	0 +/- 0	4.3 +/- 0	no - 60	0 +/- 0	4.33 +/- 0												
	TMW 1.1205	TMW 1.1205		sourdough	no	**NB**-B	**WB**-SB	NB	WB	1 +/- 0	0.4 +/- 0.06	3.97 +/- 0.09	14 +/- 7	0.08 +/- 0.04	4 +/- 0.05	no - 60	0 +/- 0	4.27 +/- 0.05	no - 60	0 +/- 0	4.35 +/- 0.02												
	TMW 1.1282	TMW 1.1282		brewery environment	yes	B	SB	B	SB	1 +/- 0	0.25 +/- 0.04	3.99 +/- 0.01	5 +/- 0	0.09 +/- 0.07	4.27 +/- 0.21	7 +/- 0	0.11 +/- 0.04	3.77 +/- 0	5 +/- 0	0.14 +/- 0.04	3.85 +/- 0												
	TMW 1.1369	TMW 1.1369		honey fermentation	no	NB	WB	NB	WB	2 +/- 0	0.34 +/- 0.01	4.26 +/- 0.01	20 +/- 1	0.15 +/- 0.01	4.09 +/- 0	no - 60	0 +/- 0	4.28 +/- 0	no - 60	0 +/- 0	4.36 +/- 0.01												
	TMW 1.1370	TMW 1.1370		honey fermentation	no	B	MB-SB	B	SB	3 +/- 1	0.4 +/- 0.2	4.12 +/- 0.08	6 +/- 1	0.13 +/- 0.02	4.16 +/- 0.08	10 +/- 3	0.12 +/- 0.05	4.16 +/- 0.12	14 +/- 0	0.1 +/- 0	4.09 +/- 0.02												
	TMW 1.2048	TMW 1.2048		sausage swell	no	**NB**-B	**WB**-MB	**NB**	WB	1 +/- 0	0.29 +/- 0.04	3.99 +/- 0.11	11 +/- 1	0.09 +/- 0.01	4.19 +/- 0	no - 60	0 +/- 0	4.37 +/- 0	no - 60	0 +/- 0	4.4 +/- 0												
	TMW 1.230	TMW 1.230		spoiled beer	yes	B	SB	B	SB	2 +/- 0	0.32 +/- 0.01	4.01 +/- 0.02	2 +/- 0	0.2 +/- 0.04	3.97 +/- 0.01	6 +/- 0	0.11 +/- 0.01	3.86 +/- 0.04	7 +/- 0	0.11 +/- 0.02	3.93 +/- 0.02												
	TMW 1.240	TMW 1.240		spoiled beer	yes	B	SB	B	SB	1 +/- 0	0.34 +/- 0.07	3.88 +/- 0	3 +/- 0	0.17 +/- 0.02	4 +/- 0.01	3 +/- 0	0.08 +/- 0	3.75 +/- 0	5 +/- 0	0.06 +/- 0.01	3.83 +/- 0												
	TMW 1.302	TMW 1.302		brewery environment	yes	NB	WB	NB	WB	2 +/- 0	0.26 +/- 0.02	4.65 +/- 0.02	6 +/- 0	0.1 +/- 0.02	4.52 +/- 0.01	no - 60	0 +/- 0	4.49 +/- 0	no - 60	0 +/- 0	4.4 +/- 0	B	SB	B	SB	B	MB	B	MB	B	MB	B	MB
	TMW 1.313	TMW 1.313		brewery environment	yes	B	SB	B	SB	2 +/- 1	0.37 +/- 0.11	4.08 +/- 0.12	2 +/- 0	0.14 +/- 0.05	4.12 +/- 0.1	4 +/- 1	0.16 +/- 0.04	3.91 +/- 0.12	5 +/- 1	0.17 +/- 0.04	3.94 +/- 0.11	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	TMW 1.315	TMW 1.315		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.46 +/- 0.03	4.08 +/- 0.03	2 +/- 0	0.24 +/- 0.01	3.9 +/- 0.01	6 +/- 0	0.16 +/- 0.01	3.79 +/- 0.02	10 +/- 2	0.14 +/- 0	3.77 +/- 0												
	TMW 1.317	TMW 1.317		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.38 +/- 0.05	3.88 +/- 0.01	2 +/- 0	0.2 +/- 0.03	4.24 +/- 0	4 +/- 0	0.22 +/- 0.03	3.87 +/- 0.04	4 +/- 0	0.15 +/- 0.03	3.83 +/- 0.02												
	TMW 1.465	TMW 1.465		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.31 +/- 0.01	4.27 +/- 0.01	3 +/- 0	0.2 +/- 0.02	4.02 +/- 0.02	5 +/- 0	0.16 +/- 0.01	4.02 +/- 0	5 +/- 0	0.13 +/- 0	4.15 +/- 0.02	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	TMW 1.473	TMW 1.473		brewery environment	yes	B	SB	B	SB	1 +/- 0	0.34 +/- 0.04	3.94 +/- 0	3 +/- 2	0.2 +/- 0.01	4.14 +/- 0.06	10 +/- 7	0.17 +/- 0.02	3.9 +/- 0.03	3 +/- 2	0.13 +/- 0.02	3.99 +/- 0.04												
	TMW 1.474	TMW 1.474		brewery environment	yes	B	SB	B	SB	1 +/- 0	0.27 +/- 0.07	4 +/- 0.01	2 +/- 0	0.09 +/- 0.02	4.41 +/- 0	5 +/- 0	0.17 +/- 0.04	3.76 +/- 0.01	5 +/- 0	0.18 +/- 0.04	3.83 +/- 0												
	TMW 1.476	TMW 1.476		brewery environment	yes	B	SB	B	SB	1 +/- 0	0.38 +/- 0.02	3.95 +/- 0.01	2 +/- 0	0.14 +/- 0.01	4.14 +/- 0	5 +/- 0	0.14 +/- 0.02	3.79 +/- 0.01	5 +/- 0	0.14 +/- 0.04	3.98 +/- 0.05												
	TMW 1.485	TMW 1.485		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.33 +/- 0.01	3.97 +/- 0	2 +/- 0	0.17 +/- 0.01	3.94 +/- 0.01	4 +/- 1	0.12 +/- 0.01	3.78 +/- 0.02	8 +/- 0	0.11 +/- 0	3.82 +/- 0												
	TMW 1.507	TMW 1.507		brewery environment	yes	B	MB-**SB**	B	SB	1 +/- 0	0.23 +/- 0.07	4.13 +/- 0.04	6 +/- 0	0.12 +/- 0.02	4 +/- 0.02	2 +/- 0	0.07 +/- 0.03	4 +/- 0.02	2 +/- 0	0.06 +/- 0	4.14 +/- 0												
