Title

Metataxonomic insights in the distribution of *Lactobacillaceae* in foods and food environments

Authors

Eugenio Parente[1*], Teresa Zotta[1], Marilisa Giavalisco[1], Annamaria Ricciardi[1]

[1] Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Potenza, Italy

***Corresponding author**

Prof. Eugenio Parente

E-mail: eugenio.parente@unibas.it

Tel.: +39-0971-205561

Abstract

Members of the family *Lactobacillaceae*, which now includes former members of the genera *Lactobacillus* and *Pediococcus*, but also member of the family *Leuconostocaceae*, are of foremost importance in food fermentations and spoilage, but also as components of animal and human microbiota and as potentially pathogenic microorganisms. Knowledge of the ecological distribution of a given species and genus is important, among other things, for the inclusion in lists of microorganisms with a Qualified Presumption of Safety or with beneficial use. The objective of this work is to use the data in FoodMicrobionet database to obtain quantitative insights (in terms of both abundance and prevalence) on the distribution of these bacteria in foods and food environments.

We first explored the reliability of taxonomic assignments using the SILVA v138.1 reference database with full length and partial sequences of the 16S rRNA gene for type strain sequences. Full length 16S rRNA gene sequences allow a reasonably good classification at the genus and species level in phylogenetic trees but shorter sequences (V1-V3, V3-V4, V4) perform much worse, with type strains of many species sharing identical V4 and V3-V4 sequences. Taxonomic assignment at the genus level of 16S rRNA genes sequences and the SILVA v138.1 reference database can be done for almost all genera of the family *Lactobacillaceae* with a high degree of confidence for full length sequences, and with a satisfactory level of accuracy for the V1-V3 regions. Results for the V3-V4 and V4 region are still acceptable but significantly worse. Taxonomic assignment at the species level for sequences for the V1-V3, V3-V4, V4 regions of the 16S rRNA gene of members of the family *Lactobacillaceae* is hardly possible and, even for full length sequences, and only 49.9% of the type strain sequences can be unambiguously assigned to species.

We then used the FoodMicrobionet database to evaluate the prevalence and abundance of *Lactobacillaceae* in food samples and in food related environments. Generalist and specialist genera were clearly evident. The lifestyle of several genera was confirmed and insights on the distribution and potential origin of rare genera (*Dellaglioa, Holzapfelia, Schleiferilactobacillus*) were obtained.

We also found that combining Amplicon Sequence Variants from different studies is indeed possible, but provides little additional information, even when strict criteria are used for the filtering of sequences.

Key words

Lactobacillaceae; metataxonomic database; microbial ecology; fermentation; FoodMicrobionet

1. Introduction

Bacteria belonging to family *Lactobacillaceae* (which now includes *Leuconostocaceae*, Zheng et al., 2020) are of foremost importance as beneficial microorganisms in food fermentations (Anagnostopoulos and Tsaltas, 2022; Arora et al., 2020; Bourdichon et al., 2012; 2019; Gänzle, 2022; Laranjo et al., 2019; Lee et al., 2020; Mayo et al., 2021; Sumby et al., 2014; Tamang et al., 2020; Walsh et al., 2020), as components of human and animal microbiota (Duar et al., 2017; Pasolli et al., 2020), as probiotics (Deng et al., 2022; Sanders et al., 2019), but also as detrimental spoilage (Doulgeraki et al., 2012; Gänzle, 2015; Pothakos et al., 2015; Xu et al., 2020) and opportunistic pathogenic microorganisms (Colautti et al., 2021; Moniente et al., 2021).

After a recent reorganization of its taxonomy (Qiao et al., 2022), the family *Lactobacillaceae* includes the vast majority of genera of lactic acid bacteria, ranging from members of the former genus *Lactobacillus*, whose species have been divided in 23 new genera (Zheng et al., 2020) and members of family *Leuconostocaceae* (*Weissella*, *Periweissella*, *Fructobacillus*, *Convivina*, *Oenococcus*, *Leuconostoc*). The new taxonomy is largely based on comparative genomics (Zheng et al., 2020). It allows to clearly separate genera and species on the basis of their physiology (heterofermentative vs. homofermentative species), ecology and lifestyle (Duar et al., 2017; Qiao et al., 2022; Zheng et al., 2020).

The genus *Leuconostoc* has also been recently reinvestigated by comparative genomic analysis (Kumar et al., 2022) and this may lead to a reclassification of species in the near future.

Lifestyle of *Lactobacillaceae* ranges from vertebrate-adapted (the majority of *Lactobacillus* *Ligilactobacillus, Limosilactobacillus*) or insect-adapted (*Apilactobacillus*, *Bombilactobacillus*, *Fructilactobacillus,* some *Lactobacillus*), environmental or plant associated (*Levilactobacillus*), free living (*Agrilactobacillus, Latilactobacillus, Lentilactobacillus, Levilactobacillus, Liquorilactobacillus, Paucilactobacillus, Schleiferilactobacillus, Secundilactobacillus*), nomadic (*Lacticaseibacillus, Lactiplantibacillus*) or with unknown ecology (*Companilactobacillus, Dellaglioa, Furfurilactobacillus, Holzapfelia, Lapidilactobacillus,* some *Ligilactobacillus, Loigolactobacillus*) (Duar et al., 2017; Zheng et al., 2020) (see also http://www.lactobacillus.uantwerpen.be/).

Members of the genus *Pediococcus* are frequently associated with vegetable fermentations, but can be isolated from a wide variety of habitats (Franz et al., 2014). The same applies for members of the genera *Leuconostoc* (Bjorkröth et al., 2014a) and *Weissella* (Bjorkröth et al., 2014b) while *Oenococcus* (Endo et al., 2014a), *Fructobacillus* (Endo et al., 2014b) and the new genus *Periweissella* (Bello et al., 2022) are more strictly associated with plants and vegetable fermentations.

Information on lifestyle and on history of safe use of microorganisms is important for their inclusion in lists of beneficial microorganisms and is one of the aspects EFSA considers for the inclusion (upon request) of a microbial species in the list of microorganisms with a Qualified Presumption of Safety (Bourdichon et al., 2012, 2019). For several reasons, this list is not a positive and comprehensive list of microorganisms used in food fermentations. On the other hand, the Inventory of Microbial Food Cultures (IMFC) with safety demonstration in fermented food products (Bourdichon et al., 2012, 2019) has this ambition and its 2022 update (Bourdichon et al., 2022) includes 314 species, 110 of which belong to the family *Lactobacillaceae*. The criteria for inclusion in IMFC list require clear identification at the

species level and demonstration, based on the scientific literature, of lack of undesirable properties (including virulence traits). Proven association of a species with a food matrix is essential.

Lifestyle and ecological niche of former members of the genus *Lactobacillus* (Duar et al., 2017; Qiao et al., 2022; Zheng et al., 2015; 2020) has been identified using an integrated approach based on source and frequency of isolation and on occurrence of genes and metabolic traits. However, especially within the genera with a nomadic lifestyle (*Lactiplantibacillus*, *Lacticaseibacillus*), niche adaptation traits have been clearly identified for different subgroups in comparative genomic studies (Douillard et al., 2013; Li et al., 2022; Wuyts et al., 2017). Further information on the lifestyle and ecology of *Lactobacillaceae* can be found in recent large metagenomic studies (Pasolli et al., 2020; Walsh et al., 2020), which also used quantitative information on prevalence and abundance to formulate and test hypotheses on lifestyle and on functional properties. Detailed prevalence and abundance data would be extremely valuable to get further insights on the distribution and specialization of *Lactobacillaceae* in foods and to identify sources of contamination.

Our research group has created and maintains the FoodMicrobionet database (Parente et al., 2016), which makes metataxonomic data on the abundance and prevalence of bacteria in foods and food related environments readily accessible. The database has been steadily growing both in size and in functions (Parente et al., 2019; 2022) and is publicly available on both GitHub (https://github.com/ep142/FoodMicrobionet/) and Mendeley data (https://data.mendeley.com/datasets/8fwwjpm79y/6). The most recent iteration of FoodMicrobionet uses SILVA v138.1 as a taxonomic reference database (https://www.arb-silva.de/documentation/release-1381/ last accessed in July 2022). This allows the taxonomic assignment of Amplicon Sequence Variants (ASV) to the genera proposed by Zheng et al. (2020) but does not allow the taxonomic assignment for the new genera *Convivina* and *Periweissella* (Bello et al., 2022; Praet et al., 2015). FoodMicrobionet includes studies which have used a large variety of target regions within the 16S gene, although those using V1-V3, V3-V4 and V4 represent the vast majority. Taxonomic assignment is performed using functions of the DADA2 package (Callahan et al., 2016) and, whenever possible, assignment down to the species level is carried out. The ability to perform species level assignment in metataxonomic studies has been questioned, but there is a consensus on the reliability of assignments at the genus level or above (Bokulich et al., 2020; Johnson et al., 2019; Pollock et al., 2018). One of the main strengths of FoodMicrobionet, however, is the level of annotation of sample and study metadata. This makes it more effective in data mining for occurrence and abundance of bacteria in food and food environments compared to other databases, like the Integrated Microbial Next Generation Sequencing database (Lagkouvardos et al., 2016), which does not have a consistent system for classification of habitats and Omnicrobe (formerly Florilege, Falentin et al., 2017), which uses text mining and does not provide directly abundance data.

