RNA-based amplicon sequencing reveals microbiota development during ripening of artisanal versus industrial Lard d'Arnad

RNA-based amplicon sequencing highlights the impact of the processing technology on microbiota composition of artisanal and industrial PDO *Lard d'Arnad*

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ABSTRACT

"Valle d'Aosta *Lard d'Arnad*" is a protected designation of origin (PDO) product produced from fat of the shoulder and back of heavy pigs. Its manufacturing process can be very diverse, especially regarding the maturation temperature and the NaCl concentration used for the brine, thereby the main goal of this study was to investigate the impact of those parameters on the microbiota developed during curing and ripening. Three farms producing *Lard d'Arnad* were selected. Two plants, reflecting the industrial process characterized either by low maturation temperature (plant A [10% NaCl; 2°C]) or by using a low NaCl concentrations (plant B [2.5% NaCl; 4°C]) were selected, while the third was characterized by an artisanal process (C [30% NaCl; 8°C]). Lard samples were obtained at time 0 and after 7, 15, 30, 60 and 90 days of maturation. From each plant 3 independent lots were analyzed. The diversity of live microbiota was evaluated by using classical plate counts and amplicon target sequencing of SSU rRNA. The main taxa identified by sequencing were *Acinetobacter johnsonii*, *Psychrobacter*, *Staphylococcus equorum*, *S. sciuri*, *Pseudomonas fragi*, *Brochothrix*, *Halomonas* and *Vibrio* and differences in their relative abundance distinguished samples from the individual plants. The composition of the microbiota was more similar among plants A and B and it was characterized by the higher presence of taxa recognized as undesired bacteria in food processing environments. Oligotype analysis of *Halomonas* and *Acinetobacter* revealed the presence of several characteristic oligotypes associated with A and B samples.

IMPORTANCE

Changes in food production process can drastically affect the microbial community structure with a possible impact on the final characteristic of the products. The industrial processes of Lard d'Arnad production are characterized by a reduction of the salt concentration in the brines to address a consumer demand for less salty products, can negatively affect the dynamics and development of the live microbiota and as a consequence can negatively impact the quality of the final product due to the highter abundance of spoilage bacteria. This study is an overview of the live microbiota

developing during lard manufacturing and highlights the importance of the use of traditional process to produce PDO product from a spoilage perspective.

KEYWORDS

Lard d'Arnad, Spoilage microbiota, meat live microbiota, RNA-based amplicon sequencing

INTRODUCTION

"Valle d'Aosta *Lard d'Arnad*" is produced from the shoulder and back of heavy pigs at least nine months old as defined in Regulation (EC) 3220/84. Slaughtering takes place at local (Valle d'Aosta) abattoirs or in the Piedmont and Lombardy regions. The lard must be cut and placed in special wooden containers (named *doils*) within 72 hours from the slaughtering. *Doils* can be made from chestnut, larch or oak wood. During the procedure, every lard layer is alternated with spices and salt until the tank is full, then the whole is topped up with salty water (concentration not defined by the regulation), previously boiled and left to cool, so as to obtain the brine required for lard storage. Wooden containers, containing about 300 kg of lard, are then put under refrigeration conditions for a period of time never shorter than 90 days. During this period of time, temperature is maintained low in order to keep the product characteristics unaltered. At the end of the maturation, the lard is washed to remove excess salt. When released for consumption, lard must be at least 3 cm thick with different shapes. Lard appears white in color with the possible presence of a thin layer of meat.

The Regulation for the production of *Lard d'Arnad* is lacking in details regarding the salt concentration as well as the ripening temperature. The traditional recipe consists in using a brine at 30% of NaCl and a ripening temperature between 8-10°C. However some producers prefer to use lower NaCl concentration (2-10%) and as a consequence lower temperatures (2-4 °C) with the aim to reduce the presence of spoilage/pathogen bacteria that can be inhibited by the temperature and to reduce the salt that can diffuse in the lard. This practice has been recently adopted as a result of consumer tests, because consumers prefer a product with lower salty taste.

However, perturbation of the food system due to different process conditions can change the development and the function of the microbiota with a possible impact on the final characteristics of a product. No information about the microbiota development during lard process is available and only one study has been conducted with the aim to characterize the microbial volatile metabolites in cured and ripened lard (1). Several researches has been witnessed over the past years aimed at

estimating the live microbial diversity in different dairy ecosystems using 16S rRNA gene as the target molecules. The use of high throughput sequencing (HTS) amplicon target sequencing was found effective to discover the presence of several live or metabolically active microbes not usually associated with that food matrix (2–5).

The aim of this study was to assess the live microbiota by HTS amplicon target sequencing (targeting the 16S rRNA gene) coupled with classical culture dependent methods to characterize the live microbiota during *Lard d'Arnad* curing and ripening. Three plants were selected, employing different NaCl-temperature combinations during maturation. Two plants reflected an industrial process (named A and B), characterized by a bigger production volume per year, and one was chosen as reflecting the traditional process (C).

MATERIALS AND METHODS

Lard manufacturing and sample collection. The lard used came from crossbred heavy pigs, live weight about 160 kg, raised on a farm where the feeding was ad libitum and the diet a standard grower one with wheat, barley and soya as the main ingredients. Slaughtering took place at the local abattoir. After slaughtering and sectioning of the meat, lard attached to the shoulder and back skin was portioned in blocks of 1-5 kg and cured with a mixture of salt, garlic and spices including rosemary, sage, bay leaf, cinnamon, pepper, juniper berries and cloves. Three different weight ratios (kg) of salt to meat were used at the three farms: crystallized sodium chloride/lard w:w = 2:100 (plants A and B); 12:100 (plant C). The curing mixture was rubbed on the lard, which was then placed in containers in alternating layers with the curing mixture. Brine containing sodium chloride was used to fill the tank and the concentration of the brine was: sodium chloride/water w:w 10:100 (plant A); 2.5:100 (plant B) and 30:100 (plant C). Ripening was performed at controlled temperature: 2 °C (A), 4 °C (B) and 8 °C (C). At the end of the process the lard was washed to remove excess salt. All the productions were carried out in the spring season.

Lard samples were collected from each farm at time 0 and after 7, 15, 30, 60 and 90 days of

maturation. From each plant 3 independent lots were analyzed and two samples were collected from each sampling time. The three lots were produced in a time span of three weeks once per week

Microbiological analysis, pH and aw determination. About 10 g from each of the two samples collect from three lots at every sampling time for the three plants (total 108 samples) were homogenized with 90 mL of Buffered Peptone Water (Oxoid, Milano, Italy) for 2 min in a stomacher (LAB Blender 400, PBI, Italy). Decimal dilutions in quarter-strength Ringer's solution were prepared, and aliquots of 0.1 ml of the appropriate dilutions were spread in triplicate on the following media: plate count agar (PCA, Oxoid) for total aerobic bacteria incubated for 48 to 72 h at 30 °C; (ii) De Man Rogosa and Sharpe agar (MRS, Oxoid) for *Lactobacillaceae* and *Lactococcaceae* (lactic acid bacteria - LAB), incubated at 30 °C for 48 h; (iii) mannitol salt agar (MSA, Oxoid) for coagulase-negative *Staphylococcaceae* (CNS) incubated at 30 °C for 48 h; (iv) violet red bile agar (VRBA, Oxoid) for *Enterobacteriaceae*, incubated at 30 °C for 24-48 h. The salt concentration in the plates was not adjusted. The pH was measured by immersing the pH probe of a digital pH meter (micropH2001, Crison, Barcelona, Spain) in a diluted and homogenized sample containing 10 g of lard and 90 ml of distilled water. Water activity (a_w) was measured with a calibrated electric hygrometer (HygroLab, Rotronic, Bassersdorf, Switzerland) according to the manufacturer's instructions.

Results were calculated as the means of log colony-forming units (CFU) for three independent lots. Data from microbiological counts were analyzed by one-way analysis of variance (ANOVA) for each individual plant, with time as the main factor, using SPSS 22.0 statistical software package (SPSS, Inc., Cary, NC, USA). When ANOVA revealed significant differences (P <0.05), the Duncan honestly significant difference (HSD) test was applied. A pairwise *t-test* was used to assess the differences in microbial loads between the three plants. Fifteen colonies from MRS and MSA media at each sampling point were randomly isolated and purified. The purified isolates were preliminarily characterized by microscopic observations, Gram straining and catalase

and oxidase reactions. Working cultures were maintained in brain heart infusion (BHI, Oxoid) or MRS broth (Oxoid) with 25% glycerol and stored at -20 °C.

Molecular typing by rep-PCR of the CNS and LAB population. LAB and CNS isolates were subjected to DNA extraction as previously reported (2). LAB and CNS fingerprints were obtained by using repetitive extragenic palindromic PCR (rep-PCR) with the primer (GTG)5 (6). The rep-PCR profiles were normalized and cluster analysis was performed using Bionumerics software (version 6.1, Applied Maths, Sint-Martens-Latem, Belgium). The dendrograms were calculated on the basis of DICE coefficient of similarity with the unweighted pair group method using arithmetic averages (UPGMA) clustering algorithm (7). The similarity distance matrix generated via Bionumerics for REP fingerprints was used to build Partial Least Squares Discriminant Analysis (PLS-DA) by using R package "mixOmics" (www.r-project.org). After cluster analysis 2 isolates from each cluster at 80 % of similarity were selected and subjected to identification. The identification of LAB and CNS was performed by amplifying the 16S rRNA gene. The oligonucleotide primers FD1 (5'-AGA GTT TGA TCC TGG CTC AG-3') and RD1 (5'-AAG GAG GTG ATC CAG CC-3') (*Escherichia coli* positions 8-17 and 1540-1524, respectively) were used (8). PCR conditions were chosen according to Ercolini et al. (9). Amplicons of 16S rRNA were sent for sequencing to GATC-Biotech (Cologne, Germany). To determine the closest known relatives of the 16S rRNA gene sequences obtained, searches were performed in public data libraries (GenBank) with the Blast search program (http://www. ncbi.nlm.nih.gov/blast/). The identity of the isolates was confirmed by phylogenetic analysis. Sequences that showed the same percentage of identity were aligned together and a Newick tree was obtained by using FastTree. The tree obtained was visualized with FigTree in order to confirm the identification.

Total RNA extraction from lard samples. At each sampling point, 1 ml of the first ten-fold serial dilution was collected and directly centrifuged at maximum speed for 30 s. Nucleic acid was extracted by pooling two biological replicates from each sampling point from each lot for the three plant (total of 54 samples). Total RNA from the samples was extracted using the MasterPure

complete DNA and RNA purification kit (Illumina Inc, San Diego, CA) following the manufacturer's instructions. Three microliters of Turbo DNase (Life Technologies, Milan, Italy) was added to digest DNA in the RNA samples, with an incubation of 3 h at 37°C. RNA was quantified using the NanoDrop and standardized at 500 ng/µl. Complete DNA digestion was confirmed using 1 µl of extracted RNA in PCR with primers FD1-RD1; when a PCR product was obtained, the DNase treatment was repeated. Reverse transcription (RT) reactions were performed using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Milan, Italy). Five hundred ng of RNA were mixed with 1 µl of random primers (Promega, 100 µM) and DNase- and RNase-free sterile water (Sigma, Milan, Italy) to a final volume of 10 µl and then incubated at 75°C for 10 min. The mix was placed on ice, and a mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 2 mM each deoxynucleoside triphosphate, 1µl of $200U \cdot \mu l^{-1}$ M-MLV, and 0.96 U of RNasin-RNase inhibitor (Promega) was transferred to the reaction tube. Reverse transcription was carried out at 42°C for 1 h.

16S rRNA amplicon target sequencing. Complementary DNA was used to assess the live microbiota by amplification of the V3-V4 region of the 16S rRNA gene using the following primers: 16S-F (5'- TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3') and 16S-R (5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3') (10). Twenty-five µl PCR reactions were prepared using 12.5 µL of the 2X KAPA HiFi HotStart Ready Mix Taq (Kapa Biosystems, Wilmington, MA), 1 µM of each primer, 2.5 µL of cDNA template and PCR grade water. 25 cycles of 30 sec of denaturation (95 °C), 30 sec of primer annealing (55 °C) and 30 sec of primer elongation (72 °C), followed by a final elongation step (72 °C) of 5 min, were carried out. The PCR products were purified by means of an Agencourt AMPure kit (Beckman Coulter, Milan, Italy), and the resulting products were tagged by using the Nextera XT Index Kit (Illumina), according to the manufacturer's instructions. Sequencing was performed with a MiSeq Illumina instrument (Illumina) with V3 chemistry and generated 250 bp paired-end reads according to the

manufacturer's instructions. The software used for the base-calling and Illumina barcode demultiplexing processes, were the MiSeq Control Soft. V2.3.0.3, the RTA v1.18.42.0 and the CASAVA v1.8.2.