Pediococcus claussenii high hazard potential	TMW 2.340	TMW 2.340	DSM 14800[T]	spoiled beer	yes	NB-B	WB-SB	B	MB	14 +/- 0	0.21 +/- 0.01	4.23 +/- 0.01	9 +/- 0	0.04 +/- 0	4.18 +/- 0.03	30 +/- 0	0.04 +/- 0.01	4.2 +/- 0.04	no - 60	0.01 +/- 0	4.39 +/- 0												
	MB 377	TMW 2.1531		unpasteurised beer	yes	NB	NB	NB	NB	14 +/- 0	0.15 +/- 0	4.35 +/- 0.01	no - 60	0 +/- 0	4.42 +/- 0.01	no - 60	0 +/- 0	4.3 +/- 0	no - 60	0.01 +/- 0	4.4 +/- 0	NB	NB	NB	NB	NB	WB	NB	WB	B	MB	NB-**B**	NB-**MB**
	MB 311	TMW 2.1545		unpasteurised beer	yes	NB-B	WB-SB	B	MB	6 +/- 4	0.19 +/- 0.03	4.12 +/- 0.08	4 +/- 1	0.06 +/- 0.03	4.36 +/- 0.09	7 +/- 0	0 +/- 0	4.24 +/- 0.01	no - 60	0 +/- 0	4.41 +/- 0.01	B	MB	B	**MB**-SB	NB	WB	NB	WB	B	MB	B	**MB**-SB
	TMW 2.50	TMW 2.50		brewery environment	yes	NB-**B**	WB-**SB**	B	SB	5 +/- 3	0.28 +/- 0.09	4.12 +/- 0.13	7 +/- 4	0.06 +/- 0.02	4.21 +/- 0.09	14 +/- 6	0.06 +/- 0.03	4.14 +/- 0.1	26 +/- 6	0.03 +/- 0.04	4.12 +/- 0.05	B	SB	B	MB-**SB**	B	SB	NB-**B**	WB-**SB**	B	SB	B	SB
	TMW 2.51	TMW 2.51		brewery environment	yes	B	SB	B	SB	4 +/- 0	0.28 +/- 0.08	3.96 +/- 0.15	3 +/- 0	0.05 +/- 0.02	4.18 +/- 0.07	3 +/- 0	0.06 +/- 0.03	4.04 +/- 0.08	3 +/- 0	0.06 +/- 0.03	4.09 +/- 0.06												
	TMW 2.53	TMW 2.53		brewery environment	yes	NB-**B**	NB-**SB**	B	SB	3 +/- 4	0.24 +/- 0.1	4.31 +/- 0.26	5 +/- 4	0.08 +/- 0.06	4.17 +/- 0.1	12 +/- 9	0.1 +/- 0.06	4.05 +/- 0.12	12 +/- 11	0.1 +/- 0.07	4.07 +/- 0.17												
	TMW 2.54	TMW 2.54		brewery environment	yes	B	SB	B	SB	5 +/- 2	0.39 +/- 0.04	4.05 +/- 0.1	3 +/- 0	0.15 +/- 0.04	3.98 +/- 0.04	6 +/- 2	0.14 +/- 0.04	3.96 +/- 0.03	7 +/- 0	0.11 +/- 0.03	4.11 +/- 0.09	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	TMW 2.56	TMW 2.56		brewery environment	yes	B	SB	B	SB	3 +/- 0	0.31 +/- 0	4.12 +/- 0.03	3 +/- 0	0.12 +/- 0.04	4.19 +/- 0.06	3 +/- 0	0.14 +/- 0.04	3.83 +/- 0.33	3 +/- 0	0.14 +/- 0.06	4.11 +/- 0.07												
	TMW 2.59	TMW 2.59		brewery environment	yes	B	SB	B	SB	4 +/- 0	0.26 +/- 0.07	4.22 +/- 0.18	5 +/- 0	0.07 +/- 0.02	4.16 +/- 0.02	4 +/- 1	0.1 +/- 0.03	3.96 +/- 0.08	3 +/- 0	0.08 +/- 0.02	4.05 +/- 0.05												
	TMW 2.60	TMW 2.60		brewery environment	yes	B	MB	B	MB	5 +/- 2	0.18 +/- 0.01	4.25 +/- 0.09	5 +/- 0	0.07 +/- 0.02	4.15 +/- 0.04	7 +/- 0	0.06 +/- 0.01	4.07 +/- 0.08	no - 60	0 +/- 0	4.4 +/- 0.01												
	TMW 2.61	TMW 2.61		brewery environment	yes	B	MB	B	MB	6 +/- 0	0.15 +/- 0.02	4.17 +/- 0.01	6 +/- 0	0.15 +/- 0.03	4.15 +/- 0	6 +/- 0	0.12 +/- 0.01	4 +/- 0.02	no - 60	0 +/- 0	4.4 +/- 0.02												
	TMW 2.62	TMW 2.62		brewery environment	yes	B	SB	B	SB	5 +/- 0	0.24 +/- 0.02	4.18 +/- 0.1	5 +/- 0	0.08 +/- 0.04	4.18 +/- 0.02	10 +/- 0	0.08 +/- 0.02	4.09 +/- 0.06	17 +/- 0	0.05 +/- 0.01	4.04 +/- 0.03												
	TMW 2.64	TMW 2.64		brewery environment	yes	B	SB	B	SB	4 +/- 0	0.17 +/- 0.02	4.39 +/- 0.01	6 +/- 1	0.08 +/- 0	4.21 +/- 0.01	3 +/- 0	0.06 +/- 0.01	4.09 +/- 0.03	7 +/- 0	0.05 +/- 0.01	4.25 +/- 0.02												
	TMW 2.65	TMW 2.65		brewery environment	yes	B	MB	B	MB	6 +/- 0	0.19 +/- 0.03	4.32 +/- 0.02	5 +/- 0	0.06 +/- 0.03	4.22 +/- 0.04	7 +/- 0	0.05 +/- 0.01	4.06 +/- 0.04	no - 60	0 +/- 0	4.4 +/- 0												
	TMW 2.67	TMW 2.67		brewery environment	yes	B	SB	B	SB	4 +/- 0	0.36 +/- 0.01	3.91 +/- 0.02	3 +/- 0	0.17 +/- 0.01	3.69 +/- 0	7 +/- 0	0.14 +/- 0.02	3.64 +/- 0.01	3 +/- 0	0.14 +/- 0	3.71 +/- 0												
Pediococcus damnosus very high hazard potential	TMW 2.4	TMW 2.4	DSM 20331[T]	lager beer yeast	yes	B	MB	B	MB	3 +/- 0	0.15 +/- 0.03	4.28 +/- 0.01	8 +/- 0	0.06 +/- 0	4.18 +/- 0	22 +/- 0	0.07 +/- 0.02	3.97 +/- 0	no - 60	0 +/- 0	4.39 +/- 0												