The objective of this work is to use the data in FoodMicrobionet to obtain quantitative insights (in terms of both abundance and prevalence) on the distribution of members of family *Lactobacillaceae* in foods and food environments. Since identification species level information is needed for the inclusion of microorganisms in the QPS or IMFC list, we also evaluated to what extent taxonomic assignment at or below the genus level was feasible for data obtained using different 16S rRNA gene hypervariable regions.

2. Materials and methods

Full length sequences of the 16S rRNA gene for type strains of members of family *Lactobacillaceae* (as defined by Zheng et al., 2020; **Supplementary Table 1**) were obtained from LPSN (https://lpsn.dsmz.de). Whenever these sequences were too short (<1,300 bp) or had more than 5 ambiguous nucleotides, replacement sequences were obtained using GenBank or NCBI RefSeq (https://www.ncbi.nlm.nih.gov/refseq/) accession number. Sequences, as .fasta files, were imported in R (R Core Team, 2022) using functions of the package phylotools (Zhang, 2017). Shorter regions (V1-V3, V3-V4, V4) frequently used as targets in 16S metagenomics were extracted by identifying the position of commonly used primers (28F, 341F, 515F, 805R).

Alignment and inference of the phylogenetic tree (for the full sequence and for regions V1-V3, V3-V4 and V4) were performed using functions of the packages DECIPHER (Wright, 2016) and phangorn (Schliep, 2011). phylogenetic tools were annotated and plotted using functions of the packages treeio (Wang et al., 2019), tidytree (Yu, 2022), and ggtree (Yu, 2020).

kmer (8 mer) distance and inference of OTUs (at 97 and 98.65% similarity) were performed using functions of the package kmer (Wilkinson, 2018).

An example script (lactobacillaceae_type_strains_1_4.R) is available on GitHub (https://github.com/ep142/FoodMicrobionet/tree/master/WIMB), together with FASTA files for sequences and metadata.

Taxonomy assignment was performed for full sequences and regions using functions assignTaxonomy() and addSpecies() of the dada2 package (Callahan et al., 2016) using SILVA v138.1 reference databases (https://zenodo.org/record/4587955#.YtGRqexBz0o). Confusion matrices were obtained using the function confusionMatrix() of package caret (Kuhn, 2022).

In an attempt to assign reference sequences from SILVA to species, kmer (5-mer) distance matrix was calculated and sequences were assigned to the species to which the type strain sequence with the shortest distance belonged. A custom reference database for assignment at the species level was built.

Taxonomy assignment was performed again on both the full sequences and on V1-V3, V3-V4 and V4 regions of type strains to test the ability of the new database to obtain reliable assignments. The allowMultiple() option was used to assign, in case of ambiguous matches, up to three of the closest species matches.

Prevalence and abundance data for all genera were extracted, tabled and plotted from FoodMicrobionet (version 4.2) for both food and food environment samples using a custom script (https://github.com/ep142/FoodMicrobionet/tree/master/WIMB). Only studies targeting V1-V3 or V3-V4 regions of 16S rRNA gene were used. Metadata used for annotation for the graphs included both the food sample/environment classification using the FoodEx2 system (EFSA, 2015) and data from the FoodMicrobionet samples table with information on occurrence of spoilage and fermentation.

For genera which are represented by only one or very few species (*Dellaglioa, Holzapfelia, Schleiferilactobacillus*) further analysis of the diversity of Amplicon Sequence Variants was performed. ASVs were extracted from phyloseq objects (available at Zenodo https://doi.org/10.5281/zenodo.6954039) using a custom script (https://github.com/ep142/FoodMicrobionet/tree/master/WIMB), and further filtered on

228 the basis of target region, length, quality etc. Annotated phylogenetic trees showing sources
229 of ASVs and their maximum relative abundance were created as described above.
230
231
232 **3. Results and discussion**
233
234 Metataxonomic studies offer great opportunities for obtaining quantitative insights in the
235 ecology of microbial taxa in foods and other environments. The marker gene which is most
236 frequently used for bacterial communities is the 16S rRNA gene. Due to the limitations of
237 next generation sequencing methods, hypervariable regions (usually V1-V3, V3-V4 and V4
238 (Parente et al., 2022; Pollock et al., 2018) are used with amplicon length (after primer
239 removal and merging of paired end sequences for Illumina sequencing) of <450 bp. In order
240 to test the limitations of this approach in the study of the ecology of *Lactobacillaceae*, we
241 retrieved from public databases sequences and the type strains of family *Lactobacillaceae*
242 and used them to infer phylogenetic trees based on full sequences and to carry out
243 taxonomic assignment using the SILVA 138.1 reference database as described in Materials
244 and Methods. The list of strains and accession number of 16S rRNA gene sequences used in
245 this study is shown in **Supplementary Table 1**, while a FASTA file with the sequences is
246 available on GitHub (https://github.com/ep142/FoodMicrobionet/tree/master/WIMB). A
247 simplified phylogenetic tree showing the relationships between type strains of the species
248 belonging to the family *Lactobacillaceae* on the basis of sequences of 16S rRNA gene is
249 shown in **Figure 1**, while a detailed tree is shown as Supplementary Material.
250 All genera were grouped in monophyletic clusters with very few exceptions (*Leuconostoc*
251 *fallax* and *Weissella muntiaci* and *W. oryzae*). As expected phylogenetic relationships differ
252 from those inferred on the basis of WGS (Qiao et al., 2022; Zheng et al., 2020) and do not
253 match lifestyle or metabolism.
254 Use of shorter regions within the 16S rRNA results in an even worse classification, with
255 sequences of many genera grouped in non-monophyletic clusters (**Supplementary Figures**
256 **1, 2, 3**) and is clearly worse for shorter sequences (V4). In addition, for both the V3-V4 and
257 V4 regions many sequences were identical or almost identical.
258 When sequences for type strains are assigned to OTUs using kmers (5-mers) the number of
259 OTUs retrieved at both the 97% and 98.65% (the similarity threshold at species level
260 suggested by Qiao et al., 2022) was lower than the number of species in the family
261 *Lactobacillaceae*. In fact, while there were 354 validly published species at the time of
262 writing of this paper, OTUs detected using function otu() of the kmer package for the list of
263 species in **Supplementary Table 1** were only 285, 315, 207 and 169 for full length
264 sequences, V1-V3, V3-V4 and V4 regions, respectively.
265 Discrimination between closely related species on the basis of full length and, to a larger
266 extent, shorter regions of the 16S rRNA gene, was problematic with genera *Apilactobacillus*,
267 *Companilactobacillus, Fructobacillus, Lacticaseibacillus, Lactiplantibacillus, Lactobacillus,*
268 *Lapidilactobacillus, Latilactobacillus, Lentilactobacillus, Leuconostoc, Levilactobacillus,*
269 *Liquorilactobacillus, Loigolactobacillus, Paucilactobacillus, Pediococcus, Periweissella,*
270 *Secundilactobacillus, Weissella, Acetilactobacillus*.