Bioinformatics and statistical analysis. Paired-end reads were first merged using FLASH software (11) with default parameters. Joint reads were further quality filtered (at Phred < Q20) using QIIME 1.9.0 software (12) through multiple_split_libraries_fastq.py script. Usearch v8.1 software (13) was adopted for chimera filtering, using the 16S reference databases v9 (RDP classifier training database). OTUs were picked at 99% of similarity by means of UCLUST clustering methods (14), and representative sequences of each cluster were used to assign taxonomy using the Greengenes 16S rRNA gene database, version 2013, by means of the RDP classifier with a minimum confidence score of 0.80 (15). Statistics and plotting were carried out in the R environment (www.r-project.org). Alpha diversity indices were calculated using the *diversity* function of the vegan package (16). The Shannon-Wiener diversity index *H'* was further analyzed using the *t*-test to assess differences between the three producers. In order to avoid biases due to different sequencing depths, all samples were rarefied at 4315 reads after raw read quality filtering.

Weighted UniFrac distance matrices and OTU table were used to perform Adonis and Anosim statistical tests in R environment. A filtered OTU table was generated at 0.5% abundance in at least 5 samples through QIIME and used to produce nodes and edge tables. The relative abundance of OTUs from the three lots from each plant was averaged. OTU table displays the higher taxonomy resolution that was reached by the 16S data, when the taxonomy assignment was not able to reach the species level, genus or the family was display. The tables were then imported in Gephi software (17), and an OTU network was built. The OTU table was used to build a principal component analysis (PCA) as a function of the plant by using the made4 package of R. Kruskal–Wallis tests were used to find significant differences in microbial taxa abundance according to the plant.

Reads assigned to *Acinetobacter* and *Halomonas* genera were extracted, and entropy analysis and oligotyping were carried out separately as described by the developers (18). High-entropy positions were chosen to compute the oligotypes (-C option): 32, 71, 109, 110, 125, 130, 242, 244, 246, 247, 253, 256, 271, 298, 300, 324, 399, 406 for *Halomonas* and 30, 32, 35, 109, 110, 111, 124, 125, 230, 241, 242, 244, 268, 290, 299, 341, 387 for *Acinetobacter*. To minimize the noise, each oligotype was required to appear in at least 1 sample, occur in more than 1.0% of the reads for at least one sample, represent a minimum of 500 reads (-M option) in all samples combined, and have a most abundant unique sequence with a minimum abundance of 50. Pairwise Wilcoxon tests were used in order to determine significant differences in specific oligotype abundance according to samples. A cladogram of representative sequences was generated using the ANVIOs software (18).

Accession number. All the sequencing data were deposited at the Sequence Read Archive of the National Center for Biotechnology Information (SRP093184).

RESULTS

Microbiological analysis and strain characterization. The results of viable counts, pH and a_w during lard ripening are shown in Table 1. pH values were stable through the ripening for samples A and C (average 6.25) while a significant reduction (from 6.65 to 6.07, P <0.05) was observed in samples B. The a_w value showed a significant reduction in all the samples analyzed from 0.97 to 0.83, 0.87 and 0.82 (for A, B and C respectively). The load of *Enterobacteriaceae* displayed a significant reduction only for samples A and B (from log 3.32 to 1.00 and from 2.53 to 0.33 respectively, P <0.05), while in samples C it did not show a significant variation throughout the ripening. No differences were observed across time in samples A and C regarding the count of CNS, however an increase in their load in samples C was observed (from 1.66 to 2.91, P <0.05). LAB as well as the total viable count did not show a significant development in all the sampling points for the three plants. Pairwise t-test (see Table S1 in the supplemental material) showed few differences when comparing the plate count data of the three plants. In detail at the end of ripening

the load of *Staphylococcaceae* and LAB were found higher in samples A, than in B and C (P <0.05). Regarding pH and a_w, the C samples displayed the lowest value compared with A and B (P <0.05).

A total of 294 isolates from MSA and 200 isolates from MRS plates were identified by REP-PCR coupled with the16S rRNA gene sequencing. From MSA plates 140 strains were isolated from samples C followed by 104 in samples A and 50 in samples B (see Fig. S1 in the supplemental material). The majority of the isolates were identified as *Staphylococcus sciuri* (123 isolates) and *S. equorum* (109 isolates). *S. xylosus* and *S. petrasii* were also identified. For the most abundant *Staphylococcaceae* (*S. sciuri* and *S. equorum*) the REP-PCR fingerprints were subject to cluster analysis performed through Bionumerics software and the similarity distance matrix generated were used to build a PLS-DA, as a function of the plant (Fig. 1). A certain degree of separation was observed in REP biotypes from plant C for both species, which appeared to group together and separate from isolates of samples A and B. Taking into account the isolates from MRS plates the majority of LAB isolates were identified as *Leuconostoc mesenteroides*, *Lactobacillus sakei*, *Lactococcus garvieae*, *Enterococcus pseudoavium* and *E. faecalis* (44, 41, 35, 29 and 24 isolates respectively) (see Fig. S1 in the supplemental material). In particular *L. mesenteroides* was mostly isolated from C while *Enteroccocci* were more often isolated from A and B.

16S data. A total of 1.197.764 raw reads (2x250bp) were obtained after sequencing. After joint, a total of 821.245 reads passed the filters applied through QIIME, with an average value of 16.062 reads/sample, and a sequence length of 457 bp. The rarefaction analysis and the Good's coverage express as percentage (Table 2) indicated that there was a satisfactory coverage for all the samples (Good's coverage average 86%). Moreover, alpha-diversity (Table 2) also showed that there was a higher level of complexity (P <0.05) in A samples when compared to C. Adonis and Anosim statistical tests based on Weighted UniFrac distance matrix showed significant differences among plants (P <0.001) and showed no significant difference among the lots from each plant. However taking into the account the single sampling point it was possible to observed few

difference between OTUs in the three lots analyzed. Differences between plants were further demonstrated by principal component analysis (PCA) based on the relative abundance of the main OTUs (Fig. 2). The PCA clearly showed that C samples were separated from A and B samples. ANOSIM statistical test confirmed this difference ($P < 0.01$). ANOSIM statistical measures of differences between the plants at different time points was also assed and the results showed significant differences among plants ($P < 0.05$).

As shown in Figure 3, the main OTUs shared among the dataset were *Acinetobacter johnsonii, A. lwoffii, Psychrobacter, S. equorum, S. sciuri, Pseudomonas fragi, Brochothrix, Halomonas, Chromohalobacter* and *Vibrio*. From the size of the edges, it was possible to see how the relative abundance of the above OTUs increased during time.

Comparing the relative abundance of the main OTUs across samples, it was possible to observe that *Halomonas* and *Acinetobacter* sp. were found to be characteristic of A samples (FDR <0.05), *Psychrobacter* and *P. fragi* characteristic from B samples (FDR <0.05) while *Salinisphaera* and *S. sciuri* characteristic for C samples (FDR <0.05). Regarding the most abundant OTUs, the different ripening condition affected the relative abundance of the main OTUs (Table 3 and Table S2 in the supplemental material). The relative abundance of OTUs from the three lots from each plant was averaged. Samples from A showed a predominance of *A. johnsonii* and *P. fragi* (from 23 to 35% and 16 to 23% of the relative abundance in respectively) in the first 15 days, and *Halomonas* and *Vibrio* from the 7th day till the end of the ripening (from 27 to 10% and from 3 to 65% at 60 days respectively). B samples displayed the dominance of *P. fragi* and *Psychrobacter* (from 12 to 40% and from 2 to 60% respectively), and *Halomonas* and *Vibrio* from the 30th day (from 4 to 30% and from 12 to 25% respectively) through the whole ripening time (Table 3). C samples were characterized by the presence of *A. johnsonii, Salinisphaera, Brochothrix* and *S. sciuri* in the first 60 days of ripening (from 20 to 40%, from 1 to 33%, from 1 to 12% and from 1 to 10% respectively). However, at the end of ripening only *Salinisphaera* and *Vibrio* dominated the C

microbiota. Several minor OTUs, such as *Methylotenera mobilis*, *S. equorum*, *Kocuria* and *Lactobacillus*, were also found randomly distributed among samples (Table 3).

The OTU co-occurrence/exclusion pattern is shown in Figure 4, where only significant correlations are reported (False Discovery Rate - FDR <0.05). Regarding the most abundant OTUs, *Halomonas* and *Vibrio* co-occur together and display the highest number of negative correlations, including a strong exclusion of *Lactobacillus* and *Pseudomonas. A. johnsonii* showed a positive correlation with *Staphylococcus* sp. and *S. sciuri* while *Brochothrix* showed the highest number of positive correlations including a strong correlation with *Pseudomonas fragi and Psychrobacter. Acinetobacter* showed including a strong co-exclusion with *Vibrio, S. succinus* and *Halomonas* while display a positive correlation with *P. fragi* (Fig. 4).

The relative abundance of *Acinetobacter* and *Halomonas* ranged from 47.15 to 0.16 and from 42.13 to 0.06 respectively in the entire dataset. A total of 29 and 14 oligotypes were identified for *Acinetobacter* and *Halomonas* respectively, but the number of different oligotypes detected was not related to the relative abundance of the genus in the dataset (see Fig. S2 in the supplemental material). A higher number of oligotypes was found within *Acinetobacter*. Oligotypes A7, A8, A9, A14, A15, A18, A22, A26 and A29 were clearly more abundant in A samples (FDR <0.05), while A1, A4, A11, A16, A19, A20, A23, A24 and A25 prevailed in B samples. Only A6, A12, A27 and A28 were characteristic of C samples (Fig. 5). *Halomonas* oligotypes H1, H2, H4, H6, H9, H12 and H14 were found more abundant in A samples while H3, H5, H8 and H10 characterized B samples. Only H7 and H13 were associated with C samples (Fig. 5). Finally, the co-occurrence/exclusion pattern (see Fig. S3 in the supplemental material) highlights that some oligotypes frequently co-exclude, e.g. *Acinetobacter* oligotypes A12 and A28 (FDR <0.05) co-excluded the presence of the other oligotypes and were associated with C samples, while *Halomonas* oligotypes H9 and H12 (FDR <0.05) are mutually exclusive with H2, H6 and H9, associated with A samples.

DISCUSSION

Lard d'Arnad is a typical PDO (Denomination of Protected Origin) product from Italy and this study aims to characterizing the live microbiota development in three production plants during its curing and ripening. The three productions differed in brine saline concentration and temperature used during ripening. For this purpose, an extensive sampling procedure of three different lots with two biological samples replicated each time was used, with the goal of limiting the inter-sample variability. The study was limited by the fact that only three plants were available for the study. The number was sufficient to guarantee statistical significance, however the analysis of more plants could improve it.

Differences in microbial composition during curing and ripening were investigated by using classical plate counts, culture dependent analysis and RNA based amplicon target sequencing. Comparing the plate count of the three plants it was possible to observe that the population monitored decreased during ripening with the exception of the *Staphylococcaceae* counts that increased during time in farm B (lower NaCl concentration). The highest percentage of CNS isolates appeared from farm C (higher concentration of NaCl). In particular the REP-biotype of *S. sciuri* as well as *S. equorum* isolated from C samples clustered separately from the the biotype isolates from A and B, thus indicating that the saline concentration during the ripening can affect the selection of these halophilic species, already identified in brine involving processes (4). *S. sciuri* has been usually isolated from fermented meat (19-20) and has been recently used as a starter culture because of its ability to produce bacteriocins able to contrast the development of spoilage bacteria (20). In addition it is also reported to display salinity tolerance in other environments (21). Similarly, the LAB population of plant C was dominated by the species *L. mesenteroides* and *Lact. garviae.* Conversely, samples A and B displayed the presence of several *Enterococci*, previously reported as an important population able to produce ammonia and other compounds possibly influencing the final flavour of the meat product (22).