	TMW 2.125	TMW 2.125		unknown	yes	NB	NB	NB	NB	3 +/- 0	0.14 +/- 0.02	4.49 +/- 0.1	no - 60	0 +/- 0	4.47 +/- 0.01	no - 60	0 +/- 0	4.32 +/- 0.01	no - 60	0 +/- 0	4.36 +/- 0												
	BLQ 116	TMW 2.1532		bottled beer	yes	NB	NB	NB	NB	3 +/- 1	0.17 +/- 0.04	4.36 +/- 0.06	no - 60	0 +/- 0	4.44 +/- 0.03	no - 60	0.01 +/- 0	4.32 +/- 0.01	no - 60	0.01 +/- 0.01	4.38 +/- 0.02	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB
	BLQ 158	TMW 2.1533		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.28 +/- 0.05	3.69 +/- 0.17	7 +/- 0	0.16 +/- 0.03	3.6 +/- 0.06	7 +/- 0	0.06 +/- 0.03	3.71 +/- 0.16	19 +/- 1	0.06 +/- 0.02	3.87 +/- 0.12	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	BLQ 185	TMW 2.1534		brewing yeast sample	yes	NB	NB	NB	NB	11 +/- 0	0.14 +/- 0	4.34 +/- 0.04	no - 60	0 +/- 0	4.43 +/- 0	no - 60	0 +/- 0	4.31 +/- 0	no - 60	0.01 +/- 0	4.4 +/- 0												
	BLQ 219	TMW 2.1535		pilsner beer	yes	B	SB	B	SB	3 +/- 1	0.4 +/- 0.06	3.64 +/- 0.09	6 +/- 1	0.12 +/- 0.03	3.8 +/- 0.05	7 +/- 0	0.11 +/- 0.01	3.77 +/- 0.11	7 +/- 0	0.09 +/- 0.01	3.89 +/- 0.07	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	B 12	TMW 2.1536		winery environment	no	NB	NB	NB	NB	14 +/- 0	0.1 +/- 0.01	4.37 +/- 0.08	no - 60	0 +/- 0	4.43 +/- 0	no - 60	0 +/- 0	4.31 +/- 0	no - 60	0.01 +/- 0	4.4 +/- 0	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB
	BLQ 189	TMW 2.1546		brewery environment	yes	NB	NB	NB	NB	17 +/- 0	0.07 +/- 0.01	4.98 +/- 0	no - 60	0 +/- 0	4.5 +/- 0	no - 60	0 +/- 0	4.49 +/- 0.01	no - 60	0 +/- 0	4.41 +/- 0												
	BLQ 348	TMW 2.1547		pilsner beer - unfiltrated	yes	NB	NB	NB	NB	13 +/- 1	0.08 +/- 0.01	4.94 +/- 0.03	no - 60	0.01 +/- 0	4.5 +/- 0	no - 60	0 +/- 0	4.48 +/- 0.01	no - 60	0 +/- 0	4.4 +/- 0												
	MB 35	TMW 2.1548		brewery environment	yes	B	MB	B	MB	7 +/- 0	0.17 +/- 0.04	4.29 +/- 0.05	7 +/- 0	0.07 +/- 0.02	4.2 +/- 0.07	30 +/- 0	0.04 +/- 0	4.21 +/- 0.01	no - 60	0 +/- 0	4.4 +/- 0.02												
	B 69	TMW 2.1549		winery environment	no	NB	**NB**-WB	NB	NB	7 +/- 0	0.05 +/- 0.01	4.96 +/- 0.01	no - 60	0.02 +/- 0.03	4.4 +/- 0.15	no - 60	0 +/- 0	4.49 +/- 0.01	no - 60	0 +/- 0	4.41 +/- 0.01												
	BLQ 103	TMW 2.1635		Kellerbier	yes	NB	NB	NB	NB	2 +/- 0	0.1 +/- 0.01	4.66 +/- 0.14	no - 60	0 +/- 0	4.46 +/- 0.01	no - 60	0 +/- 0	4.31 +/- 0.01	no - 60	0 +/- 0	4.36 +/- 0												
	BLQ 182	TMW 2.1636		Kellerbier	yes	B	SB	B	SB	3 +/- 1	0.22 +/- 0.02	4.34 +/- 0.07	6 +/- 1	0.08 +/- 0.02	4.18 +/- 0.01	6 +/- 2	0.06 +/- 0.02	4.07 +/- 0.02	7 +/- 4	0.04 +/- 0.01	4.13 +/- 0.01												
	BLQ 205	TMW 2.1637		brewing environment	yes	B	SB	B	SB	3 +/- 0	0.23 +/- 0.02	4.35 +/- 0.07	6 +/- 1	0.08 +/- 0.03	4.22 +/- 0.03	6 +/- 1	0.06 +/- 0.02	4.11 +/- 0.03	5 +/- 2	0.04 +/- 0.01	4.16 +/- 0.04												
	BLQ 1498	TMW 2.1638		brewery fermentation tank	yes	NB	NB	NB	NB	3 +/- 0	0.19 +/- 0.01	4.27 +/- 0.12	no - 60	0.01 +/- 0	4.47 +/- 0.01	no - 60	0 +/- 0	4.32 +/- 0.01	no - 60	0 +/- 0	4.34 +/- 0.04												
	BLQ 1522	TMW 2.1639		brewery environment	yes	NB	NB	NB	NB	3 +/- 1	0.31 +/- 0.06	3.64 +/- 0.02	no - 60	0 +/- 0	4.5 +/- 0.03	no - 60	0 +/- 0	4.37 +/- 0.04	no - 60	0 +/- 0	4.4 +/- 0.04												
	BLQ 1523	TMW 2.1640		wheat beer	yes	NB	NB	NB	NB	3 +/- 1	0.36 +/- 0.06	3.57 +/- 0.1	no - 60	0 +/- 0	4.47 +/- 0.01	no - 60	0 +/- 0	4.32 +/- 0.01	no - 60	0 +/- 0	4.36 +/- 0.01												
	BLQ 1530	TMW 2.1641		brewery environment	yes	B	SB	B	SB	3 +/- 0	0.21 +/- 0.01	4.35 +/- 0.09	12 +/- 6	0.06 +/- 0.02	4.2 +/- 0.03	11 +/- 4	0.09 +/- 0.11	4.1 +/- 0.03	11 +/- 4	0.04 +/- 0.01	4.16 +/- 0.03												
	BLQ 1008	TMW 2.1642		pilsner beer - unfiltrated	yes	NB	NB	NB	NB	4 +/- 0	0.2 +/- 0.01	4.22 +/- 0.1	no - 60	0 +/- 0	4.45 +/- 0.03	no - 60	0 +/- 0	4.33 +/- 0.01	no - 60	0 +/- 0	4.35 +/- 0												