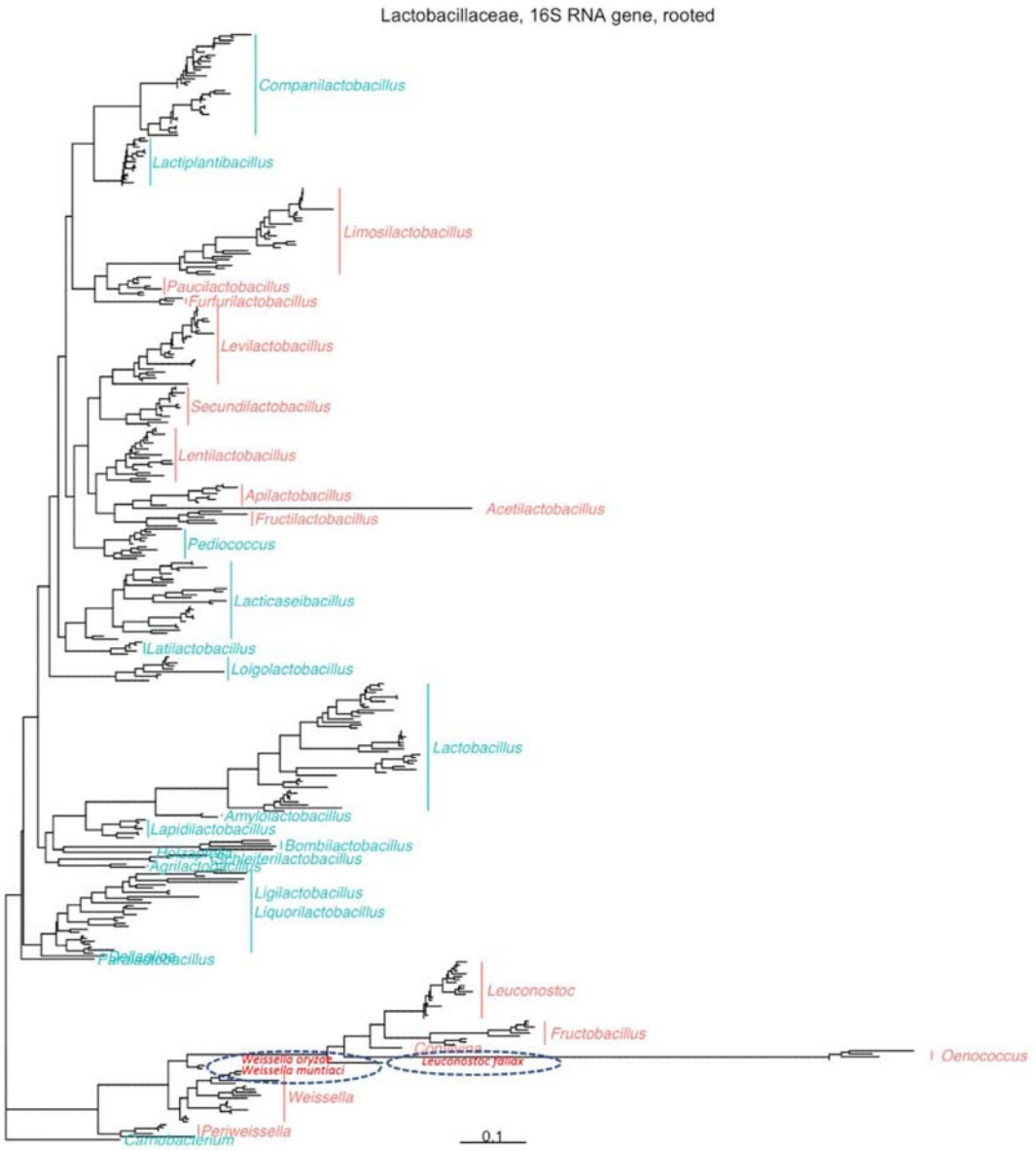

Figure 1. Maximum-likelihood phylogenetic tree showing the evolutionary relationships among sequences of the 16S rRNA gene for type strains of species belonging to the family *Lactobacillaceae*. Clades for each genus are outlined in blue (homofermentative species) or red (heterofermentative species) and the three species which do not cluster with the corresponding genera. The scale (number of nucleotide substitutions per nucleotide) is shown. Bootstrap values were 100% for all genus-level clades (data not shown).

To obtain further information on the feasibility of taxonomic assignment at the genus and species level for full length and V1-V3, V3-V4 and V4 regions of 16S rRNA gene sequences for the family *Lactobacillaceae*, we used the SILVA v138.1 (Glöckner et al., 2017) reference database. The sensitivity and specificity of genus level assignments are shown in **Figure 2**. Data by region and genus are shown in **Supplementary Table 2**.

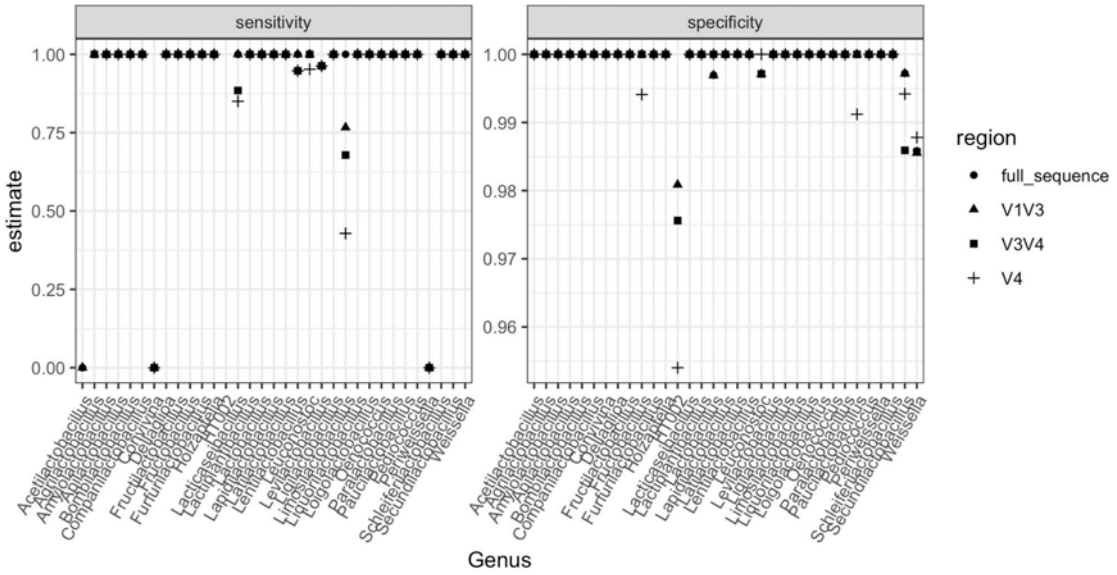

Figure 2. Sensitivity and specificity for taxonomic assignment of full length and V1-V3, V3-V4 and V4 region sequences of the 16S rRNA gene for type strains of species belonging to family *Lactobacillaceae*. Taxonomic assignment was performed using the function assignTaxonomy() of the dada2 package using the SILVA v1381 reference database.

The overall accuracy values for genus level assignment were: full sequence 0.979 (95% confidence interval 0.958-0.991); V1-V3 0.959 (0.933-0.977), V3-V4 0.946 (0.918-0.966), V4 0.922 (0.889-0.948). Accuracy clearly decreased with the length of the target sequence and, for comparable lengths, was worse for the V3-V4 region, compared to the V1-V3 region.

The 0 value for sensitivity for general *Acetilactobacillus*, *Convivina* and *Periweissella* reflects the lack of sequences assigned to these genera in the SILVA v138.1 database. Low level of sensitivity for shorter sequences of genus *Limosilactobacillus* and low levels of specificity for sequences labeled as "HT002" in the SILVA database, were due to taxonomic assignment of sequences of some type strains of *Limosilactobacillus* to "HT002". Relatively low values for specificity in taxonomic assignment of short sequences for *Secundilactobacillus* was due to the erroneous identification of some sequence of type strains of *Levilactobacillus*, *Lacticaseibacillus*, and *Lentilactobacillus* as belonging to *Secundilactobacillus*. Low specificity for *Weissella* was systematically due to the species which now belong to genus *Periweissella*.

We conclude that, with few exceptions, taxonomic assignment at the genus level of 16S rRNA genes sequences using the naïve Bayes classifier and the SILVA v138.1 reference database can be done for almost all genera of the family *Lactobacillaceae* with a very high degree of confidence for full length sequences, and with a satisfactory level of accuracy for

the V1-V3 regions. Results for the V3-V4 and V4 regions are still acceptable but significantly worse.

Lifestyle appears to be relatively constant within genera of the family *Lactobacillaceae* (Duar et al., 2017; Qiao et al., 2022; Zheng et al., 2020). On the other hand, several important genera have been found in a wide variety of niches, and, although their lifestyle has been defined as nomadic, evidence of niche adaptation exists for well-defined clades within important species (Douillard et al., 2013; Li et al., 2022; Wuyts et al., 2017). If species assignment was possible with a good degree of confidence in metataxonomic studies, one may exploit databases such as FoodMicrobionet to get more insight on the distribution of individual species within the family *Lactobacillaceae*.

Since the SILVA v138.1 database does not allow species assignment for sequences belonging to the former genus *Lactobacillus* we created a custom database in which the reference sequences for the 23 genera proposed by Zheng et al. (2020) were attributed to the same species of the type strain sequence with the smallest kmer distance. We then performed taxonomic assignment at the genus and species level for type strain sequences using functions of package dada2 (Callahan et al., 2016). The allowMultiple option was used to obtain multiple matches whenever a single, unambiguous match was not possible. The proportion of matches, partial matches and failure to match species identification is shown in **Figure 3**.

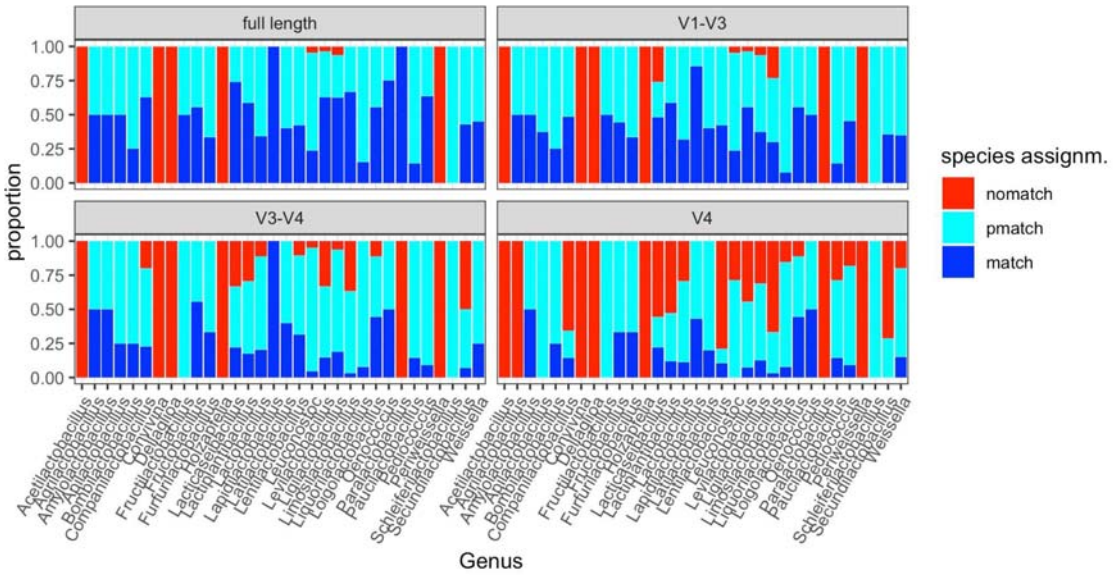

Figure 3. Proportion of correct taxonomic assignments for full-length and partial sequences (V1-V3, V3-V4, V4) of the 16S rRNA gene for type strains of the family *Lactobacillaceae*. Species assignment was performed using the addSpecies() function of the dada2 package, using a custom reference database derived from SILVA v138.1. match: the species assignment was unambiguous; pmatch the species assignment was ambiguous (more than one match returned by the naïve Bayes classifier) but included the correct identification; nomatch: no species assignment was returned.