The OTU network clearly showed that the core of OTUs was dominated by the presence of *A. johnsonii, Psychrobacter, S. equorum, S. sciuri, P. fragi, Brochothrix, Halomonas* and *Vibrio.*

However the relative abundance of these core taxa drove the separation of the samples as explained by beta diversity calculation. The results clearly showed that the composition of the microbiota was more similar in A and B compared to C samples. This indicates that the higher temperature and saline concentration (used in plant C) can affect the microbiota composition selecting specific taxa. Further, it was possible to deduce significant associations between specific OTUs and production plant. In addition those differences could depend of the microbial richness and diversity in pork meat and spices among the different plants.

Samples from plant A were characterized by the dominance of psychrotrophic spoilage taxa *Vibrio, Halomonas, Acinetobacter* sp. and *P. fragi* and their presence excluded other OTUs. *Acinetobacter* has been isolated during cold storage in food matrices and usually reported as spoilage agent, together with *Pseudomonas* (23–25), due to their strong lipolytic activity (26, 27). *Halomonas* has been reported to be characteristic microorganism in brine with a lipolytic potential in the food matrix (28). This microorganism, originating from marine environments, has been associated with short-ripened cheeses (29). Moreover a controversial role of *Halomonas* in food matrix has been reported and it was suggested to be an indicator for hygienic deficiencies in cheese producing facilities (30). Oligotype analysis of *Halomonas* and *Acinetobacter* revealed the presence of several characteristic *Halomonas* and *Acinetobacter* oligotypes associated with A samples. Samples from plant B were characterized by the dominance of *P. fragi*, *Psychrobacter* and *Vibrio*. *Psychrobacter* and *Vibrio* were clearly associated with several matrices including pasteurized milk (31) and meat (32). *Psychrobacter* can be involved in food spoilage, and is recognized as undesired genus in food processing environments (33). It was recently found as one of the main spoilage in fermented meat. It is probably introduced by salt or spices or originating during the slicing process, and its development is favoured at refrigerated conditions, indicating that refrigerated fermented meat can be an ideal niche for this species (34). *P. fragi* is often associated with spoilage ability in several food matrices (e.g. milk, cheese, meat, seafood) (9, 35–37) and aerobic storage of meat at low temperatures can provide an ecological advantage to *P. fragi* (26).

Brochothrix, *S. sciuri* and *Salinisphera* were clearly associated with C samples. *Brochothrix* found to be present in C samples during the first 60 days of storage has already been associated with meat and cheese products (38–40). *Acinetobacter johnsonii* was also found in C samples but was not detected at the end of the ripening. Moreover the higher concentrations of sodium chloride affected the oligotype composition of *Acinetobacter*. Few *Acinetobacter* oligotypes characterized C samples and a strong co-exclusion with the other ones was observed. The evidence presented in this study shows that the traditional method used to produce cured and ripened lard is effective in reducing the presence of several spoilage bacteria associated with industrial manufacturing. Several spoilage bacteria as well as several oligotypes were found in the industrial samples (e.g. *Enterococci*, *Halomonas*) most likely associated with the lower percentage of salt used for the production with a probable impact on the final characteristics of the product.

The results provide an overview of the live microbiota developing during lard manufacturing and highlight the importance of using traditional methods to preserve the safety and quality of the final product. This study provides an important set of information contributing to a better characterization of DPO products.

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Figure legends

Fig 1 PLS-DA models based on *Staphylococcus equorum* REP fingerprints (plot A) and *S. sciuri* (plot B) isolates from Lard d'Arnad samples. The samples are color coded according to the plant (A [10% brine; 2°C]: blue, B [2.5% brine; 4°C]: yellow, and C [30% brine; 8°C]: red).

Fig 2 PCA based on the OTUs abundance of the plant: A [10% brine; 2°C]: blue, B [2.5% brine; 4°C]:yellow, and C [30% brine; 8°C]: red.

Fig 3 OTU network summarizing the relationships between taxa and samples. Only OTUs occurring at 0.5% in at least 5 samples are shown. The abundance of OTUs in the three lots for each plant was averaged. Sizes of the OTUs are made proportional to weighted degree (i.e., for OTUs, this measures the total occurrence of an OTU in the whole data set) using a power spline. OTUs and samples are connected with a line (i.e., edge) to a sample node, and its thickness is made proportional to the abundance of an OTU in the connected sample. Samples are color coded as a function of the plant: A [10% brine; 2°C]: light blue, B [2.5% brine; 4°C]: yellow, and C [30% brine; 8°C]: red.

Fig 4 Significant co-occurrence and co-exclusion relationships between bacterial OTUs. Spearman's rank correlation matrix of OTUs with > 0.2% abundance in at least 5 samples. Strong correlations are indicated by large circles, whereas weak correlations are indicated by small circles. The color of the scale bar denote the nature of the correlation, with 1 indicating a perfectly positive correlation (dark blue) and -1 indicating a perfectly negative correlation (dark red). Only significant correlations (FDR <0.05) are shown.

Fig 5 Differences in representative *Acinetobacter* and *Halomonas* oligotype sequence distribution between three lard samples manufactured with different saline concentration of the brine and temperature (A [10% brine; 2°C], B [2.5% brine; 4°C], and C [30% brine; 8°C]). Inner bars indicate the presence of an oligotype in a given sample (A: blue, B: yellow and C: red). Outlier circle if colored denote oligotypes abundance significantly present in a given plant (P <0.05, Pairwise comparisons using Wilcoxon rank sum test).

Table 1 pH, a_w and viable counts of different microbial groups in lard samples during ripening for 90 days. Data are averages of results of three lots ± standard deviations.

		Mean* ± SD log CFU g^{-1}					
Plants	days	pH	a_w	*Enterobacteriaceae*	CNS	LAB	Total Viable Count
A	0	6.62 ± 0.53 b	0.97± 0.04 c	3.32 ± 2.65 b	3.48 ± 1.22 a	2.57 ± 0.94 a	5.72 ± 1.84 a
	7	6.17 ± 0.29 a	0.91 ± 0.00 b	3.77 ± 0.93 b	3.76 ± 2.92 a	3.02 ± 0.48 a	5.98 ± 1.64 a
	15	6.30 ± 0.30 ab	0.90 ± 0.01 b	2.87 ± 0.54 b	3.53 ± 2.82 a	3.00 ± 0.52 a	6.06 ± 0.75 a
	30	6.32 ± 0.21 ab	0.90 ± 0.00 b	3.06 ± 0.45 b	4.86 ± 0.52 a	3.16 ± 0.75 a	5.46 ± 0.92 a
	60	6.25 ± 0.22 ab	0.91 ± 0.01 b	2.47 ± 0.92 ab	2.56 ± 2.12 a	3.21 ± 0.43 a	5.92 ± 1.74 a
	90	6.38 ± 0.10 ab	0.83 ± 0.02 a	1.00 ± 0.91 a	4.22 ± 1.29 a	2.54 ± 0.72 a	4.59 ± 1.40 a
B	0	6.65 ± 0.10 d	0.98 ± 0.00 c	2.53 ± 1.48 bc	1.66 ± 0.97 a	2.14 ± 0.60 a	4.09 ± 1.03 a
	7	6.30 ± 0.17 c	0.90 ± 0.03 b	2.91 ± 0.24 bc	3.59 ± 0.33 b	2.30 ± 0.22 a	5.52 ± 0.57 b
	15	6.27 ± 0.14 c	0.87 ± 0.03 a	3.20 ± 0.40 c	3.66 ± 0.28 b	2.10 ± 0.81 a	5.47 ± 0.35 b
	30	5.93 ± 0.10 a	0.87 ± 0.01 a	2.87 ± 0.56 bc	3.68 ± 0.32 b	2.34 ± 0.29 a	5.22 ± 0.45 b
	60	6.18 ± 0.15 bc	0.87 ± 0.01 a	1.86 ± 1.09 b	2.81 ± 1.12 b	2.70 ± 0.69 a	4.80 ± 0.28 ab
	90	6.07 ± 0.10 ab	0.87 ± 0.01 a	0.33 ± 0.52 a	2.91 ± 0.59 b	2.25 ± 0.58 a	4.13 ± 0.55 a
C	0	6.47 ± 0.47 c	0.98 ± 0.00 c	0.52 ± 1.28 ab	2.60 ± 0.92 a	1.84 ± 1.04 ab	3.55 ± 1.26 a
	7	6.10 ± 0.25 ab	0.86 ± 0.04 b	2.34 ± 2.00 b	3.21 ± 0.98 ab	2.79 ± 0.49 b	4.79 ± 1.32 a
	15	6.08 ± 0.15 ab	0.83 ± 0.04 ab	1.87 ± 1.48 ab	3.66 ± 0.36 b	2.15 ± 0.49 ab	4.45 ± 1.28 a
	30	5.93 ± 0.24 a	0.80 ± 0.04 a	1.96 ± 1.60 ab	3.13 ± 0.79 ab	2.17 ± 1.15 ab	4.15 ± 1.48 a
	60	6.12 ± 0.15 ab	0.79 ± 0.03 a	1.18 ± 1.01 ab	3.02 ± 0.24 ab	1.90 ± 0.68 ab	4.25 ± 0.40 a
	90	6.40 ± 0.13 bc	0.82 ± 0.05 ab	0.22 ± 0.53 a	2.53 ± 0.53 a	1.09 ± 1.26 a	3.42 ± 0.87 a

* Different letters in the same column for each plant indicate significant differences among times (P <0.05).

Table 2 Observed diversity and Good's coverage (%) for the 16S rRNA amplicons. Samples are labeled according to time (0, 7, 15, 30, 60 and 90 days); plant (A, B and C) and lots (_A; _B; _C)

Code	Time	Lot	Plant	Good's coverage (%)	chao1	observed_species	shannon	Number of sequence
A_0_A	0	1	A	87.01	951.28	285	4.73	29200
A_7_A	7	1	A	85.88	1494.00	294	5.06	6800
A_15_A	15	1	A	89.46	633.14	254	5.00	10124
A_30_A	30	1	A	89.66	733.14	253	5.60	12124
A_60_A	60	1	A	89.34	993.05	237	4.39	16346
A_90_A	90	1	A	84.19	1216.76	362	6.14	13906
A_0_B	0	2	A	77.23	1607.05	512	7.05	40895
A_7_B	7	2	A	91.59	514.28	207	4.02	15962
A_15_B	15	2	A	92.28	627.35	186	4.20	39534
A_30_B	30	2	A	92.66	475.04	180	4.04	23230
A_60_B	60	2	A	92.66	493.14	170	3.31	32758
A_90_B	90	2	A	83.25	1255.78	368	6.10	19319
A_0_C	0	3	A	93.41	576.00	156	3.56	61972
A_7_C	7	3	A	89.15	819.23	247	4.98	16792
A_15_C	15	3	A	90.59	660.00	213	4.05	10636
A_30_C	30	3	A	90.65	589.53	222	4.19	16578
A_60_C	60	3	A	88.46	721.67	254	4.74	17061
A_90_C	90	3	A	88.21	894.79	267	5.35	15840
B_0_A	0	1	B	86.57	958.17	307	5.43	15035
B_7_A	7	1	B	91.59	635.55	190	3.57	11577
B_15_A	15	1	B	89.46	803.12	242	4.69	15877
B_30_A	30	1	B	89.90	660.33	231	3.98	16632
B_60_A	60	1	B	90.00	670.33	232	3.78	18632
B_90_A	90	1	B	89.71	679.53	234	3.91	19348
B_0_B	0	2	B	86.01	714.01	350	5.27	7112
B_7_B	7	2	B	86.07	919.28	306	4.97	12123
B_15_B	15	2	B	85.32	1119.89	341	5.91	6910