	B 98	TMW 2.1643		winery environment	no	NB	NB	NB	NB	4 +/- 0	0.13 +/- 0.08	4.4 +/- 0.36	no - 60	0 +/- 0	4.46 +/- 0.01	no - 60	0 +/- 0	4.31 +/- 0.01	no - 60	0 +/- 0	4.37 +/- 0.03												
Lactobacillus backii very high hazard potential	TMW 1.1299	TMW 1.1299	DSM 18080[T]	spoiled lager beer	yes	B	SB	B	SB	2 +/- 0	0.16 +/- 0.01	4.86 +/- 0	5 +/- 0	0.08 +/- 0	4.37 +/- 0.01	6 +/- 1	0.08 +/- 0.01	4.25 +/- 0.01	5 +/- 0	0.06 +/- 0	4.3 +/- 0.01												
	TMW 1.1430	TMW 1.1430		spoiled beer	yes	B	SB	B	SB	4 +/- 0	0.2 +/- 0	4.56 +/- 0.01	4 +/- 0	0.06 +/- 0.01	4.3 +/- 0.01	4 +/- 0	0.06 +/- 0	4.29 +/- 0	11 +/- 0	0.05 +/- 0	4.33 +/- 0												
	TMW 1.1432	TMW 1.1432		spoiled beer	yes	B	SB	B	SB	2 +/- 0	0.18 +/- 0	4.84 +/- 0	5 +/- 0	0.27 +/- 0.29	4.33 +/- 0	5 +/- 0	0.08 +/- 0.02	4.23 +/- 0.01	6 +/- 1	0.07 +/- 0	4.28 +/- 0.01												
	TMW 1.1883	TMW 1.1883		spoiled beer	yes	B	SB	B	SB	2 +/- 0	0.5 +/- 0	3.87 +/- 0	7 +/- 0	0.08 +/- 0	4.46 +/- 0	7 +/- 0	0.24 +/- 0.01	3.8 +/- 0.01	7 +/- 0	0.2 +/- 0.02	3.75 +/- 0.02												
	BLQ 40	TMW 1.1988		light wheat beer	yes	B	SB	B	SB	2 +/- 0	0.19 +/- 0	4.5 +/- 0.01	5 +/- 0	0.07 +/- 0.01	4.27 +/- 0	7 +/- 0	0.06 +/- 0	4.24 +/- 0.01	11 +/- 0	0.06 +/- 0	4.32 +/- 0												
	BLQ 179	TMW 1.1989		beer	yes	B	SB	B	SB	3 +/- 0	0.17 +/- 0.01	4.67 +/- 0	7 +/- 0	0.08 +/- 0	4.36 +/- 0	9 +/- 0	0.07 +/- 0	4.27 +/- 0	11 +/- 0	0.06 +/- 0	4.32 +/- 0.01	B	SB	B	MB-**SB**	B	SB	B	MB-**SB**	B	SB	B	SB
	BLQ 212	TMW 1.1990		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.3 +/- 0.01	4.31 +/- 0.01	5 +/- 0	0.12 +/- 0.01	4.1 +/- 0	11 +/- 0	0.08 +/- 0	4.08 +/- 0	11 +/- 0	0.06 +/- 0	4.28 +/- 0.02												
	BLQ 225	TMW 1.1991		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.31 +/- 0	4.3 +/- 0	4 +/- 0	0.09 +/- 0	4.07 +/- 0	10 +/- 0	0.08 +/- 0	4.06 +/- 0.02	10 +/- 0	0.05 +/- 0.01	4.29 +/- 0.02	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	BLQ 395	TMW 1.1992		brewery environment	yes	B	MB-**SB**	B	SB	5 +/- 3	0.22 +/- 0.04	4.58 +/- 0.05	14 +/- 4	0.05 +/- 0.01	4.34 +/- 0.09	19 +/- 2	0.04 +/- 0.01	4.28 +/- 0.05	18 +/- 7	0.04 +/- 0	4.36 +/- 0.03												
	BLQ 95	TMW 1.2002		brewery environment	yes	NB-B	WB-SB	B	MB	4 +/- 2	0.21 +/- 0.04	4.14 +/- 0.19	11 +/- 4	0.11 +/- 0.06	4.28 +/- 0.17	9 +/- 0	0.07 +/- 0.01	4.34 +/- 0.01	16 +/- 0	0.06 +/- 0.01	4.38 +/- 0	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	BLQ 100	TMW 1.2003		brewery environment	yes	B	SB	B	SB	5 +/- 0	0.17 +/- 0.02	4.21 +/- 0.19	6 +/- 0	0.16 +/- 0.02	4.4 +/- 0.04	14 +/- 0	0.12 +/- 0.02	4.3 +/- 0.02	17 +/- 0	0.07 +/- 0.02	4.22 +/- 0.01												
	BLQ 135	TMW 1.2004		wheat beer	yes	NB	**NB**-WB	NB	NB	4 +/- 1	0.32 +/- 0.2	3.86 +/- 0.29	no - 60	0 +/- 0	4.54 +/- 0.02	no - 60	0 +/- 0	4.45 +/- 0.03	no - 60	0 +/- 0	4.38 +/- 0.06	NB	WB	NB	NB-**WB**	NB	NB	NB	NB	B	MB	B	MB
	MB 479	TMW 1.2005		pilsner beer	yes	NB-B	WB-SB	B	MB	4 +/- 2	0.17 +/- 0.07	4.37 +/- 0.24	4 +/- 3	0.05 +/- 0.01	4.4 +/- 0.02	11 +/- 1	0.02 +/- 0	4.3 +/- 0.01	3 +/- 0	0.02 +/- 0	4.33 +/- 0.01												
	BLQ 55	TMW 1.2070		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.16 +/- 0.01	4.87 +/- 0	9 +/- 1	0.05 +/- 0.02	4.36 +/- 0	7 +/- 0	0.04 +/- 0.01	4.26 +/- 0.01	7 +/- 0	0.04 +/- 0.01	4.31 +/- 0												
	BLQ 61	TMW 1.2071		brewery environment	yes	B	**MB**-SB	B	MB	2 +/- 0	0.12 +/- 0	5.02 +/- 0.01	5 +/- 0	0.04 +/- 0	4.46 +/- 0.03	8 +/- 2	0.03 +/- 0.01	4.42 +/- 0	no - 60	0 +/- 0	4.48 +/- 0.01												
	BLQ 87	TMW 1.2072		dark lager beer	yes	B	SB	B	SB	2 +/- 0	0.47 +/- 0.03	3.87 +/- 0.01	3 +/- 0	0.07 +/- 0	4.42 +/- 0	4 +/- 0	0.11 +/- 0	4.13 +/- 0	4 +/- 0	0.1 +/- 0	4.23 +/- 0												