The distribution within-genus of the proportion of matching species identifications is shown in **Supplementary Figure 4**. The median value for the within-genus proportion of matching species identification was 0.475, 0.366, 0.182 and 0.098 for full length sequences, V1-V3, V3-V4 and V4, respectively.

We conclude that taxonomic assignment down to the species level using the naïve Bayes classifier for sequences for the V1-V3, V3-V4, V4 regions of the 16S rRNA gene of members of the family *Lactobacillaceae* is hardly possible and that, even for full length sequences, only 49.9% of the type strain sequences can be unambiguously assigned to species. This low percentage of successful identification may be due to the relatively large number of closely related species in some genera *(Lactiplantibacillus*, *Lacticaseibacillus*, etc.) in which the within-genus similarity of 16S rRNA gene sequences is very high.

This confirms that use of 16S rRNA gene targets in metataxonomic studies can reliably resolve genus, but not species, unless the full-length gene is used (Bokulich et al., 2020; Johnson et al., 2019; Pollock et al., 2018). Custom reference databases have been claimed to improve the accuracy of taxonomic assignment (Meola et al., 2019) and, recently, the *cpn60* gene has demonstrated potential in the identification at the species level of short sequences of *Lactobacillaceae* (Links et al., 2012; Shukla and Hill, 2022). Alternatives to the naïve Bayes classifier (Wang et al., 2007) have also been claimed to improve taxonomic assignment. Further testing is needed to identify the best tool for the identification of *Lactobacillaceae* at the species level using metataxonomic data.

Third generation sequencing of the full length 16S rRNA gene is increasingly being used in metataxonomic studies and can indeed result in species level identification (Callahan et al., 2019; Johnson et al., 2019). However, the vast majority of metataxonomic studies still use short sequences of the 16S rRNA gene, and these data, when properly organized, can be exploited to evaluate the distribution of genera of *Lactobacillaceae* in foods and food environments.

We therefore exploited FoodMicrobionet for this purpose. *Lactobacillaceae* are found in 194 studies out of 211 and in 8,007 food or food environment samples out of 12,748. This confirms that they are a ubiquitous group. A prevalence and abundance table is provided in Supplementary Material.

Further analysis was carried out separately for food samples and food environment samples.

In a first step, we limited the analysis to food samples, with longer sequences (>300 bp) targeting the V1-V3 and V3-V4 regions, 125 studies and 5,393 samples and calculated prevalence and abundance for the L1 category of the EFSA FoodEx2 classification (EFSA, 2015, see **Supplementary Table 3**), which groups foods in large categories. This is a very conservative choice, because, for many genera, even shorter sequences (V4 region) would guarantee a reliable genus level identification. The results are shown in **Figure 4**.

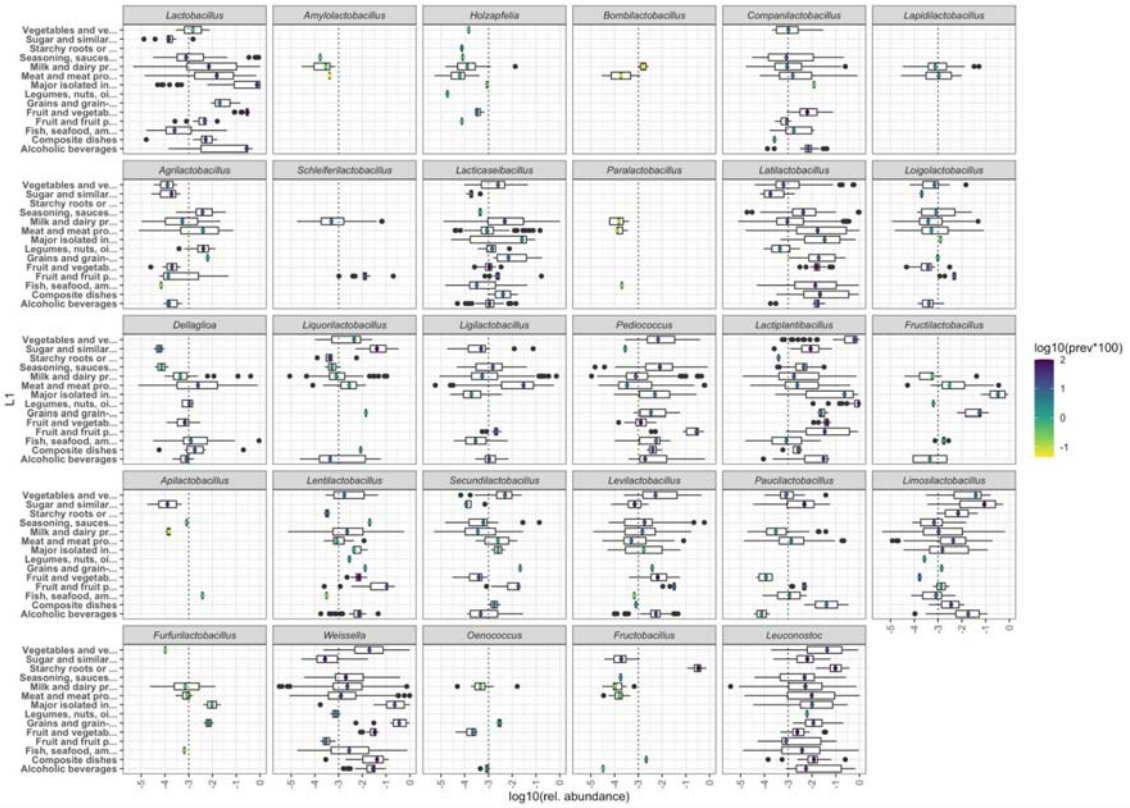

Figure 4. Boxplot showing the distribution of abundance of sequences (V1-V3 and V3-V4, length >350 bp) of genera of the family *Lactobacillaceae* in food samples. The prevalence is shown as the colour scale of a marker centred on the median. Foods are grouped on the basis of the L1 category of the EFSA FoodEx2 classification (EFSA, 2015). Names of the categories have been shortened in some cases. Refer to **Supplementary Table 3**. Genera in facets were ordered on the basis of their taxonomic relationships (Qiao et al., 2022; Zheng et al., 2020).

The level of aggregation provided by L1 may be too coarse for understanding the ecology of individual genera. We therefore recalculated prevalence and statistics for abundance for the L4 level of the FoodEx 2 classification. Obtaining a plot for all genera using this aggregation level is prohibitive because there are too many categories. However, a prevalence and abundance table is provided as Supplementary Material. Example plots for a few generalist (*Lacticaseibacillus, Latilactobacillus, Dellaglioa, Levilactobacillus, Weissella*) and specialist (*Holzapfelia*, *Schleiferilactobacillus, Liquorilactobacillus, Fructilactobacillus, Fructobacillus*) genera are shown in **Supplementary Figures 5** to **14**.

A few genera of the family *Lactobacillaceae* rarely appear in the sample set after filtering and almost never exceed a relative abundance of 0.01. These include *Amylolactobacillus* (unknown lifestyle in Zheng et al., 2020), *Holzapfelia* (unknown lifestyle in Zheng et al., 2020, but found with low prevalence and abundance in bovine muscle, cattle milk and cheese), *Bombilactobacillus* (insect associated), *Paralactobacillus* (unknown lifestyle), *Apilactobacillus* (insect associated). Since the original source of isolation of *Holzapfelia floricola* (the only species of this genus) was a flower and since *Bombilactobacillus* is insect associated, their presence in bovine meat or milk samples may be associated with pasture.

A few genera were clearly associated with specific food environments, in which they occasionally appeared with relatively high abundance. *Lapidilactobacillus* was found in dairy and meat products, although its lifestyle was described as unknown by Zheng et al. (2020). Type strains of this genus were isolated from pickles, distilled spirits, silage (as found in the BacDive database https://bacdive.dsmz.de/). Its origin may therefore be vegetable sources. *Schleiferilactobacillus* occurred in milk and fermented milk products, with occasionally high relative abundance (0.1-0.2) in firm ripened cheeses which were also spoiled and in fermented fruit products (**Supplementary Figure *7***). Its lifestyle has been described as free-living and type strains have been isolated from fermented vegetable products. *Dellaglioa* sequences were retrieved sometimes with a high prevalence and abundance from a variety of sources, and their relative abundance was highest in meat and meat products, with high abundances often associated with spoiled products (**Supplementary Figure 10**). The only species of this genus is *Dellaglioa algida*, which has an unknown lifestyle but whose strains, in accordance with our findings, have been isolated from vacuum packaged meat. This genus may therefore be animal associated. *Fructilactobacillus* sequences were found in a variety of food categories, with highest abundances in yeast cultures and sourdoughs, which is compatible with the ecology of *Fructilactobacillus sanfranciscensis* (De Vuyst et al., 2014). This genus has been described as insect associated (Zheng et al., 2020). The significance of the occurrence, at low abundance, in milk, meat and smoked fish products is unclear and may due to contaminating sequences.