B_30_B	30	2	B	84.13	1057.00	364	5.98	18349
B_60_B	60	2	B	90.03	610.53	218	4.00	14621
B_90_B	90	2	B	89.65	762.20	221	4.04	15488
B_0_C	0	3	B	84.25	1282.79	360	6.21	27782
B_7_C	7	3	B	83.56	1157.07	380	6.28	6849
B_15_C	15	3	B	87.77	720.89	291	5.11	35191
B_30_C	30	3	B	79.86	1386.82	453	6.91	19158
B_60_C	60	3	B	93.66	568.83	148	3.83	18236
B_90_C	90	3	B	92.28	551.15	176	4.06	20743
C_0_A	0	1	C	81.81	992.24	455	7.01	4733
C_7_A	7	1	C	78.86	1248.95	504	7.18	4374
C_15_A	15	1	C	72.15	1750.57	633	8.01	9585
C_30_A	30	1	C	91.09	595.04	210	4.14	13114
C_60_A	60	1	C	79.86	1489.16	441	6.43	4713
C_90_A	90	1	C	85.32	910.02	330	5.20	7516
C_0_B	0	2	C	83.81	1463.10	358	6.02	7236
C_7_B	7	2	C	89.40	802.00	256	4.81	6294
C_15_B	15	2	C	90.09	705.04	228	4.47	4315
C_30_B	30	2	C	90.65	790.32	210	4.13	8374
C_60_B	60	2	C	91.65	780.22	211	4.23	7384
C_90_B	90	2	C	89.40	936.80	227	3.95	4688
C_0_C	0	3	C	75.35	1648.40	548	7.64	24441
C_7_C	7	3	C	85.19	865.52	352	5.85	10082
C_15_C	15	3	C	82.43	1181.88	416	6.73	22410
C_30_C	30	3	C	90.34	1043.50	202	4.00	4595
C_60_C	60	3	C	83.69	1230.33	367	6.11	17724
C_90_C	90	3	C	82.37	916.76	437	5.97	7067

Table 3 Incidence of the major taxonomic groups detected by 16S amplicon target sequencing. Only OTUs with an incidence above 0.2% in at least 5 samples are shown. Abundance of OTUs in the 3 lots for each plant was averaged and standard deviations was also displayed. Samples are labeled according to time (0, 7, 15, 30, 60 and 90 days) and plant (A, B and C).

OTU ID	A_0 ± SD		A_7 ± SD		A_15 ± SD		A_30 ± SD		A_60 ± SD		A_90 ± SD	
Acinetobacter	4.12	5.94	5.67	9.44	2.49	3.99	0.04	0.04	1.07	1.85	2.40	3.73
Acinetobacter guillouiae	0.10	0.13	0.25	0.43	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Acinetobacter johnsonii	35.02	0.67	23.40	36.74	31.85	42.40	3.01	2.80	0.61	0.89	8.01	12.15
Acinetobacter lwoffii	1.53	1.95	2.70	4.56	1.55	2.21	0.18	0.15	0.00	0.00	0.36	0.56
Aeromonadaceae	0.77	0.91	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus	0.15	0.25	0.04	0.07	0.02	0.04	0.00	0.00	0.19	0.33	0.04	0.04
Brochothrix	3.21	0.36	0.38	0.65	0.27	0.47	0.00	0.00	0.31	0.54	2.70	4.62
Caulobacteraceae	1.84	3.19	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.54
Chromohalobacter	0.00	0.00	0.10	0.13	0.08	0.14	1.75	0.92	0.38	0.39	17.29	16.46
Clostridium	1.07	1.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.07
Halomonadaceae	0.00	0.00	0.00	0.00	0.15	0.13	0.44	0.33	0.17	0.18	0.40	0.35
Halomonas	0.00	0.00	27.00	45.89	15.43	7.63	21.53	12.68	9.77	5.27	10.35	6.57
Kocuria	0.13	0.22	0.00	0.00	0.04	0.07	0.04	0.03	0.00	0.00	0.06	0.11
Lactobacillus	0.29	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.04	0.17	0.13
Listeriaceae	0.86	1.44	0.17	0.19	0.06	0.11	0.10	0.04	0.06	0.06	3.24	3.95
Macrococcus caseolyticus	0.61	0.94	0.19	0.22	0.19	0.23	0.00	0.00	0.00	0.00	0.44	0.76
Methylotenera mobilis	1.36	2.30	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.83
Micrococcus	0.19	0.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.04
Paracoccus	0.63	1.09	0.04	0.04	0.00	0.00	0.03	0.03	0.02	0.04	0.44	0.60
Propionibacterium acnes	0.29	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Pseudomonas	2.43	3.17	1.69	1.76	0.23	0.24	0.05	0.04	3.01	5.22	0.13	0.06
Pseudomonas fragi	23.71	3.14	23.53	38.64	16.42	28.16	0.05	0.05	6.13	10.61	12.61	21.35
Psychrobacter	0.73	0.59	1.80	1.09	1.74	2.90	0.18	0.11	2.66	4.49	9.60	11.10
Salinisphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.36	2.35
Sphingomonas	0.29	0.51	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.22
Staphylococcus	0.04	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.40	1.96
Staphylococcus equorum	0.02	0.04	3.76	3.45	3.55	1.66	1.78	1.97	0.77	0.63	12.17	7.21
Staphylococcus sciuri	0.23	0.22	1.21	1.89	1.21	1.16	0.71	0.62	0.02	0.04	5.29	9.06
Staphylococcus succinus	0.00	0.00	0.17	0.24	0.08	0.10	0.08	0.07	0.02	0.04	0.52	0.44
Vibrio	0.06	0.11	3.60	6.23	22.92	39.59	64.50	6.16	65.73	16.10	3.79	6.34

OTU ID	B_0 ± SD		B_7 ± SD		B_15 ± SD		B_30 ± SD		B_60 ± SD		B_90 ± SD	
Acinetobacter	16.86	0.82	0.92	0.64	0.15	0.14	0.73	0.79	0.00	0.00	0.13	0.22
Acinetobacter guillouiae	0.27	0.42	0.00	0.00	0.04	0.07	0.00	0.00	0.00	0.00	0.00	0.00
Acinetobacter johnsonii	13.26	22.31	0.21	0.26	0.04	0.07	0.17	0.24	0.00	0.00	0.02	0.04
Acinetobacter lwoffii	1.59	2.27	1.02	1.77	0.04	0.04	1.44	2.50	0.00	0.00	0.02	0.04
Aeromonadaceae	4.90	4.45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bacillus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.84	1.45
Brochothrix	0.23	0.16	1.71	2.23	3.74	6.16	0.88	0.33	0.12	0.11	0.13	0.17
Caulobacteraceae	0.06	0.00	0.00	0.00	0.06	0.11	0.04	0.07	0.00	0.00	0.04	0.04
Chromohalobacter	0.00	0.00	0.00	0.00	0.00	0.00	0.52	0.64	21.33	20.48	1.94	0.71
Clostridium	0.04	0.07	0.02	0.04	0.15	0.25	0.04	0.07	0.00	0.00	0.02	0.04
Halomonadaceae	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.19	0.69	1.20	0.08	0.10
Halomonas	0.00	0.00	0.04	0.07	0.15	0.14	4.33	2.45	26.78	21.04	9.49	6.84
Kocuria	0.02	0.04	0.00	0.00	0.02	0.04	0.02	0.04	0.00	0.00	0.02	0.04
Lactobacillus	1.11	1.65	0.17	0.16	1.92	3.22	0.13	0.06	0.08	0.07	0.15	0.16
Listeriaceae	0.98	1.04	0.13	0.17	1.25	1.42	0.54	0.36	0.06	0.00	0.13	0.13
Macrococcus caseolyticus	0.61	1.05	0.04	0.07	0.13	0.22	0.38	0.60	0.00	0.00	0.00	0.00
Methylotenera mobilis	21.64	36.51	0.10	0.13	0.19	0.27	1.97	3.30	0.04	0.04	0.27	0.25
Micrococcus	0.04	0.07	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.02	0.04
Paracoccus	0.08	0.10	0.02	0.04	0.00	0.00	0.19	0.27	0.00	0.00	0.06	0.06
Propionibacterium acnes	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00
Pseudomonas	2.63	3.04	3.79	4.11	1.07	0.66	1.13	1.58	0.03	0.03	12.92	22.38
Pseudomonas fragi	24.84	30.39	28.69	10.74	40.99	2.26	12.71	8.22	0.15	0.07	0.27	0.13
Psychrobacter	6.98	2.50	59.43	4.31	39.57	13.61	46.26	28.70	2.95	2.13	27.10	43.13
Salinisphaera	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sphingomonas	0.44	0.76	0.00	0.00	0.04	0.07	0.04	0.07	0.00	0.00	0.00	0.00
Staphylococcus	0.08	0.10	0.06	0.06	0.02	0.04	0.19	0.23	0.00	0.00	0.06	0.11
Staphylococcus equorum	0.61	1.00	1.78	2.92	0.73	1.21	7.32	6.40	2.21	0.60	0.92	1.13
Staphylococcus sciuri	0.08	0.10	0.02	0.04	0.21	0.36	0.17	0.10	0.00	0.00	0.02	0.04
Staphylococcus succinus	0.00	0.00	0.06	0.06	0.25	0.43	1.57	1.58	0.40	0.07	0.23	0.30
Vibrio	0.00	0.00	0.00	0.00	0.00	0.00	12.48	18.51	35.67	30.06	25.55	34.23

OTU ID	C_0 ± SD		C_7 ± SD		C_15 ± SD		C_30 ± SD		C_60 ± SD		C_90 ± SD	
Acinetobacter	4.12	2.27	0.29	0.19	0.61	0.53	4.12	0.35	0.48	0.83	0.06	0.06
Acinetobacter guillouiae	0.10	0.63	0.29	0.45	0.50	0.45	0.10	0.25	0.13	0.22	0.02	0.04
Acinetobacter johnsonii	35.02	11.73	35.88	32.36	51.70	27.26	35.02	49.38	20.72	20.33	0.59	0.85

Acinetobacter lwoffii	1.53	3.77	2.01	2.11	7.66	2.40	1.53	3.72	0.33	0.58	0.02	0.04
Aeromonadaceae	0.77	0.07	0.00	0.00	0.00	0.00	0.77	0.00	0.02	0.04	0.40	0.69
Bacillus	0.15	1.29	0.27	0.32	0.10	0.13	0.15	0.04	1.20	0.94	0.23	0.22
Brochothrix	3.21	0.00	0.15	0.25	1.23	2.03	3.21	0.07	0.12	0.11	0.02	0.04
Caulobacteraceae	1.84	0.82	0.23	0.40	0.27	0.37	1.84	0.04	0.53	0.47	0.02	0.04
Chromohalobacter	0.00	1.92	0.90	0.82	1.44	1.58	0.00	32.89	11.99	12.07	0.04	0.07
Clostridium	1.07	3.87	1.51	1.94	0.31	0.54	1.07	0.20	0.50	0.11	0.50	0.87
Halomonadaceae	0.00	0.04	0.23	0.30	1.40	1.48	0.00	0.29	0.29	0.04	0.06	0.11
Halomonas	0.00	0.07	0.10	0.10	2.47	2.20	0.00	4.65	0.82	0.11	0.06	0.11
Kocuria	0.13	0.78	1.15	1.39	0.48	0.51	0.13	0.00	0.59	0.18	0.06	0.11
Lactobacillus	0.29	10.82	0.98	1.49	0.54	0.42	0.29	0.07	2.11	1.63	0.94	0.76
Listeriaceae	0.86	4.10	7.03	10.79	0.87	0.82	0.86	0.24	9.71	7.04	1.13	1.96
Macrococcus caseolyticus	0.61	0.66	2.72	4.49	1.17	1.07	0.61	0.13	0.36	0.18	0.04	0.07
Methylotenera mobilis	1.36	1.10	0.08	0.14	0.61	0.94	1.36	0.07	0.19	0.00	0.27	0.42
Micrococcus	0.19	0.35	0.48	0.68	0.61	0.48	0.19	0.00	0.73	0.47	0.02	0.04
Paracoccus	0.63	1.66	1.25	1.66	1.88	2.29	0.63	0.06	1.59	0.95	1.36	2.14
Propionibacterium acnes	0.29	0.10	0.19	0.33	0.25	0.29	0.29	0.00	1.05	0.91	0.31	0.44
Pseudomonas	2.43	4.22	1.44	2.01	0.27	0.37	2.43	0.07	0.08	0.07	1.42	2.46
Pseudomonas fragi	23.71	0.04	4.39	7.33	1.63	2.61	23.71	10.61	0.04	0.04	0.50	0.45
Psychrobacter	0.73	0.38	0.73	0.53	2.61	0.64	0.73	40.95	6.48	9.49	6.32	8.67
Salinisphaera	0.00	0.18	6.46	10.07	3.74	3.30	0.00	17.46	21.00	19.35	33.44	32.84
Sphingomonas	0.29	0.50	0.10	0.13	0.50	0.43	0.29	0.06	0.19	0.11	0.10	0.13
Staphylococcus	0.04	0.69	0.21	0.19	0.46	0.55	0.04	0.00	0.36	0.40	0.02	0.04
Staphylococcus equorum	0.02	1.07	0.19	0.19	2.82	4.67	0.02	0.27	4.55	3.11	1.13	1.90
Staphylococcus sciuri	0.23	2.69	10.64	17.84	4.08	4.22	0.23	0.57	2.61	1.67	0.00	0.00
Staphylococcus succinus	0.00	0.11	0.08	0.14	3.79	6.56	0.00	0.07	1.19	0.76	0.06	0.11
Vibrio	0.06	2.17	0.08	0.10	0.00	0.00	0.06	0.11	0.04	0.04	37.07	35.54