	BLQ 216	TMW 1.2073		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.3 +/- 0.01	4.44 +/- 0	6 +/- 0	0.12 +/- 0	4.23 +/- 0.01	6 +/- 0	0.13 +/- 0.01	4.11 +/- 0	6 +/- 0	0.11 +/- 0.01	4.17 +/- 0												
	BLQ 1410	TMW 1.2077		brewery environment	yes	B	MB	B	MB	2 +/- 0	0.14 +/- 0.01	4.54 +/- 0.45	10 +/- 0	0.06 +/- 0.01	4.44 +/- 0	8 +/- 3	0.06 +/- 0.02	4.3 +/- 0	no - 60	0 +/- 0	4.36 +/- 0												
	MB 350	TMW 1.2078		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.17 +/- 0	4.85 +/- 0	5 +/- 0	0.05 +/- 0	4.35 +/- 0	7 +/- 0	0.03 +/- 0.01	4.26 +/- 0	7 +/- 0	0.04 +/- 0.02	4.32 +/- 0												
	MB 421	TMW 1.2079		unpasteurised beer	yes	NB	NB-**WB**	NB	WB	3 +/- 0	0.08 +/- 0.01	4.84 +/- 0	10 +/- 0	0.03 +/- 0.01	4.41 +/- 0.04	no - 60	0 +/- 0	4.33 +/- 0	no - 60	0 +/- 0	4.34 +/- 0.01												
Lactobacillus lindneri very high hazard potential	TMW 1.88	TMW 1.88	DSM 20690[T]	spoiled beer	yes	**NB**-B	**NB**-MB	**NB**	NB	5 +/- 0	0.06 +/- 0.03	4.64 +/- 0.03	no - 60	0 +/- 0	4.48 +/- 0.01	no - 60	0 +/- 0	4.47 +/- 0.03	no - 60	0 +/- 0	4.33 +/- 0.06	B	SB	B	SB	B	SB	B	SB	B	SB	NB-**B**	NB-**SB**
	TMW 1.1285	TMW 1.1285	DSM 20692	spoiled beer	yes	B	MB	B	MB	8 +/- 0	0.06 +/- 0.01	4.72 +/- 0.01	5 +/- 0	0.01 +/- 0	4.42 +/- 0.01	5 +/- 0	0.01 +/- 0	4.31 +/- 0	no - 60	0 +/- 0	4.38 +/- 0												
	TMW 1.1286	TMW 1.1286	DSM 20692[NB]	spoiled beer	yes	B	MB	B	MB	7 +/- 1	0.06 +/- 0.01	4.71 +/- 0.02	5 +/- 0	0.01 +/- 0	4.41 +/- 0	5 +/- 0	0.01 +/- 0	4.31 +/- 0	no - 60	0 +/- 0	4.39 +/- 0												
	TMW 1.1433	TMW 1.1433		spoiled beer	yes	B	MB	B	MB	3 +/- 0	0.07 +/- 0	4.69 +/- 0	4 +/- 0	0.02 +/- 0	4.4 +/- 0	7 +/- 0	0.01 +/- 0	4.3 +/- 0	no - 60	0 +/- 0	4.38 +/- 0												
	MB 282	TMW 1.1993		beer	yes	NB-B	NB-SB	B	MB	3 +/- 0	0.09 +/- 0.02	4.69 +/- 0.08	14 +/- 0	0.02 +/- 0	4.46 +/- 0.01	30 +/- 0	0.01 +/- 0	4.34 +/- 0	no - 60	0.01 +/- 0	4.4 +/- 0												
	BLQ 612	TMW 1.2006		brewery environment	yes	**NB**-B	**NB**-SB	**NB**	NB	5 +/- 0	0.03 +/- 0.01	4.66 +/- 0.04	no - 60	0 +/- 0	4.47 +/- 0	no - 60	0 +/- 0	4.46 +/- 0.02	no - 60	0 +/- 0	4.34 +/- 0.05	B	SB	B	SB	B	SB	B	SB	NB	WB	**NB**-B	**WB**-MB
	V 11	TMW 1.2007		brewery environment	yes	NB-**B**	NB-**SB**	B	SB	5 +/- 0	0.04 +/- 0.03	4.66 +/- 0.05	16 +/- 7	0.04 +/- 0.03	4.42 +/- 0.04	11 +/- 1	0.03 +/- 0.01	4.2 +/- 0.05	12 +/- 1	0.04 +/- 0.02	4.16 +/- 0												
	MB 324	TMW 1.2008		brewery environment	yes	B	MB-**SB**	B	SB	5 +/- 0	0.05 +/- 0.02	4.61 +/- 0.03	10 +/- 3	0.03 +/- 0.02	4.39 +/- 0.09	7 +/- 1	0.06 +/- 0.03	4.14 +/- 0.08	7 +/- 1	0.06 +/- 0.02	4.05 +/- 0	B	SB	B	SB	B	SB	B	SB	NB	NB	NB	NB
	BS 38	TMW 1.2009		brewery environment	yes	NB-**B**	WB-**SB**	B	SB	5 +/- 0	0.06 +/- 0.04	4.66 +/- 0.01	12 +/- 2	0.03 +/- 0.02	4.47 +/- 0.02	7 +/- 1	0.02 +/- 0.02	4.21 +/- 0.05	8 +/- 1	0.03 +/- 0.03	4.08 +/- 0	B	SB	B	SB	B	SB	B	SB	NB	WB	NB	WB
	V 7	TMW 1.2080		brewery environment	yes	B	SB	B	SB	3 +/- 0	0.25 +/- 0.05	3.98 +/- 0	no - 60	0.01 +/- 0	4.46 +/- 0	4 +/- 1	0.08 +/- 0.02	3.75 +/- 0.01	5 +/- 0	0.07 +/- 0.02	3.82 +/- 0												
	V 9	TMW 1.2081		brewery environment	yes	B	SB	B	SB	3 +/- 0	0.26 +/- 0.03	3.99 +/- 0	no - 60	0 +/- 0	4.45 +/- 0	5 +/- 0	0.06 +/- 0.01	3.75 +/- 0.01	3 +/- 0	0.06 +/- 0.02	3.8 +/- 0												
	MB 33	TMW 1.2082		brewery environment	yes	B	SB	B	SB	4 +/- 0	0.31 +/- 0.02	4.03 +/- 0.01	30 +/- 0	0.01 +/- 0	4.58 +/- 0.01	4 +/- 0	0.16 +/- 0	3.88 +/- 0	4 +/- 0	0.15 +/- 0.03	3.94 +/- 0												
	TMW 1.456	TMW 1.456		brewery environment	yes	B	SB	B	SB	3 +/- 0	0.21 +/- 0.05	3.94 +/- 0.02	no - 60	0 +/- 0	4.44 +/- 0.01	5 +/- 0	0.06 +/- 0.01	3.71 +/- 0.01	11 +/- 0	0.13 +/- 0.01	3.77 +/- 0												