Furfurilactobacillus is another genus whose sequences have a restricted distribution. Its lifestyle is unknown, although the source of type strains is invariably sourdough. In agreement with this, we found that its highest median abundance (which was close to 0.01) was found in flours and sourdoughs. Again, occurrence of sequences in products of animal origin has an unclear significance. *Oenococcus* is a genus which is strictly adapted to alcoholic beverages (Badotti et al., 2014; Bech-Terkilsen et al., 2020; Endo and Okada, 2006); the occurrence of sequences assigned to this genus in foods of animal origin may be certainly suspicious. Members of the genus *Fructobacillus* are fructophilic LAB which are associated with fructose-rich environments (Dicks and Endo, 2022; Endo and Salminen, 2013; Endo et al., 2018) including fruits and honeybees and honey. The abundance of their sequences in vegetable products, with high abundance in sugar plants is therefore not surprising, while their occasional abundance in milk and dairy products is unclear.

Many other genera are definitely generalist, and their sequences occur with sometimes high prevalence and abundance in a large variety of foods of plant and animal origin (**Figure 4**). These include *Lactobacillus* (lifestyle described as vertebrate, animal or insect associated), a genus including several species used as starters in dairy fermentations (Bourdichon et al., 2022), *Companilactobacillus*, *Lacticaseibacillus, Latilactobacillus, Ligilactobacillus, Pediococcus, Lactiplantibacillus, Lentilactobacillus, Secundilactobacillus, Levilactobacillus, Paucilactobacillus, Leuconostoc, Weissella*. This is in good agreement with their nomadic or free-living lifestyles. *Loigolactobacillus* and *Limosilactobacillus* have been described as vertebrate associated, but sequences assigned to these genera are widely distributed in food samples, including those of vegetable origin.

The sources of *Lactobacillaceae* in foods can be many and diverse. Although source tracking has been used for a few studies, and environmental microbiomes, especially in traditional food productions, are colonized by stable communities which shape the microbial landscape of different areas of food factories (da Silva Vale et al., 2021; De Filippis et al., 2020; Sun and D'Amico, 2021), unambiguous attribution of sources of contamination can be difficult due to

the low taxonomic resolution of metataxonomic studies. As a proof of concept, we performed a search in the FoodMicrobionet database using the same filtering criteria described above. Only 19 studies with 908 samples matched these criteria and the results are shown in **Figure 5**.

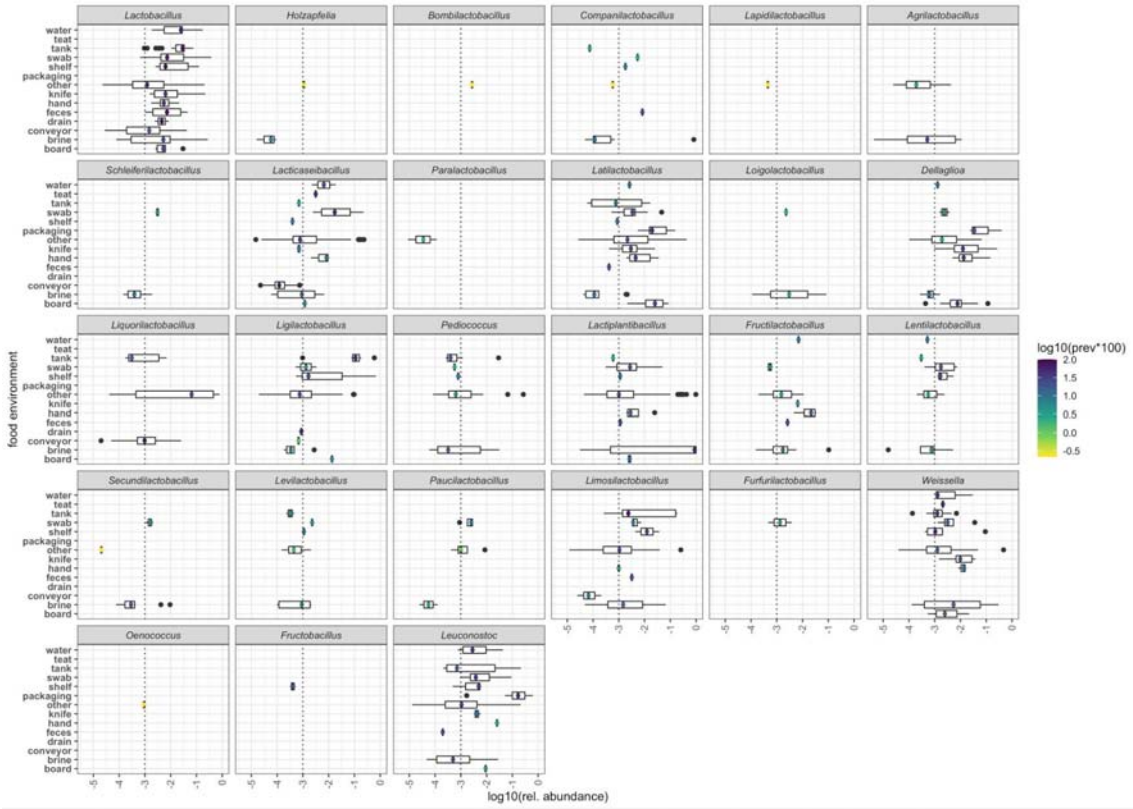

Figure 5. Boxplot showing the distribution of abundance of sequences (V1-V3 and V3-V4, length >350 bp) of genera of the family *Lactobacillaceae* in food related environmental samples. The prevalence is shown as the colour scale of a marker centred on the median. Genera in facets were ordered on the basis of their taxonomic relationships (Qiao et al., 2022; Zheng et al., 2020).

The number of food environment samples in FoodMicrobionet is much lower than that of food samples (only 2,197 out of 12,145) and the coverage of food categories is far less homogeneous. In addition, the description of food environmental samples is not standardized. Here, we have tried to extract some common naming patterns, but the number of samples is too low to create further subcategories: although "teat" is always referred to dairy cow's teat surfaces, all the other items may refer to environments related to different food industries or raw material production contexts. Anyway, while it is quite clear that generalist like *Lactobacillus*, *Lacticaseibacillus, Latilactobacillus*, *Lactiplantibacillus, Weissella* and *Leuconostoc* are widely distributed in food environments and sometime are abundant components (>0.1 relative abundance) of the microbiota the number of variety of samples is too low to evaluate associations of genera with potential sources of contamination.

Starting from version 4.1, FoodMicrobionet includes, as an external resource (https://doi.org/10.5281/zenodo.6954040), R lists containing the ASV for all studies processed using the dada2 pipeline (Parente et al., 2022). Although the ecological significance of ASV is not clear (i.e. there is no convincing proof that they represent different subpopulations within a taxonomic group) they have been used to evaluate microdiversity in some studies (Quijada et al., 2018; Skeie et al., 2019; Zwirzitz et al., 2020). Their main advantage, however, is that results from different studies can be compared, something which is impossible when using Operational Taxonomic Units (Callahan et al., 2017). We used ASV analysis to analyze the distribution of ASVs for the three genera of family *Lactobacillaceae* which have only one or very few species and which are relatively prevalent or abundant in at least some food groups (*Dellaglioa, Holzapfelia, Schleiferilactobacillus*). The results are shown in **Supplementary Figures 15 to 20**. The results confirm the findings obtained in previous sections. Only occasionally ASV did not cluster with the corresponding reference sequences, thus confirming the reliability of the taxonomic assignment. On the other hand, the diversity of partial sequences was usually very low and discriminating ASVs on the basis of their clustering with the type strains of a given species is difficult if not impossible (see for example **Supplementary Figures 19 and 20**, for *Schleiferilactobacillus*). In one instance (**Supplementary Figure 18**, *Holzapfelia*, V3-V4) a number of ASVs provided a hint of belonging to a different, possibly unknown species, but the supporting evidence is too scant. In some instances, the grouping of ASVs was clearly confounded with the study from which they were obtained. No clear evidence of association of sub-clusters of ASVs with a given food source was obtained.

4. Conclusions

We have demonstrated that the FoodMicrobionet database can be used to retrieve detailed quantitative information on the distribution of economically important taxa (here genera belonging to the family *Lactobacillaceae*). We used a very conservative approach, based on the analysis of samples for which sequencing was carried out using target regions (V1-V3 and V3-V4) for which reliable taxonomic assignment at the genus level was possible. The rich metadata structure of the studies and sample tables of FoodMicrobionet v4 (Parente et al., 2022) allows informed users to fine tune queries and to retrieve information at the desired level of aggregation for both taxa and samples. Our results provided support to the description of lifestyles of *Lactobacillaceae* (Duar et al., 2017; Zheng et al., 2020) and provides hints on the ecological niches of rarely isolated genera. The approach can be easily replicated for other taxonomic groups.