33

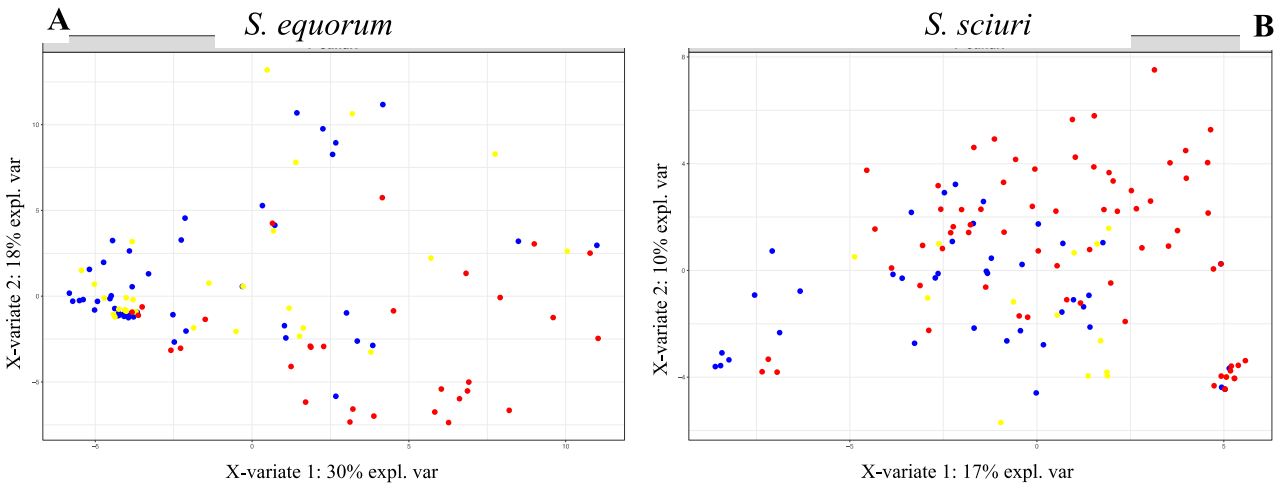

A
S. equorum
X-variate 2: 18% expl. var
X-variate 1: 30% expl. var
S. sciuri
B
X-variate 2: 10% expl. var
X-variate 1: 17% expl. var

Fig 1

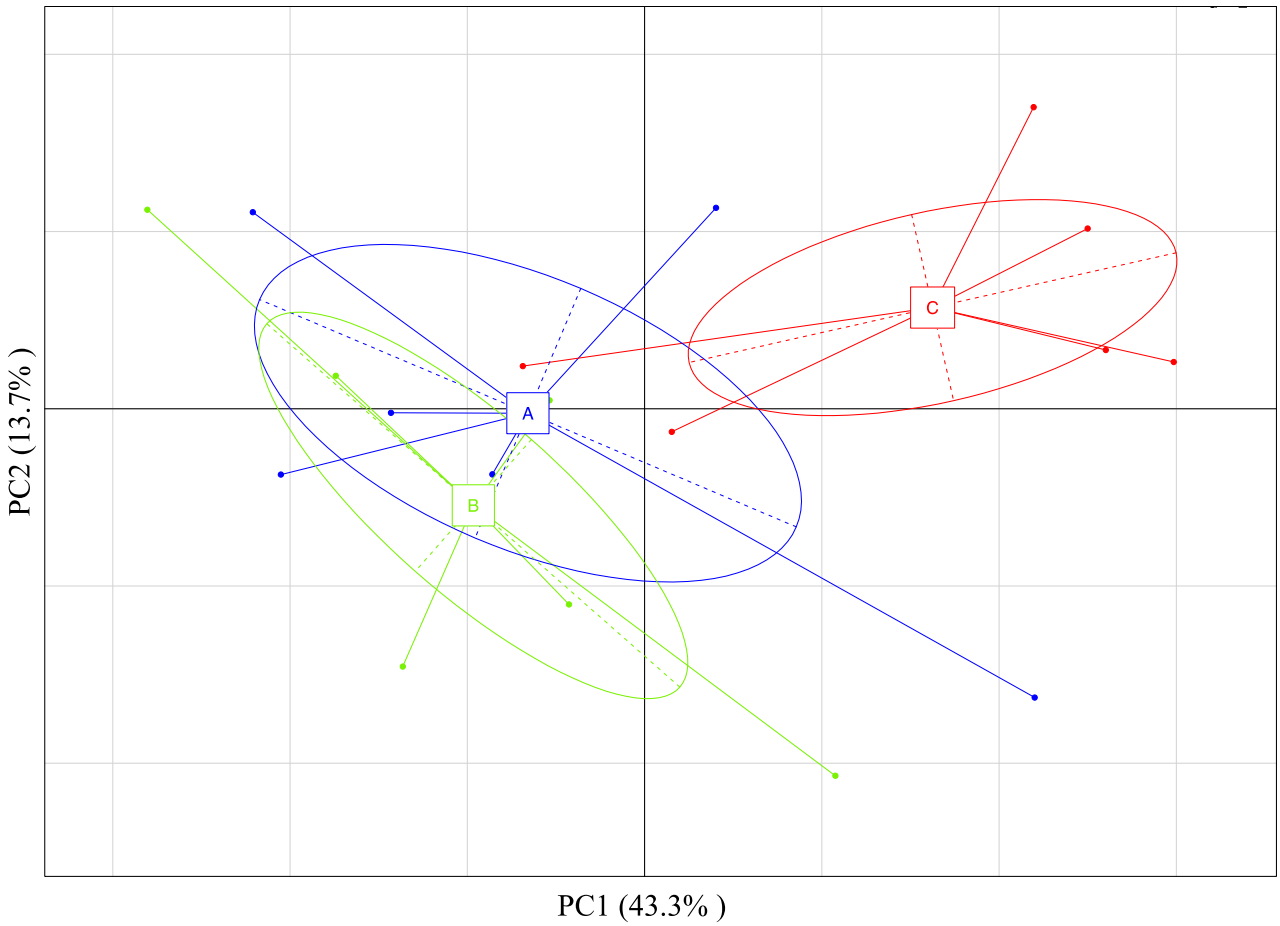
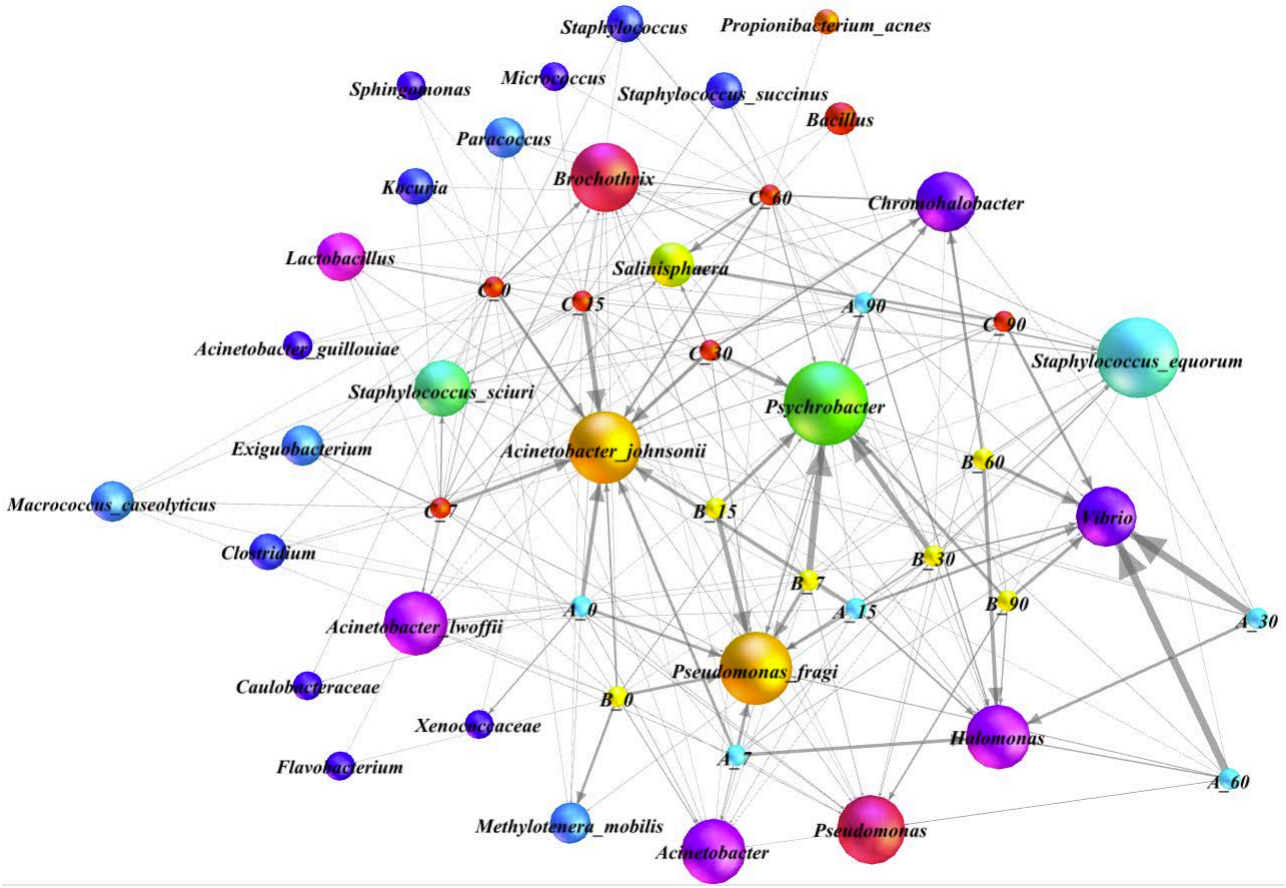