	TMW 1.481	TMW 1.481		brewery environment	yes	B	MB	B	MB	2 +/- 0	0.04 +/- 0	4.77 +/- 0.01	6 +/- 0	0.02 +/- 0	4.44 +/- 0	8 +/- 0	0.01 +/- 0	4.35 +/- 0	no - 60	0 +/- 0	4.44 +/- 0												
Lactobacillus paracollinoides very high hazard potential	TMW 1.696	TMW 1.696	DSM 15502[T]	brewery environment	yes	NB	WB	NB	WB	12 +/- 2	0.2 +/- 0.02	4.16 +/- 0.11	7 +/- 0	0.19 +/- 0.01	3.77 +/- 0.01	no - 60	0 +/- 0	4.29 +/- 0.01	no - 60	0 +/- 0	4.38 +/- 0.02												
	TMW 1.1979	TMW 1.1979	DSM 20197	beer	yes	NB	NB	NB	NB	no - 60	0.02 +/- 0	4.88 +/- 0	no - 60	0 +/- 0	4.41 +/- 0.02	no - 60	0.01 +/- 0	4.3 +/- 0	no - 60	0.01 +/- 0	4.4 +/- 0	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB	NB
	V 8	TMW 1.1994		brewery environment	yes	B	SB	B	SB	2 +/- 0	0.21 +/- 0	4.06 +/- 0.01	4 +/- 0	0.07 +/- 0	4.12 +/- 0.01	6 +/- 0	0.04 +/- 0	3.94 +/- 0.01	10 +/- 0	0.01 +/- 0	4.24 +/- 0.01	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	MB 480	TMW 1.1995		pilsner beer	yes	B	SB	B	SB	4 +/- 0	0.41 +/- 0.01	3.89 +/- 0.02	3 +/- 0	0.29 +/- 0.01	3.78 +/- 0	7 +/- 0	0.22 +/- 0.01	3.82 +/- 0.02	7 +/- 0	0.2 +/- 0.02	3.83 +/- 0	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
	MB 426	TMW 1.2010		pilsner beer	yes	B	SB	B	SB	4 +/- 1	0.34 +/- 0.04	3.64 +/- 0.12	3 +/- 1	0.19 +/- 0.02	3.6 +/- 0.16	6 +/- 1	0.14 +/- 0.02	3.67 +/- 0.05	6 +/- 1	0.2 +/- 0.04	3.75 +/- 0.1												

Supplementary Table 2 **Genomes included for comparitive genomics. For each strain the corresponding assembly accession is given, as well as assembly level and the isolation source (only differentiating between brewery / non-brewery). Note that we analyzed and compared the genomes based on RAST annotations in order to get comparable annotations**

Species	Strain	Assembly accession	Assembly level	Isolation source (brewery / non-brewery)
Lactobacillus acetotolerans	NBRC 13120	GCA_001042405.1	Complete Genome	non-brewery
Lactobacillus acidophilus	30SC	GCA_000191545.1	Complete Genome	non-brewery
Lactobacillus acidophilus	FSI4	GCA_000934625.1	Complete Genome	non-brewery
Lactobacillus acidophilus	La-14	GCA_000389675.2	Complete Genome	non-brewery
Lactobacillus acidophilus	NCFM	GCA_000011985.1	Complete Genome	non-brewery
Lactobacillus amylovorus	GRL 1112	GCA_000182855.2	Chromosome	non-brewery
Lactobacillus amylovorus	GRL1118	GCA_000194115.1	Complete Genome	non-brewery
Lactobacillus backii	TMW 1.1988	GCA_001663655.1	Complete Genome	brewery
Lactobacillus backii	TMW 1.1989	GCA_001663675.1	Complete Genome	brewery
Lactobacillus backii	TMW 1.1991	GCA_001663715.1	Complete Genome	brewery
Lactobacillus backii	TMW 1.1992	GCA_001663735.1	Complete Genome	brewery
Lactobacillus backii	TMW 1.2002	GCA_001663755.1	Complete Genome	brewery
Lactobacillus brevis	TMW 1.465	GCA_000833395.1	Contig	brewery
Lactobacillus brevis	TMW 1.313	GCA_000833405.1	Contig	brewery
Lactobacillus brevis	TMW 1.6	GCA_000833415.1	Contig	non-brewery
Lactobacillus brevis	ATCC 367	GCA_000014465.1	Complete Genome	non-brewery
Lactobacillus brevis	BSO 464	GCA_000807975.1	Chromosome	brewery
Lactobacillus brevis	KB290	GCA_000359625.1	Complete Genome	non-brewery
Lactobacillus buchneri	CD034	GCA_000298115.2	Complete Genome	non-brewery
Lactobacillus buchneri	NRRL B-30929	GCA_000211375.1	Complete Genome	non-brewery
Lactobacillus casei	12A	GCA_000309565.2	Complete Genome	non-brewery
Lactobacillus casei	ATCC 334	GCA_000014525.1	Complete Genome	non-brewery
Lactobacillus casei	BD-II	GCA_000194765.1	Complete Genome	non-brewery
Lactobacillus casei	BL23	GCA_000026485.1	Complete Genome	non-brewery
Lactobacillus casei	LC2W	GCA_000194785.1	Complete Genome	non-brewery