The biological significance of conclusion based on metataxonomic approaches targeting the DNA should be evaluated with caution. It is well known that the active fraction of the food microbiota may be significantly different from the total microbiota, whose composition is evaluated using DNA as a target (Erkus et al., 2013; 2016) and that contamination during wet laboratory procedures traces of DNA from ingredients may influence the composition of the microbiota (Dahlberg et al., 2019; Duthoo et al., 2022). Although recent studies sometimes include reagent blanks, the information in sample metadata tables obtained from NCBI Short Read Archive is not sufficient for statistical removal of contaminating sequences (Davis et al., 2018). One conservative approach to address the second problem might be to remove samples in which the relative abundance of a target taxon is very low

($<10^{-3}$). However, this may significantly reduce the sensitivity of the analysis and arbitrarily remove rare taxa.

Due to the limited taxonomic resolution of partial 16S rRNA gene sequences, taxonomic assignment at the species level was not reliable. We also found that combining ASVs from different studies (Callahan et al., 2017) is indeed possible, but provides little additional information, even when strict criteria are used for the filtering of sequences.

Acknowledgements

This work was partially funded by Ministero dell'Università e Ricerca (MUR Ministry), Project "Strengthening the MIRRI Italian Research Infrastructure for Sustainable Bioscience and Bioeconomy", n. "IR0000005 (within the NRRP plan actions).

References

Anagnostopoulos, D.A., Tsaltas, D., 2022. Current status, recent advances, and main challenges on table olive fermentation: the present meets the future. Front. Microbiol. 12, 797295. https://doi.org/10.3389/fmicb.2021.797295

Arora, K., Ameur, H., Polo, A., Di Cagno, R., Rizzello, C.G., Gobbetti, M., 2020. Thirty years of knowledge on sourdough fermentation: A systematic review. Trends Food Sci. Tech. 108, 71-83. https://doi.org/10.1016/j.tifs.2020.12.008

Badotti, F., Moreira, A.P.B., Tonon, L.A.C., de Lucena, B.T.L., Gomes, F.C.O., Kruger, R., Thompson, C.C., de Morais, M.A., Rosa, C.A., Thompson, F.L., 2014. *Oenococcus alcoholitolerans* sp. nov., a lactic acid bacteria isolated from cachaça and ethanol fermentation processes. Antonie Van Leeuwenhoek. 106, 1259-1267. https://doi.org/10.1007/s10482-014-0296-z

Bech-Terkilsen, S., Westman, J.O., Swiegers, J.H., Siegumfeldt, H., 2020. *Oenococcus oeni*, a species born and moulded in wine: a critical review of the stress impacts of wine and the physiological responses. Aust. J. Grape Wine Res. 26, 188-206. https://doi.org/10.1111/ajgw.12436

Bello, S., Rudra, B., Gupta, R.S., 2022. Phylogenomic and comparative genomic analyses of *Leuconostocaceae* species: identification of molecular signatures specific for the genera *Leuconostoc*, *Fructobacillus* and *Oenococcus* and proposal for a novel genus *Periweissella* gen. nov. Int. J. Syst. Evol. Microbiol. 72, 005284. https://doi.org/10.1099/ijsem.0.005284

Björkroth, J., Dicks, L.M.T., Endo, A., Holzapfel, W.H., 2014a. The genus *Leuconostoc*. In: Holzapfel, W.H., Woods, B.J.B. (Eds.), Lactic Acid Bacteria: Biodiversity and Taxonomy. Wiley Blackwell, Oxford, UK, pp. 391–404. https://doi.org/10.1002/9781118655252.ch23

Björkroth, J., Dicks, L.M.T., Endo, A., 2014b. The genus *Weissella*. In: Holzapfel, W.H., Woods, B.J.B. (Eds.), Lactic Acid Bacteria: Biodiversity and Taxonomy. Wiley Blackwell, Oxford, UK, pp. 417-428. https://doi.org/10.1002/9781118655252.ch25

Bokulich, N.A., Ziemski, M., Robeson, M.S., Kaehler, B.D., 2020. Measuring the microbiome: best practices for developing and benchmarking microbiomics methods. Comput. Struct. Biotechnol. J. 18, 4048-4062. https://doi.org/10.1016/j.csbj.2020.11.049

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J.C., Gerds, M.L., Hammes, W.P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I.B., Prajapati, J.B., Seto, Y., Schure, E.T., Boven, A.V., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S., Hansen, E.B., 2012.

579 Food fermentations: microorganisms with technological beneficial use. Int. J. Food
580 Microbiol. 154, 87-97. https://doi.org/10.1016/j.ijfoodmicro.2011.12.030
581 Bourdichon, F., Laulund, S., Tenning, P., 2019. Inventory of microbial species with a
582 rationale: a comparison of the IDF/EFFCA inventory of microbial food cultures with the
583 EFSA Biohazard Panel qualified presumption of safety. FEMS Microbiol. Lett. 366,
584 fnz048. https://doi.org/10.1093/femsle/fnz048
585 Bourdichon F, Budde-Niekel, A; Dubois A, Fritz, D, Hatte, J-L, Laulund S., McAuliffe, O,
586 Ouwehand AC, Yao, S., Zgoda A., Zuliani, V., Morelli L. Inventory of microbial food
587 cultures with safety demonstration in fermented food products. Update of the Bulletin
588 of the IDF 377-2002, 455–2012 and 495-2018. Bulletin of IDF 514, 2022.
589 Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016.
590 DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods.
591 13, 581-583. https://doi.org/10.1038/nmeth.3869
592 Callahan, B.J., McMurdie, P.J., Holmes, S.P., 2017. Exact sequence variants should replace
593 operational taxonomic units in marker-gene data analysis. ISME J. 11, 2639-2643.
594 https://doi.org/10.1038/ismej.2017.119
595 Callahan, B.J., Wong, J., Heiner, C., Oh, S., Theriot, C.M., Gulati, A.S., McGill, S.K., Dougherty,
596 M.K., 2019. High-throughput amplicon sequencing of the full-length 16S rRNA gene
597 with single-nucleotide resolution. Nucleic Acids Res. 47, e103.
598 https://doi.org/10.1093/nar/gkz569
599 Colautti, A., Arnoldi, M., Comi, G., Iacumin, L., 2021. Antibiotic resistance and virulence
600 factors in lactobacilli: something to carefully consider. Food Microbiol. 103, 103934.
601 https://doi.org/10.1016/j.fm.2021.103934
602 da Silva Vale, A., de Melo Pereira, G.V., de Carvalho Neto, D.P., Sorto, R. D., Goés-Neto, A.,
603 Kato, R., Soccol, C. R., 2021. Facility-specific 'house' microbiome ensures the
604 maintenance of functional microbial communities into coffee beans fermentation:
605 implications for source tracking. Environ. Microbiol. Rep. 13, 470-481.
606 https://doi.org/10.1111/1758-2229.12921
607 Dahlberg, J., Sun, L., Waller, K.P., Östensson, K., McGuire, M., Agenäs, S., Dicksved, J., 2019.
608 Microbiota data from low biomass milk samples is markedly affected by laboratory
609 and reagent contamination. PLoS One. 14, e0218257.
610 https://doi.org/10.1371/journal.pone.0218257
611 Davis, N.M., Proctor, D.M., Holmes, S.P., Relman, D.A., Callahan, B.J., 2018. Simple statistical
612 identification and removal of contaminant sequences in marker-gene and
613 metagenomics data. Microbiome. 6, 226. https://doi.org/10.1186/s40168-018-0605-2
614 De Filippis, F., Valentino, V., Alvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2020.
615 Environmental microbiome mapping as a strategy to improve quality and safety in the
616 food industry. Curr. Opin. Food Sci. 38, 168-176.
617 https://doi.org/10.1016/j.cofs.2020.11.012
618 Deng, Z., Hou, K., Zhao, J., Wang, H., 2022. The probiotic properties of lactic acid bacteria
619 and their applications in animal husbandry. Curr. Microbiol. 79, 22.
620 https://doi.org/10.1007/s00284-021-02722-3
621 De Vuyst, L., Kerrebroeck, S.V., Harth, H., Huys, G., Daniel, H.M., Weckx, S., 2014. Microbial
622 ecology of sourdough fermentations: diverse or uniform? Food Microbiol. 37, 11-29.
623 https://doi.org/10.1016/j.fm.2013.06.002
624 Dicks, L.M.T., Endo, A., 2022. Are fructophilic lactic acid bacteria (FLAB) beneficial to
625 humans? Benef. Microbes. 13, 3-11. https://doi.org/10.3920/bm2021.0044