Fig 2


Staphylococcus
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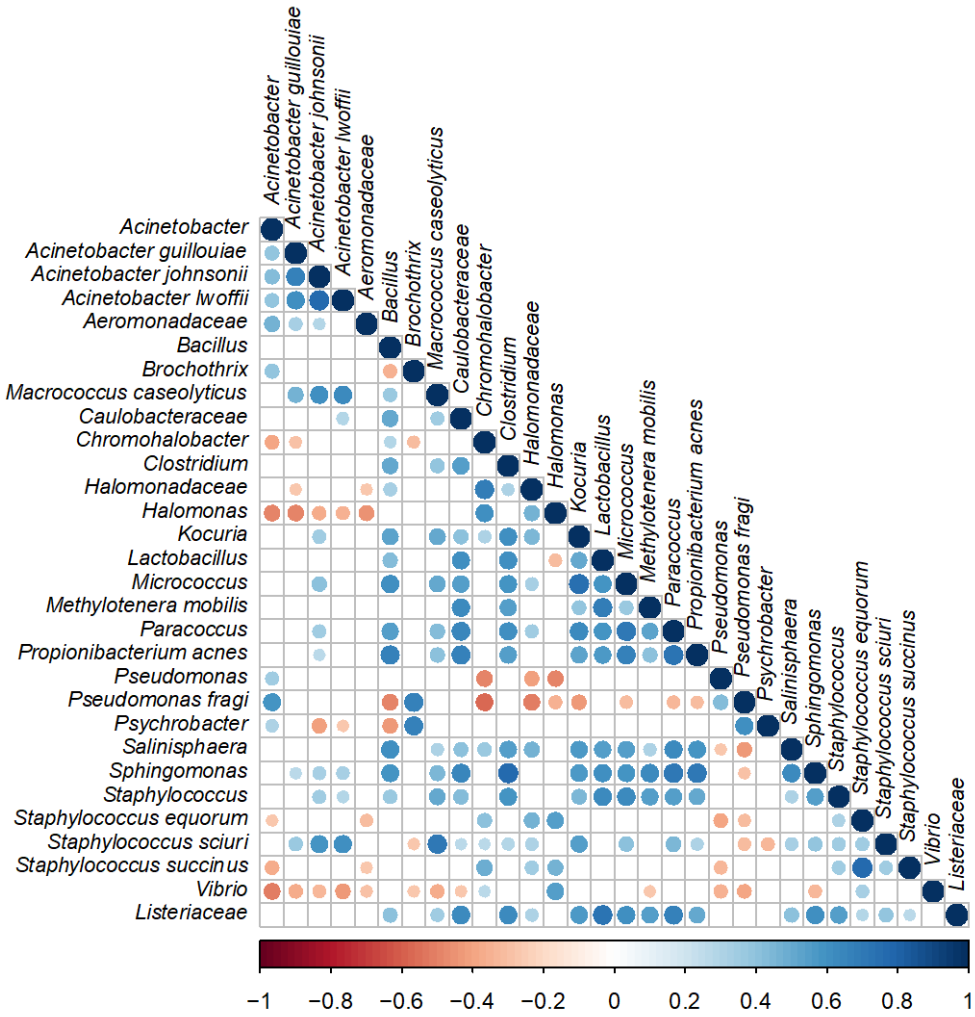
Sphingomonas
Micrococcus
Staphylococcus_succinus
Bacillus
Paracoccus
Kocuria
Brochothrix
C_60
Chromohalobacter
Lactobacillus
Salinisphaera
C_0
C_15
A_90
C_90
Acinetobacter_guillouiae
C_30
Staphylococcus_equorum
Staphylococcus_sciuri
Psychrobacter
Exiguobacterium
Acinetobacter_johnsonii
B_60
Macrococcus_caseolyticus
C_7
B_15
Vibrio
Clostridium
B_30
B_7
A_0
A_15
B_90
A_30
Acinetobacter_lwoffii
Pseudomonas_fragi
Caulobacteraceae
B_0
Xenococcaceae
Halomonas
A_7
Flavobacterium
A_60
Methylotenera_mobilis
Pseudomonas
Acinetobacter

Fig 3


Acinetobacter
Acinetobacter guillouiae
Acinetobacter johnsonii
Acinetobacter lwoffii
Aeromonadaceae
Bacillus
Brochothrix
Macrococcus caseolyticus
Caulobacteraceae
Chromohalobacter
Clostridium
Halomonadaceae
Halomonas
Kocuria
Lactobacillus
Micrococcus
Methylotenera mobilis
Paracoccus
Propionibacterium acnes
Pseudomonas
Pseudomonas fragi
Psychrobacter
Salinisphaera
Sphingomonas
Staphylococcus
Staphylococcus equorum
Staphylococcus sciuri
Staphylococcus succinus
Vibrio
Listeriaceae
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−0.6
−0.4
−0.2
0
0.2
0.4
0.6
0.8
1

Fig 4

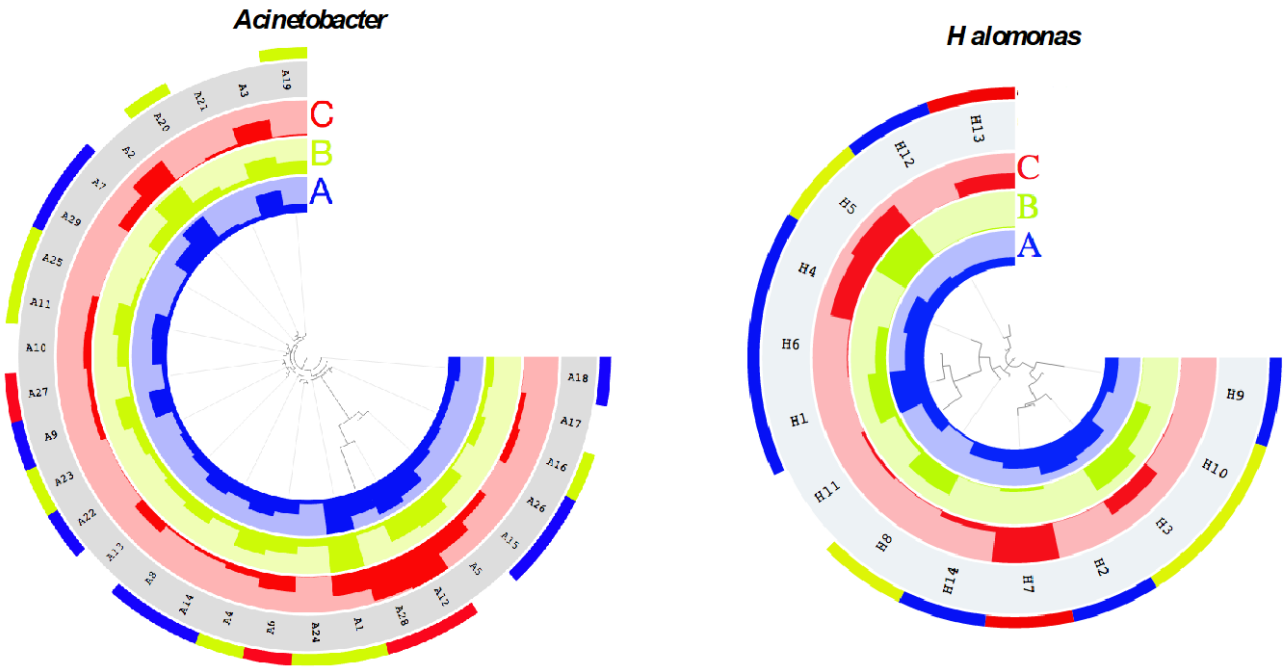
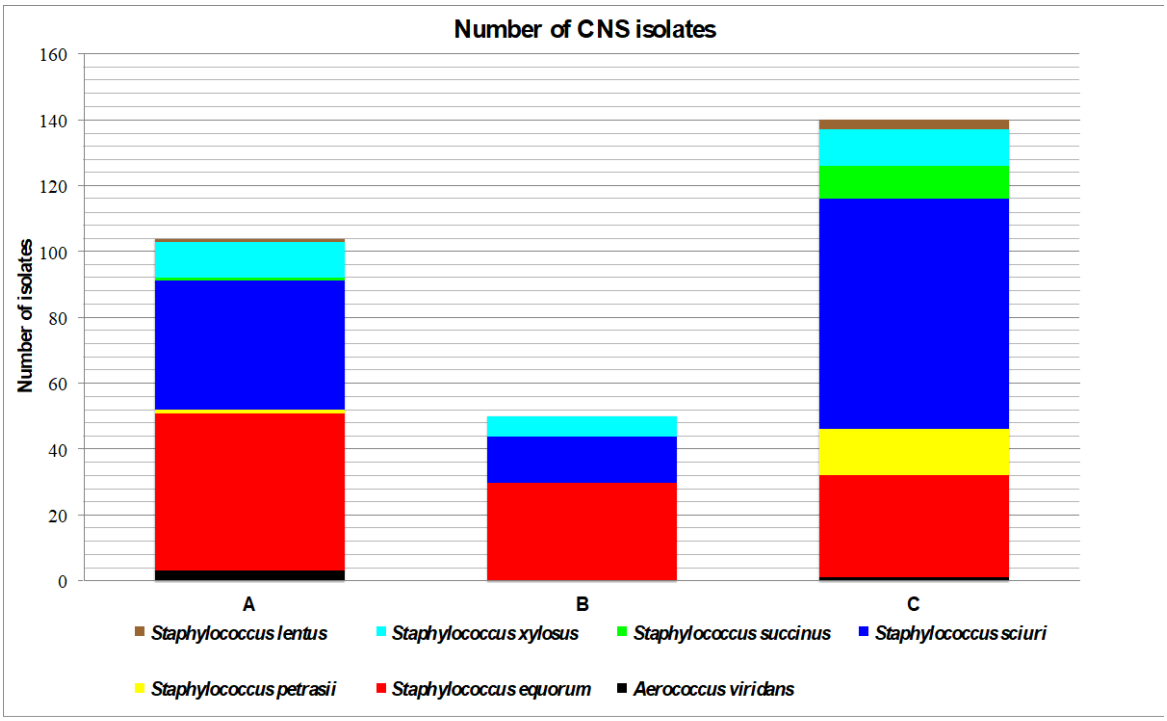

Acinetobacter
C
B
A
A19
A3
A21
A20
A2
A7
A29
A25
A11
A10
A27
A9
A23
A22
A13
A8
A14
A4
A6
A24
A1
A28
A12
A5
A15
A26
A16
A17
A18
Halomonas
C
B
A
H13
H12
H5
H4
H6
H1
H11
H8
H14
H7
H2
H3
H10
H9

Fig 5



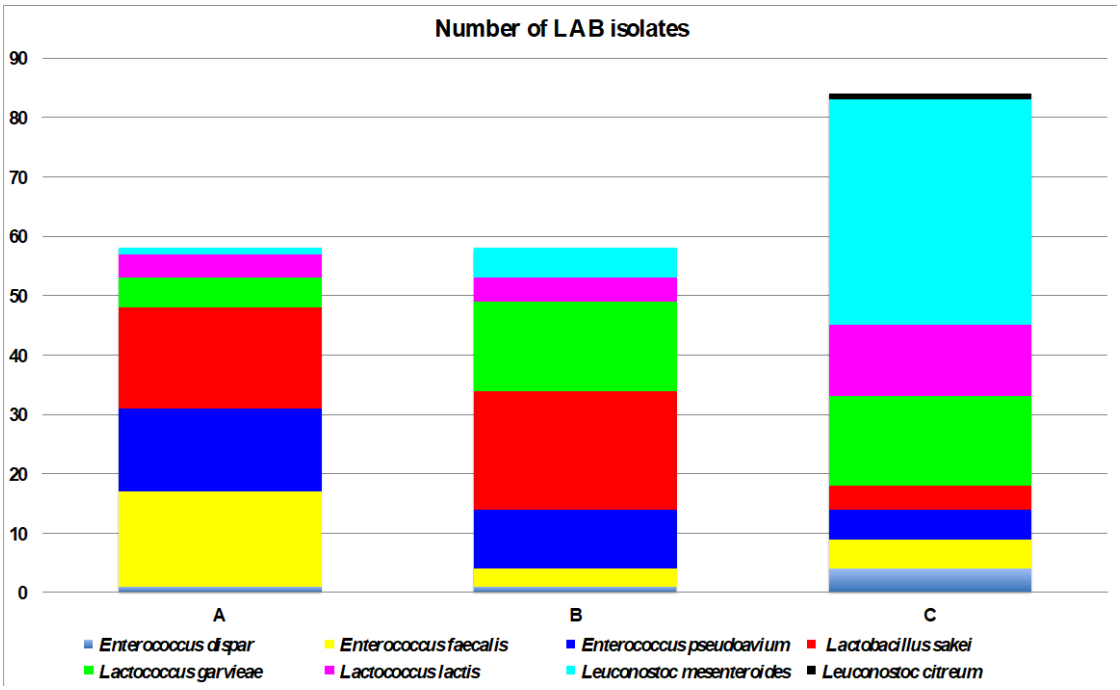


FIG S1 Identification of the CNS and LAB isolates. The number of isolates for each species associated with a plant is shown.