Lactobacillus casei	LcA	GCA_000400585.1	Chromosome	non-brewery
Lactobacillus casei	LcY	GCA_000388095.2	Chromosome	non-brewery
Lactobacillus casei	LOCK919	GCA_000418515.1	Complete Genome	non-brewery
Lactobacillus casei	Zhang	GCA_000019245.3	Complete Genome	non-brewery
Lactobacillus casei	ATCC 393	GCA_000829055.1	Complete Genome	non-brewery
Lactobacillus casei	W56	GCA_000318035.1	Complete Genome	non-brewery
Lactobacillus coryniformis	KCTC 3167	GCA_000166795.1	Scaffold	non-brewery
Lactobacillus crispatus	ST1	GCA_000091765.1	Chromosome	non-brewery
Lactobacillus bulgaricus	2038	GCA_000191165.1	Complete Genome	non-brewery
Lactobacillus bulgaricus	ATCC 11842	GCA_000056065.1	Complete Genome	non-brewery
Lactobacillus bulgaricus	ATCC BAA-365	GCA_000014405.1	Complete Genome	non-brewery
Lactobacillus bulgaricus	ND02	GCA_000182835.1	Complete Genome	non-brewery
Lactobacillus farciminis	CNCM-I-3699-S	GCA_001046795.1	Chromosome	non-brewery
Lactobacillus farciminis	CNCM-I-3699-R	GCA_001188635.1	Chromosome	non-brewery
Lactobacillus fermentum	3872	GCA_000466785.3	Complete Genome	non-brewery
Lactobacillus fermentum	CECT 5716	GCA_000210515.1	Complete Genome	non-brewery
Lactobacillus fermentum	F-6	GCA_000397165.1	Complete Genome	non-brewery
Lactobacillus fermentum	IFO 3956	GCA_000010145.1	Complete Genome	non-brewery
Lactobacillus gasseri	130918	GCA_000814885.1	Complete Genome	non-brewery
Lactobacillus gasseri	ATCC 33323	GCA_000014425.1	Complete Genome	non-brewery
Lactobacillus ginsenosidimutans	EMML 3141	GCA_001050475.1	Complete Genome	non-brewery
Lactobacillus helveticus	CNRZ32	GCA_000422165.1	Complete Genome	non-brewery
Lactobacillus helveticus	DPC 4571	GCA_000015385.1	Complete Genome	non-brewery
Lactobacillus helveticus	H10	GCA_000189515.1	Complete Genome	non-brewery
Lactobacillus helveticus	H9	GCA_000525715.1	Complete Genome	non-brewery
Lactobacillus helveticus	KLDS1.8701	GCA_000961015.1	Complete Genome	non-brewery
Lactobacillus helveticus	MB2-1	GCA_001006025.1	Complete Genome	non-brewery
Lactobacillus helveticus	R0052	GCA_000165775.3	Complete Genome	non-brewery
Lactobacillus hokkaidonensis	LOOC260	GCA_000829395.1	Complete Genome	non-brewery
Lactobacillus jensenii	JV-V16	GCA_000159335.1	Chromosome	non-brewery

Species	Strain	Assembly accession	Assembly level	Isolation source (brewery / non-brewery)
Lactobacillus johnsonii	DPC 6026	GCA_000204985.1	Complete Genome	non-brewery
Lactobacillus johnsonii	FI9785	GCA_000091405.1	Complete Genome	non-brewery
Lactobacillus johnsonii	N6.2	GCA_000498675.1	Complete Genome	non-brewery
Lactobacillus johnsonii	NCC 533	GCA_000008065.1	Complete Genome	non-brewery
Lactobacillus kefiranofaciens	ZW3	GCA_000214785.1	Complete Genome	non-brewery
Lactobacillus koreensis	26-25	GCA_001050435.1	Complete Genome	non-brewery
Lactobacillus lindneri	TMW 1.481	GCA_001702135.1	Complete Genome	brewery
Lactobacillus lindneri	TMW 1.1993	GCA_001702115.1	Complete Genome	brewery
Lactobacillus mucosae	LM1	GCA_000248095.3	Complete Genome	non-brewery
Lactobacillus paracasei	CAUH35	GCA_001191565.1	Complete Genome	non-brewery
Lactobacillus paracasei	L9	GCA_001244395.1	Complete Genome	non-brewery
Lactobacillus paracasei	N1115	GCA_000582665.1	Complete Genome	non-brewery
Lactobacillus paracasei	8700:02:00	GCA_000155515.2	Complete Genome	non-brewery
Lactobacillus paracasei	JCM 8130	GCA_000829035.1	Complete Genome	non-brewery
Lactobacillus paracollinoides	TMW 1.1979	GCA_001702155.1	Complete Genome	brewery
Lactobacillus paracollinoides	TMW 1.1994	GCA_001702175.1	Complete Genome	brewery
Lactobacillus paracollinoides	TMW 1.1995	GCA_001702195.1	Complete Genome	brewery
Lactobacillus pentosus	KCA1	GCA_000271445.1	Chromosome	non-brewery