626 Douillard, F.P., Ribbera, A., Kant, R., Pietilä, T.E., Järvinen, H.M., Messing, M., Randazzo, C.,
627 Paulin, L., Laine, P., Ritari, J., Caggia, C., Lähteinen, T., Brouns, S.J.J., Satokari, R., von
628 Ossowski, I., Reunanen, J., Palva, A., de Vos, W.M., 2013. Comparative genomic and
629 functional analysis of 100 *Lactobacillus rhamnosus* strains and their comparison with
630 strain GG. PLoS Genet. 9, e1003683. https://doi.org/10.1371/journal.pgen.1003683
631 Doulgeraki, A.I., Ercolini, D., Villani, F., Nychas, G.J.E., 2012. Spoilage microbiota associated
632 to the storage of raw meat in different conditions. Int. J. Food Microbiol. 157, 130-141.
633 https://doi.org/10.1016/j.ijfoodmicro.2012.05.020
634 Duar, R.M., Lin, X.B., Zheng, J., Martino, M.E., Grenier, T., Pérez-Muñoz, M.E., Leulier, F.,
635 Gänzle, M., Walter, J., 2017. Lifestyles in transition: evolution and natural history of
636 the genus *Lactobacillus*. FEMS Microbiol. Rev. 41, S27-S48.
637 https://doi.org/10.1093/femsre/fux030
638 Duthoo, E., De Reu, K., Leroy, F., Weckx, S., Heyndrickx, M., Rasschaert, G., 2022. To culture
639 or not to culture: careful assessment of metabarcoding data is necessary when
640 evaluating the microbiota of a modified-atmosphere-packaged vegetarian meat
641 alternative throughout its shelf-life period. BMC Microbiol. 22, 34.
642 https://doi.org/10.1186/s12866-022-02446-9
643 EFSA, 2015. The food classification and description system FoodEx 2 (revision 2). In: EFSA
644 Supporting Publications, Vol. 12. pp. 1-90. https://doi.org/10.2903/sp.efsa.2015.EN-
645 804.
646 Endo, A., Okada, S., 2006. *Oenococcus kitaharae* sp. nov., a non-acidophilic and non-
647 malolactic-fermenting *oenococcus* isolated from a composting distilled shochu
648 residue. Int. J. Syst. Evol. Microbiol. 56, 2345-2348.
649 https://doi.org/10.1099/ijs.0.64288-0
650 Endo, A., Salminen, S., 2013. Honeybees and beehives are rich sources for fructophilic lactic
651 acid bacteria. Syst. Appl. Microbiol. 36, 444-448.
652 https://doi.org/10.1016/j.syapm.2013.06.002
653 Endo, A., Dicks, L.M.T., 2014a. The genus *Oenococcus*. In: Holzapfel, W.H., Woods, B.J.B.
654 (Eds.), Lactic Acid Bacteria: Biodiversity and Taxonomy. Wiley Blackwell, Oxford, UK,
655 pp. 405-415. https://doi.org/10.1002/9781118655252.ch24
656 Endo, A., Dicks, L.M.T., 2014b. The genus *Fructobacillus*. In: Holzapfel, W.H., Woods, B.J.B.
657 (Eds.), Lactic Acid Bacteria: Biodiversity and Taxonomy. Wiley Blackwell, Oxford, UK,
658 pp. 380-389. https://doi.org/10.1002/9781118655252.ch22
659 Endo, A., Maeno, S., Tanizawa, Y., Kneifel, W., Arita, M., Dicks, L., Salminen, S., 2018.
660 Fructophilic Lactic Acid Bacteria, a unique group of fructose-fermenting microbes.
661 Appl. Environ. Microbiol. 84, e01290-18. https://doi.org/10.1128/aem.01290-18
662 Erkus, O., de Jager, V.C.L., Spus, M., van Alen-Boerrigter, I.J., van Rijswijck, I.M., Hazelwood,
663 L., Janssen, P.W.M., van Hijum, S.A.F.T., Kleerebezem, M., Smid, E.J., 2013.
664 Multifactorial diversity sustains microbial community stability. ISME J. 7, 2126-2136.
665 https://doi.org/10.1038/ismej.2013.108
666 Erkus, O., de Jager, V.C.L., de Geene, R.T.C.M., van Alen-Boerrigter, I., Hazelwood, L., van
667 Hijum, S.A.F.T., Kleerebezem, M., Smid, E.J., 2016. Use of propidium monoazide for
668 selective profiling of viable microbial cells during Gouda cheese ripening. Int. J. Food
669 Microbiol. 228, 1–9. https://doi.org/10.1016/j.ijfoodmicro.2016.03.027
670 Falentin, H., Chaix, E., Dridi, B., Bessieres, P., Buchin, S., Deutsch, S.M., Weber, M., Bossy, R.,
671 Derozier, S., Perret, B., Aubin, S., Deleger, L., Dibie, J., Delbes, C., Irlinger, F., Valence,
672 F., Casaregola, S., Thierry, A., Zagorec, M., Champomier-Verges, M.C., Ba, M., Ferré, A.,

Renault, P., Loux, V., Nédellec, C., Sicard, D., 2017,. Florilege: a database gathering microbial phenotypes of food interest, In: Proceedings of the 4th International Conference on Microbial Diversity 2017-10-24, 2017-10-26. ITA, Bari. https://hal.archives-ouvertes.fr/hal-01651302

Franz, C.M.A.P., Endo, A., Abriouel, H., Van Reenen, C.A., Gálvez, A., Dicks, L.M.T., 2014. The genus *Pediococcus*. In: Holzapfel, W.H., Woods, B.J.B. (Eds.), Lactic Acid Bacteria: Biodiversity and Taxonomy. Wiley Blackwell, Oxford, UK, pp. 359-376. https://doi.org/10.1002/9781118655252.ch21

Gänzle, M., 2015. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr. Opin. Food Sci. 2, 106-117. https://doi.org/10.1016/j.cofs.2015.03.001

Gänzle, M., 2022. The periodic table of fermented foods: limitations and opportunities. Appl. Microbiol. Biotechnol. 106, 2815-2826. https://doi.org/10.1007/s00253-022-11909-y

Glöckner, F.O., Yilmaz, P., Quast, C., Gerken, J., Beccati, A., Ciuprina, A., Bruns, G., Yarza, P., Peplies, J., Westram, R., Ludwig, W., 2017. 25 years of serving the community with ribosomal RNA gene reference databases and tools. J. Biotechnol. 261, 169-176. https://doi.org/10.1016/j.jbiotec.2017.06.1198

Johnson, J.S., Spakowicz, D.J., Hong, B.Y., Petersen, L.M., Demkowicz, P., Chen, L., Leopold, S.R., Hanson, B.M., Agresta, H.O., Gerstein, M., Sodergren, E., Weinstock, G.M., 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat. Commun. 10, 5029. https://doi.org/10.1038/s41467-019-13036-1

Kuhn, M., 2022. caret: Classification and Regression Training. R package version 6.0-92. https://CRAN.R-project.org/package=caret

Kumar, S., Bansal, K., Sethi, S.K., 2022. Comparative genomics analysis of genus *Leuconostoc* resolves its taxonomy and elucidates its biotechnological importance. Food Microbiol. 106, 104039. https://doi.org/10.1016/j.fm.2022.104039

Lagkouvardos, I., Joseph, D., Kapfhammer, M., Giritli, S., Horn, M., Haller, D., Clavel, T., 2016. IMNGS: A comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies. Sci. Rep. 6, 33721. https://doi.org/10.1038/srep33721

Laranjo, M., Potes, M.E., Elias, M., 2019. Role of starter cultures on the safety of fermented meat products. Front. Microbiol. 10, 454. https://doi.org/10.3389/fmicb.2019.00853

Lee, S.H., Whon, T.W., Roh, S.W., Jeon, C.O., 2020. Unraveling microbial fermentation features in kimchi: from classical to meta-omics approaches. Appl. Microbiol. Biotechnol. 104, 7731-7744. https://doi.org/10.1007/s00253-020-10804-8

Li, K., Wang, S., Liu, W., Kwok, L.Y., Bilige, M., Zhang, W., 2022. Comparative genomic analysis of 455 *Lactiplantibacillus plantarum* isolates: habitat-specific genomes shaped by frequent recombination. Food Microbiol. 104, 103989. https://doi.org/10.1016/j.fm.2022.103989

Links, M.G., Dumonceaux, T.J., Hemmingsen, S.M., Hill, J.E., 2012. The chaperonin-60 universal target is a barcode for bacteria that enables de novo assembly of metagenomic sequence data. Plos One 7, e49755. https://doi.org/10.1371/journal.pone.0049755

Mayo, B., Rodríguez, J., Vázquez, L., Flórez, A.B., 2021. Microbial interactions within the cheese ecosystem and their application to improve quality and safety. Foods 10, 602. https://doi.org/10.3390/foods10030602