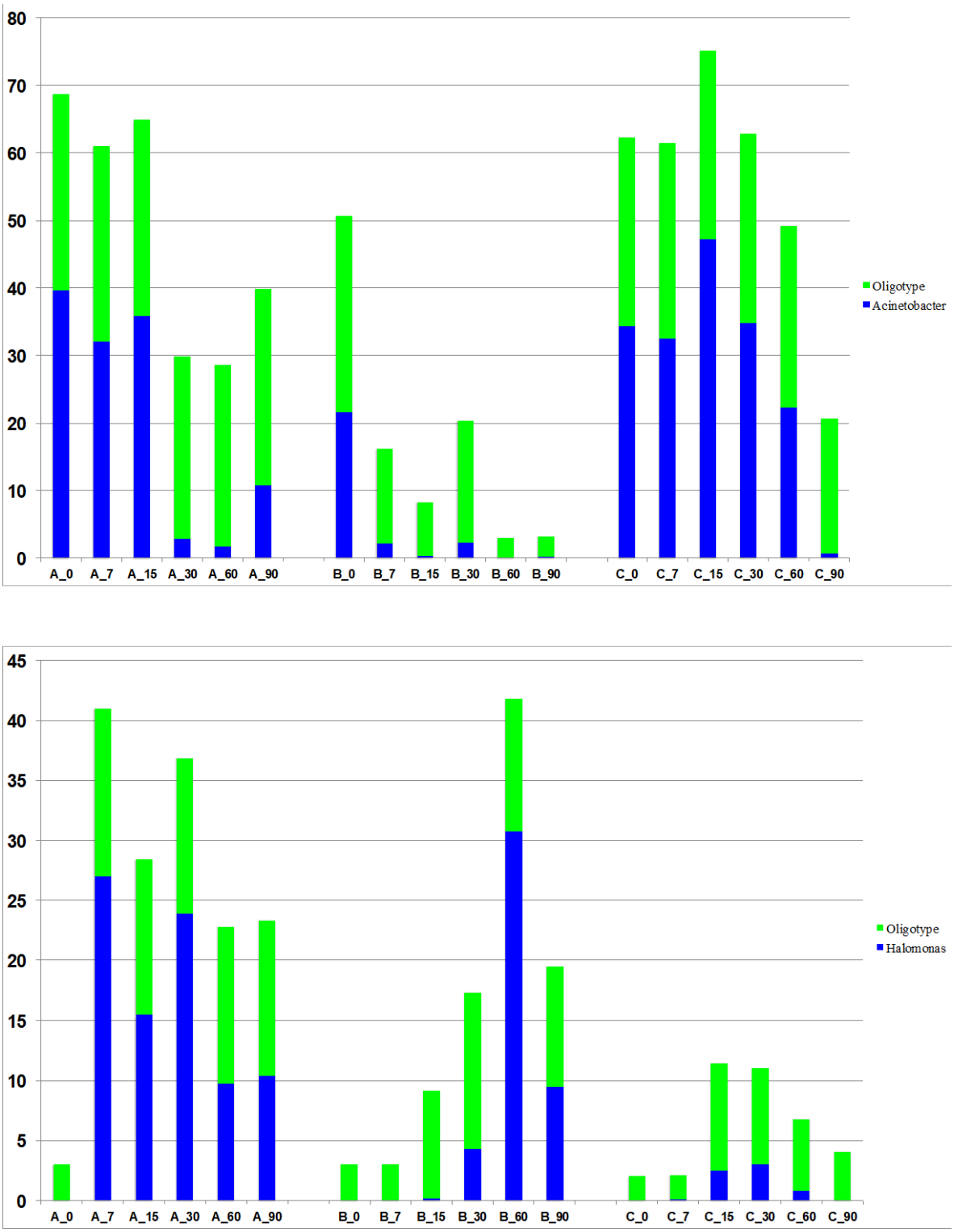


FIG S2 Proportion of sequence reads (blue bar) in each lard sample that mapped to the genus *Acinetobacter* (plot A) and *Halomonas* (plot B) and proportions of abundant oligotypes (green bar) generated from sequence reads that mapped to the genus *Acinetobacter* (plot A) and *Halomonas* (plot B) using the oligotyping pipeline

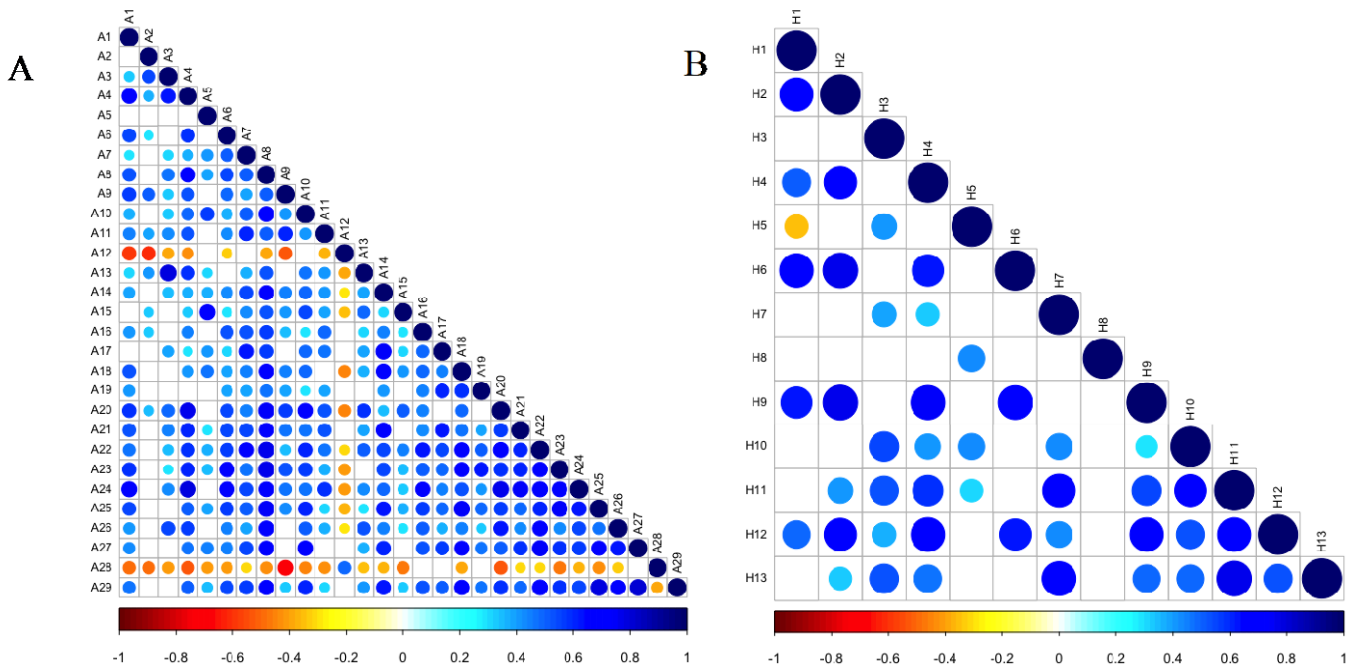


FIG S3 Significant co-occurrence and co-exclusion relationships between *Acinetobacter* (plot A) and *Halomonas* (plot B) oligotype. Spearman's rank correlation matrix of OTUs with > 0.2% abundance in at least 5 samples. Large circles indicate strong correlations. whereas small circles indicate weak correlations. The colors of the scale bar denote the nature of the correlation. with 1 indicating a perfectly positive correlation (dark blue) and -1 indicating a perfectly negative correlation (dark red). Only significant correlations (FDR < 0.05) are shown.

Table S1 *P*-value of pairwise t-test among the three plant based on pH. a_w and viable counts of different meat spoilage microbial groups in lard samples during ripening for 90 days. Only significant value are highlights (P <0.05).

	days	pH	a_w	*Entreobacteriaceae*	*Staphyloococcaceae*	LAB	Total Viable Count
A vs. B	0	0.88	0.31	0.54	0.02	0.37	0.09
	7	0.36	0.32	0.06	0.89	0.01	0.53
	15	0.81	0.01	0.26	0.92	0.04	0.11
	30	0.00	0.00	0.52	0.00	0.03	0.58
	60	0.55	0.00	0.32	0.80	0.15	0.15
	90	0.00	0.00	0.15	0.05	0.47	0.47
A vs. C	0	0.62	0.33	0.04	0.19	0.23	0.04
	7	0.68	0.01	0.14	0.67	0.42	0.20
	15	0.81	0.01	0.26	0.92	0.04	0.11
	30	0.02	0.00	0.14	0.00	0.11	0.09
	60	0.24	0.00	0.04	0.61	0.00	0.04
	90	0.80	0.48	0.10	0.01	0.03	0.11
B vs. C	0	0.37	0.90	0.03	0.12	0.55	0.44
	7	0.14	0.09	0.50	0.40	0.05	0.24

15	0.05	0.08	0.06	0.98	0.89	0.09
30	1.00	0.00	0.22	0.14	0.73	0.12
60	0.45	0.00	0.29	0.67	0.07	0.02
90	0.00	0.02	0.71	0.28	0.07	0.12

Table S2 Incidence of the major taxonomic groups detected by 16S amplicon target sequencing. Only OTUs with an incidence above 0.2% in at least 5 samples are shown. Samples are labeled according to time (0. 7. 15. 30. 60 and 90 days) and plant (A. B and C) and lots (_A; _B; _C)

OTU_ID	A_0_A	A_0_B	A_0_C	A_7_A	A_7_B	A_7_C	A_15_A	A_15_B	A_15_C	A_30_A	A_30_B	A_30_C	A_60_A	A_60_B	A_60_C	A_90_A	A_90_B	A_90_C
Acinetobacter	10,98	0,75	0,63	16,56	0,06	0,38	7,09	0,38	0,00	0,00	0,06	0,06	3,20	0,00	0,00	6,71	0,31	0,19
Acinetobacter guillouiae	0,00	0,25	0,06	0,00	0,00	0,75	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Acinetobacter johnsonii	35,67	34,33	35,07	4,39	0,06	65,75	7,09	80,80	7,65	0,00	5,52	3,52	1,63	0,00	0,19	1,69	0,31	22,02
Acinetobacter lwoffii	0,19	0,63	3,76	0,06	0,06	7,97	0,00	4,08	0,56	0,00	0,25	0,28	0,00	0,00	0,00	0,00	0,06	1,00
Aeromonadaceae	0,25	0,25	1,82	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Bacillus	0,00	0,44	0,00	0,13	0,00	0,00	0,00	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,56	0,00	0,06	0,06
Brochothrix	3,63	3,00	3,00	1,13	0,00	0,00	0,82	0,00	0,00	0,00	0,00	0,00	0,94	0,00	0,00	8,03	0,06	0,00
Macrococcus caseolyticus	0,00	1,69	0,13	0,06	0,06	0,44	0,13	0,00	0,44	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,32
Caulobacteraceae	0,00	5,52	0,00	0,00	0,06	0,13	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,94	0,00
Chromohalobacter	0,00	0,00	0,00	0,06	0,25	0,00	0,00	0,00	0,25	0,69	2,38	2,17	0,25	0,06	0,82	0,13	32,94	18,82
Clostridium	0,00	3,20	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,13	0,13	0,00
Halomonadaceae	0,00	0,00	0,00	0,00	0,00	0,00	0,25	0,00	0,19	0,06	0,63	0,63	0,38	0,06	0,06	0,38	0,75	0,06
Halomonas	0,00	0,00	0,00	0,88	79,99	0,13	24,15	9,97	12,17	35,88	11,86	16,86	3,83	13,86	11,61	2,76	14,12	14,18
Kocuria	0,00	0,38	0,00	0,00	0,00	0,00	0,00	0,00	0,13	0,00	0,06	0,04	0,00	0,00	0,00	0,00	0,19	0,00
Lactobacillus	0,00	0,88	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,06	0,06	0,00	0,06	0,31	0,13
Micrococcus	0,00	0,56	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,13	0,06	0,06
Methylobacterium mobilis	0,06	4,02	0,00	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,44	0,00
Paracoccus	0,00	1,88	0,00	0,00	0,06	0,06	0,00	0,00	0,00	0,00	0,06	0,02	0,00	0,06	0,00	0,19	1,13	0,00
Propionibacterium acnes	0,00	0,88	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Pseudomonas	6,09	0,69	0,50	3,70	0,44	0,94	0,50	0,06	0,13	0,00	0,06	0,07	0,00	0,00	9,03	0,19	0,06	0,13
Pseudomonas fragi	20,14	25,00	26,00	68,13	2,13	0,31	48,93	0,31	0,00	0,00	0,06	0,09	18,38	0,00	0,00	37,76	0,25	0,31
Psychrobacter	1,19	0,94	0,06	2,63	2,20	0,56	5,08	0,13	0,00	0,06	0,19	0,29	7,84	0,13	0,00	22,27	4,96	1,57
Salinisphaera	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	4,08	0,00
Sphingomonas	0,00	0,88	0,00	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,38	0,00
Staphylococcus	0,00	0,13	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,56	0,00	3,64
Staphylococcus equorum	0,00	0,06	0,00	0,00	4,52	6,78	3,89	1,76	5,02	0,31	4,02	1,02	1,25	1,00	0,06	3,95	17,44	15,12
Staphylococcus sciuri	0,00	0,25	0,44	0,00	0,25	3,39	0,00	1,32	2,32	0,00	1,07	1,07	0,00	0,06	0,00	0,00	0,13	15,75
Staphylococcus succinus	0,00	0,00	0,00	0,00	0,44	0,06	0,00	0,19	0,06	0,00	0,13	0,13	0,00	0,06	0,00	0,94	0,06	0,56
Vibrio	0,00	0,19	0,00	0,00	0,00	10,79	0,06	0,06	68,63	60,35	71,58	61,58	61,10	83,63	52,45	11,10	0,00	0,25
Listeriaceae	0,06	2,53	0,00	0,00	0,38	0,13	0,00	0,00	0,19	0,06	0,13	0,13	0,06	0,13	0,00	1,38	7,78	0,56