Lactobacillus plantarum	16	GCA_000412205.1	Complete Genome	non-brewery
Lactobacillus plantarum	B21	GCA_000931425.1	Complete Genome	non-brewery
Lactobacillus plantarum	CMPG5300	GCA_000762955.1	Chromosome	non-brewery
Lactobacillus plantarum	JDM1	GCA_000023085.1	Complete Genome	non-brewery
Lactobacillus plantarum	CGMCC 1.557	GCA_001272315.1	Chromosome	non-brewery
Lactobacillus plantarum	P8	GCA_000392485.2	Complete Genome	non-brewery
Lactobacillus plantarum	ST-III	GCA_000148815.2	Complete Genome	non-brewery
Lactobacillus plantarum	WCFS1	GCA_000203855.3	Complete Genome	non-brewery
Lactobacillus plantarum	ZJ316	GCA_000338115.2	Complete Genome	non-brewery
Lactobacillus reuteri	IRT	GCA_001046835.1	Complete Genome	non-brewery
Lactobacillus reuteri	DSM 20016	GCA_000016825.1	Complete Genome	non-brewery
Lactobacillus reuteri	I5007	GCA_000410995.1	Complete Genome	non-brewery
Lactobacillus reuteri	JCM 1112	GCA_000010005.1	Complete Genome	non-brewery
Lactobacillus reuteri	SD2112	GCA_000159455.2	Complete Genome	non-brewery
Lactobacillus reuteri	TD1	GCA_000439275.1	Complete Genome	non-brewery
Lactobacillus rhamnosus	ATCC 8530	GCA_000233755.1	Complete Genome	non-brewery
Lactobacillus rhamnosus	ATCC 53103	GCA_000011045.1	Complete Genome	non-brewery
Lactobacillus rhamnosus	Lc 705	GCA_000026525.1	Complete Genome	non-brewery
Lactobacillus rhamnosus	LOCK900	GCA_000418475.1	Complete Genome	non-brewery
Lactobacillus rhamnosus	LOCK908	GCA_000418495.1	Complete Genome	non-brewery
Lactobacillus rossiae	DSM 15814	GCA_000428925.1	Scaffold	non-brewery
Lactobacillus ruminis	ATCC 27782	GCA_000224985.1	Complete Genome	non-brewery
Lactobacillus sakei	23K	GCA_000026065.1	Complete Genome	non-brewery
Lactobacillus salivarius	JCM1046	GCA_000758365.1	Complete Genome	non-brewery
Lactobacillus salivarius	CECT 5713	GCA_000143435.1	Complete Genome	non-brewery
Lactobacillus salivarius	Ren	GCA_001011095.1	Complete Genome	non-brewery
Lactobacillus salivarius	UCC118	GCA_000008925.1	Complete Genome	non-brewery
Lactobacillus sanfranciscensis	TMW 1.1304	GCA_000225325.1	Complete Genome	non-brewery
Lactobacillus sp.	wkB8	GCA_000761135.1	Complete Genome	non-brewery
Lactococcus lactis	IL1403	GCA_000006865.1	Complete Genome	non-brewery
Leuconostoc mesenteroides	ATCC 8293	GCA_000014445.1	Complete Genome	non-brewery
Pediococcus claussenii	ATCC BAA-344	GCA_000237995.2	Complete Genome	brewery
Pediococcus claussenii	TMW 2.53	GCA_001702215.1	Complete Genome	brewery
Pediococcus claussenii	TMW 2.54	GCA_001702235.1	Complete Genome	brewery
Pediococcus damnosus	TMW 2.1532	GCA_001611035.1	Complete Genome	brewery
Pediococcus damnosus	TMW 2.1533	GCA_001611075.1	Complete Genome	brewery
Pediococcus damnosus	TMW 2.1534	GCA_001611115.1	Complete Genome	brewery
Pediococcus damnosus	TMW 2.1535	GCA_001611135.1	Complete Genome	brewery
Pediococcus damnosus	TMW 2.1536	GCA_001611155.1	Complete Genome	non-brewery
Pediococcus pentosaceus	ATCC 25745	GCA_000014505.1	Complete Genome	non-brewery
Pediococcus pentosaceus	SL4	GCA_000496265.1	Complete Genome	non-brewery

Supplementary Table 3 **Primer sequences for relevant diagnostic marker genes (corresponding to primer names). All sequences are given 5' to 3'.**

Primer pair ID(S)	Forward Primer	Reverse primer
616V/609R (16S rDNA)	AGACTTTGATYMTGGCTCAG	ACTACYVGGGTATCTAAKCC
M05 (fabZ, FAS cluster)	ATTGAGGCAATGGCTCAGAC	CGATCCGTGACCTAATCCAATG
hitA	TTGCAATCAATGGCTGCTCG	TGCGGTCCCGCTAAGAATAC
horA	AATCTTAACCCTGCCGGTGG	TGGATTCGAGTGGTTGAGCC
horC	TACACAGAAACCCGTTCACC	CTGTGCGCTAATTCGTGATG
M37	GGCTTRTTTGCCGATRTG	ACGTCGCCTTGATGRTGG
M42 (npx)	TCCAGCAGGTAAGCCAATG	CGCAGTAGCGAAGTGATAGTC