720 Meola, M., Rifa, E., Shani, N., Delbès, C., Berthoud, H., Chassard, C., 2019. DAIRYdb: a
721 manually curated reference database for improved taxonomy annotation of 16S rRNA
722 gene sequences from dairy products. BMC genomics. 20, 560.
723 https://doi.org/10.1186/s12864-019-5914-8
724 Moniente, M., García-Gonzalo, D., Ontañón, I., Pagán, R., Botello-Morte, L., 2021. Histamine
725 accumulation in dairy products: Microbial causes, techniques for the detection of
726 histamine-producing microbiota, and potential solutions. Compr. Rev. Food Sci. Food
727 Saf.20, 1481-1523. https://doi.org/10.1111/1541-4337.12704
728 Parente, E., Cocolin, L., De Filippis, F., Zotta, T., Ferrocino, I., O'sullivan, O., Neviani, E., De
729 Angelis, M., Cotter, P.D., Ercolini, D., 2016. FoodMicrobionet: A database for the
730 visualisation and exploration of food bacterial communities based on network
731 analysis. Int. J. Food Microbiol. 219, 28-37.
732 https://doi.org/10.1016/j.ijfoodmicro.2015.12.001
733 Parente, E., De Filippis, F., Ercolini, D., Ricciardi, A., Zotta, T., 2019. Advancing integration of
734 data on food microbiome studies: FoodMicrobionet 3.1, a major upgrade of the
735 FoodMicrobionet database. Int. J. Food Microbiol. 305, 108249.
736 https://doi.org/10.1016/j.ijfoodmicro.2019.108249
737 Parente, E., Zotta, T., Ricciardi, A., 2022. FoodMicrobionet v4: A large, integrated, open and
738 transparent database for food bacterial communities. Int. J. Food Microbiol. 372,
739 109696. https://doi.org/10.1016/j.ijfoodmicro.2022.109696
740 Pasolli, E., De Filippis, F., Mauriello, I.E., Cumbo, F., Walsh, A.M., Leech, J., Cotter, P.D.,
741 Segata, N., Ercolini, D., 2020. Large-scale genome-wide analysis links lactic acid
742 bacteria from food with the gut microbiome. Nat. Commun. 11, 2610.
743 https://doi.org/10.1038/s41467-020-16438-8
744 Pollock, J., Glendinning, L., Wisedchanwet, T., Watson, M., 2018. The madness of
745 microbiome: attempting to find consensus "best practice" for 16S microbiome studies.
746 Appl. Environ. Microbiol. 84, 3225. https://doi.org/10.1128/aem.02627-17
747 Pothakos, V., Stellato, G., Ercolini, D., Devlieghere, F., 2015. Processing environment and
748 ingredients are both sources of *Leuconostoc gelidum*, which emerges as a major
749 spoiler in ready-to-eat meals. Appl. Environ. Microbiol. 81, 3529-3541.
750 https://doi.org/10.1128/aem.03941-14
751 Praet, J., Meeus, I., Cnockaert, M., Houf, K., Smagghe, G., Vandamme, P., 2015. Novel lactic
752 acid bacteria isolated from the bumble bee gut: *Convivina intestini* gen. nov., sp. nov.,
753 *Lactobacillus bombicola* sp. nov., and *Weissella bombi* sp. nov. Antonie Van
754 Leeuwenhoek 107, 1337-1349. https://doi.org/10.1007/s10482-015-0429-z
755 Qiao, N., Wittouck, S., Mattarelli, P., Zheng, J., Lebeer, S., Felis, G.E., Gänzle, M., 2022. After
756 the storm-Perspectives on the taxonomy of *Lactobacillaceae*. JDS Commun. 3, 222-
757 227. https://doi.org/10.3168/jdsc.2021-0183
758 Quijada, N.M., De Filippis, F., Sanz, J.J., García-Fernández, M.D.C., Rodríguez-Lázaro, D.,
759 Ercolini, D., Hernández, M., 2018. Different *Lactobacillus* populations dominate in
760 "Chorizo de León" manufacturing performed in different production plants. Food
761 Microbiol. 70, 94-102. https://doi.org/10.1016/j.fm.2017.09.009
762 R Core Team, 2022. R: A language and environment for statistical computing. R Foundation
763 for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
764 Sanders, M.E., Merenstein, D.J., Reid, G., Gibson, G.R., Rastall, R.A., 2019. Probiotics and
765 prebiotics in intestinal health and disease: from biology to the clinic. Nat. Rev.
766 Gastroenterol. Hepatol. 16, 605-616. https://doi.org/10.1038/s41575-019-0173-3

767 Schliep, K.P., 2011. phangorn: phylogenetic analysis in R. Bioinform. 27, 592-593.
768 https://doi.org/10.1093/bioinformatics/btq706
769 Shukla, I., Hill, J.E., 2022. cpn60 barcode sequences accurately identify newly defined genera
770 within the *Lactobacillaceae*. Can. J. Microbiol. 99, 1-8. https://doi.org/10.1139/cjm-
771 2021-0296
772 Skeie, S.B., Håland, M., Thorsen, I.M., Narvhus, J., Porcellato, D., 2019. Bulk tank raw milk
773 microbiota differs within and between farms: a moving goalpost challenging quality
774 control. J. Dairy Sci. 102, 1959-1971. https://doi.org/10.3168/jds.2017-14083
775 Sumby, K.M., Grbin, P.R., Jiranek, V., 2014. Implications of new research and technologies
776 for malolactic fermentation in wine. Appl. Microbiol. Biotechnol. 98, 8111-8132.
777 https://doi.org/10.1007/s00253-014-5976-0
778 Sun, L., D'Amico, D.J., 2021. Composition, succession, and source tracking of microbial
779 communities throughout the traditional production of a farmstead cheese. Food
780 Microbiol. 6, e00830-21. https://doi.org/10.1128/msystems.00830-21
781 Tamang, J.P., Cotter, P.D., Endo, A., Han, N.S., Kort, R., Liu, S.Q., Mayo, B., Westerik, N.,
782 Hutkins, R., 2020. Fermented foods in a global age: East meets West. Compr. Rev.
783 Food Sci. Food Saf. 19, 184-217. https://doi.org/10.1111/1541-4337.12520
784 Walsh, A.M., Macori, G., Kilcawley, K.N., Cotter, P.D., 2020. Meta-analysis of cheese
785 microbiomes highlights contributions to multiple aspects of quality. Nat. Food. 1, 500-
786 510. https://doi.org/10.1038/s43016-020-0129-3
787 Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid
788 assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ.
789 Microbiol. 73, 5261-5267. https://doi.org/10.1128/aem.00062-07
790 Wang, L.G., Lam, T.T.Y., Xu, S., Dai, Z., Zhou, L., Feng, T., Guo, P., Dunn, C.W., Jones, B.R.,
791 Bradley, T., Zhu, H., Guan, Y., Jiang, Y., Yu, G., 2019. Treeio: an R package for
792 phylogenetic tree input and output with richly annotated and associated data. Mol.
793 Biol. Evol. 37, 599-603. https://doi.org/10.1093/molbev/msz240
794 Wilkinson, S.P., 2018. kmer: an R package for fast alignment-free clustering of biological
795 sequences. R package version 1.0.0. https://cran.r-project.org/package=kmer
796 Wright, E.S. 2016. "Using DECIPHER v2.0 to Analyze Big Biological Sequence Data in R." R J.,
797 81, 352-359. https://journal.r-project.org/archive/2016/RJ-2016-025/index.html
798 Wuyts, S., Wittouck, S., Boeck, I.D., Allonsius, C.N., Pasolli, E., Segata, N., Lebeer, S., 2017.
799 Large-Scale phylogenomics of the *Lactobacillus casei* group highlights taxonomic
800 inconsistencies and reveals novel clade-associated features. MSystems. 2, e00061-17.
801 https://doi.org/10.1128/msystems.00061-17
802 Xu, Z., Luo, Y., Mao, Y., Peng, R., Chen, J., Soteyome, T., Bai, C., Chen, L., Liang, Y., Su, J.,
803 Wang, K., Liu, J., Kjellerup, B.V., 2020. Spoilage lactic acid bacteria in the brewing
804 industry. J. Microbiol. Biotechnol. 30, 955-961.
805 https://doi.org/10.4014/jmb.1908.08069
806 Yu, G., 2020. Using ggtree to Visualize Data on Tree-Like Structures. Curr. Protoc. Bioinform.
807 69, e96. https://doi.org/10.1002/cpbi.96
808 Yu, G., 2022. tidytree: A Tidy Tool for Phylogenetic Tree Data Manipulation. R package
809 version 0.3.9. https://CRAN.R-project.org/package=tidytree
810 Zhang, J., 2017. phylotools: Phylogenetic Tools for Eco-Phylogenetics. R package version
811 0.2.2. https://CRAN.R-project.org/package=phylotools

Zheng, J., Ruan, L., Sun, M., Gänzle, M., 2015. A genomic view of Lactobacilli and Pediococci demonstrates that phylogeny matches ecology and physiology. Appl. Environ. Microbiol. 81, 7233-7243. https://doi.org/10.1128/aem.02116-15

Zheng, J., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P., O'Toole, P.W., Pot, B., Vandamme, P., Walter, J., Watanabe, K., Wuyts, S., Felis, G.E., Gänzle, M.G., Lebeer, S., 2020. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. Int. J. Syst. Evol. Micr. 70, 2782-2858. https://doi.org/10.1099/ijsem.0.004107

Zwirzitz, B., Wetzels, S.U., Dixon, E.D., Stessl, B., Zaiser, A., Rabanser, I., Thalguter, S., Pinior, B., Roch, F.-F., Strachan, C., Zanghellini, J., Dzieciol, M., Wagner, M., Selberherr, E., 2020. The sources and transmission routes of microbial populations throughout a meat processing facility. Npj Biofilms Microbiomes. 6, 26. https://doi.org/10.1038/s41522-020-0136-z