OTU_ID	B_0_A	B_0_B	B_0_C	B_7_A	B_7_B	B_7_C	B_15_A	B_15_B	B_15_C	B_30_A	B_30_B	B_30_C	B_60_A	B_60_B	B_60_C	B_90_A	B_90_B	B_90_C
Acinetobacter	17,63	16,94	16,00	1,19	0,19	1,38	0,31	0,06	0,06	0,63	0,00	1,57	0,00	0,00	0,00	0,38	0,00	0,00
Acinetobacter guillouiae	0,00	0,06	0,75	0,00	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Acinetobacter johnsonii	0,06	0,69	39,02	0,00	0,13	0,50	0,00	0,13	0,00	0,00	0,06	0,44	0,00	0,00	0,00	0,00	0,06	0,00
Acinetobacter lwoffii	0,06	0,50	4,20	0,00	0,00	3,07	0,00	0,06	0,06	0,00	0,00	4,33	0,00	0,00	0,00	0,00	0,00	0,06
Aeromonadaceae	0,00	6,00	8,70	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Bacillus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,51
Brochothrix	0,25	0,06	0,38	0,75	4,27	0,13	0,31	10,85	0,06	1,25	0,75	0,63	0,13	0,00	0,23	0,31	0,06	0,00
Macrococcus caseolyticus	0,00	0,00	1,82	0,00	0,00	0,13	0,00	0,38	0,00	0,00	0,06	1,07	0,00	0,00	0,00	0,00	0,00	0,00
Caulobacteraceae	0,06	0,06	0,06	0,00	0,00	0,00	0,00	0,19	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,06	0,00	0,06
Chromohalobacter	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,25	0,06	1,25	1,00	41,97	21,00	2,32	2,38	1,13
Clostridium	0,00	0,13	0,00	0,00	0,06	0,00	0,00	0,44	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,06	0,00	0,00
Halomonadaceae	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,13	0,38	0,00	2,07	0,00	0,06	0,19
Halomonas	0,00	0,00	0,00	0,00	0,13	0,00	0,06	0,31	0,06	3,07	2,76	7,15	10,85	50,63	18,85	9,41	16,37	2,70
Kocuria	0,00	0,00	0,06	0,00	0,00	0,00	0,00	0,06	0,00	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,06	0,00
Lactobacillus	0,25	3,01	0,06	0,00	0,19	0,31	0,00	0,13	5,65	0,19	0,13	0,06	0,13	0,00	0,11	0,31	0,13	0,00
Micrococcus	0,00	0,00	0,13	0,00	0,00	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,06	0,00
Methylobacterium mobilis	0,75	63,80	0,38	0,00	0,06	0,25	0,00	0,50	0,06	0,00	0,13	5,77	0,06	0,00	0,06	0,50	0,31	0,00
Paracoccus	0,19	0,00	0,06	0,00	0,00	0,06	0,00	0,00	0,00	0,00	0,06	0,50	0,00	0,00	0,00	0,13	0,06	0,00
Propionibacterium acnes	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,00
Pseudomonas	5,96	0,00	1,94	2,13	0,75	8,47	1,00	1,76	0,44	0,38	0,06	2,95	0,06	0,00	0,03	0,00	0,00	38,77
Pseudomonas fragi	58,91	0,50	15,12	39,71	28,11	18,26	42,79	41,72	38,46	12,55	4,58	21,02	0,19	0,06	0,19	0,38	0,31	0,13
Psychrobacter	9,72	6,38	4,84	55,65	64,12	58,53	55,14	33,63	29,95	79,36	28,36	31,05	4,64	0,56	3,64	76,85	4,14	0,31
Salinisphaera	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Sphingomonas	0,00	1,32	0,00	0,00	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00	0,00
Staphylococcus	0,00	0,19	0,06	0,00	0,13	0,06	0,00	0,06	0,00	0,00	0,13	0,44	0,00	0,00	0,00	0,19	0,00	0,00
Staphylococcus equorum	0,00	1,76	0,06	0,00	0,19	5,14	0,00	2,13	0,06	0,82	13,61	7,53	2,89	1,76	1,99	0,50	2,20	0,06
Staphylococcus sciuri	0,19	0,00	0,06	0,00	0,00	0,06	0,00	0,63	0,00	0,06	0,19	0,25	0,00	0,00	0,00	0,00	0,06	0,00
Staphylococcus succinus	0,00	0,00	0,00	0,00	0,13	0,06	0,00	0,75	0,00	0,25	3,32	1,13	0,44	0,31	0,44	0,13	0,56	0,00
Vibrio	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,63	33,81	3,01	57,78	1,44	47,78	6,84	65,06	4,77
Listeriaceae	2,07	0,88	0,00	0,00	0,06	0,31	0,06	0,88	2,82	0,13	0,75	0,75	0,06	0,06	0,06	0,25	0,13	0,00

OTU_ID	C_0_A	C_0_B	C_0_C	C_7_A	C_7_B	C_7_C	C_15_A	C_15_B	C_15_C	C_30_A	C_30_B	C_30_C	C_60_A	C_60_B	C_60_C	C_90_A	C_90_B	C_90_C
Acinetobacter	0,25	0,56	4,33	0,25	0,50	0,13	0,00	0,94	0,88	0,06	0,69	0,13	0,00	1,44	0,00	0,00	0,06	0,13
Acinetobacter guillouiae	0,00	1,25	0,69	0,06	0,82	0,00	0,00	0,88	0,63	0,00	0,44	0,00	0,00	0,38	0,00	0,00	0,06	0,00
Acinetobacter johnsonii	40,56	63,86	54,62	19,57	73,15	14,93	30,88	82,56	41,66	0,00	88,52	6,34	0,50	41,15	20,50	0,06	1,57	0,13
Acinetobacter lwoffii	0,88	6,96	7,78	0,00	4,20	1,82	10,13	5,33	7,53	0,00	6,96	1,19	0,00	1,00	0,00	0,00	0,06	0,00
Aeromonadaceae	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,06	0,00	0,00	0,00	1,19
Bacillus	2,38	0,25	0,06	0,63	0,00	0,19	0,25	0,00	0,06	0,00	0,00	0,06	1,88	0,13	1,58	0,25	0,00	0,44
Brochothrix	0,00	0,00	0,00	0,00	0,44	0,00	3,58	0,13	0,00	0,13	0,00	0,00	0,06	0,25	0,05	0,00	0,06	0,00
Macrococcus caseolyticus	0,00	0,50	1,32	0,06	0,19	7,90	2,20	0,06	1,25	0,00	0,25	0,06	0,25	0,56	0,25	0,13	0,00	0,00
Caulobacteraceae	1,38	0,75	2,38	0,69	0,00	0,00	0,69	0,00	0,13	0,00	0,00	0,06	0,69	0,00	0,89	0,06	0,00	0,00
Chromohalobacter	0,00	0,00	3,32	1,69	0,06	0,94	1,19	0,00	3,14	0,75	0,00	57,34	0,63	24,65	10,70	0,13	0,00	0,00
Clostridium	0,56	0,00	6,96	3,70	0,00	0,82	0,94	0,00	0,00	0,00	0,06	0,38	0,56	0,38	0,56	1,51	0,00	0,00
Halomonadaceae	0,00	0,00	0,06	0,56	0,00	0,13	2,95	0,00	1,25	0,00	0,00	0,50	0,31	0,25	0,31	0,19	0,00	0,00
Halomonas	0,13	0,00	0,00	0,13	0,00	0,19	4,20	0,00	3,20	8,34	0,00	0,63	0,88	0,69	0,88	0,00	0,19	0,00
Kocuria	0,06	0,00	1,38	0,75	0,00	2,70	1,07	0,13	0,25	0,00	0,00	0,00	0,69	0,38	0,69	0,19	0,00	0,00
Lactobacillus	19,95	0,31	2,26	2,70	0,00	0,25	1,00	0,19	0,44	0,00	0,00	0,13	2,51	0,31	3,51	1,63	1,07	0,13
Micrococcus	0,25	0,19	0,82	1,25	0,00	0,19	0,94	0,06	0,82	0,00	0,00	0,00	1,00	0,19	1,00	0,06	0,00	0,00
Methylobacterium mobilis	1,76	0,06	2,13	0,25	0,00	0,00	0,13	0,00	1,69	0,00	0,13	0,13	0,19	0,19	0,19	0,06	0,75	0,00
Paracoccus	3,20	1,07	4,33	3,14	0,00	0,63	1,13	0,06	4,45	0,00	0,06	0,13	2,45	0,56	1,75	0,25	0,00	3,83
Propionibacterium acnes	0,13	0,19	0,31	0,56	0,00	0,00	0,56	0,00	0,19	0,00	0,00	0,00	1,57	0,00	1,57	0,13	0,00	0,82
Pseudomonas	0,88	8,03	0,56	0,38	3,76	0,19	0,13	0,69	0,00	0,13	0,13	0,00	0,13	0,00	0,13	0,00	0,00	4,27
Pseudomonas fragi	0,13	0,13	0,06	0,13	12,86	0,19	0,00	4,64	0,25	18,82	0,63	0,25	0,06	0,00	0,06	0,00	0,88	0,63
Psychrobacter	0,06	0,13	0,75	0,13	1,13	0,94	2,89	1,88	3,07	71,08	0,25	0,06	1,00	17,44	1,00	0,88	16,32	1,76
Salinisphaera	0,00	0,00	0,31	18,07	0,00	1,32	5,02	0,00	6,21	0,00	0,00	30,24	40,40	1,69	20,90	67,57	2,06	30,70
Sphingomonas	0,50	0,06	1,07	0,25	0,00	0,06	0,75	0,00	0,75	0,00	0,06	0,13	0,13	0,31	0,13	0,06	0,00	0,25
Staphylococcus	1,00	0,25	1,63	0,38	0,00	0,25	0,31	0,00	1,07	0,00	0,00	0,00	0,13	0,82	0,13	0,00	0,06	0,00
Staphylococcus equorum	0,56	0,00	2,07	0,38	0,00	0,19	8,22	0,00	0,25	0,00	0,06	0,50	7,21	1,13	5,31	0,06	3,32	0,00
Staphylococcus sciuri	0,00	0,38	4,83	0,38	0,31	31,24	3,70	0,06	8,47	0,00	1,13	0,38	3,58	0,69	3,58	0,00	0,00	0,00
Staphylococcus succinus	0,00	0,00	0,19	0,25	0,00	0,00	11,36	0,00	0,00	0,00	0,00	0,13	1,63	0,31	1,63	0,00	0,19	0,00
Vibrio	3,76	0,00	0,00	0,19	0,00	0,06	0,00	0,00	0,00	0,19	0,00	0,00	0,06	0,00	0,06	0,13	71,02	40,06
Listeriaceae	1,95	8,28	0,60	19,45	0,00	1,63	0,41	0,38	1,82	0,13	0,06	0,50	14,87	1,69	12,57	3,39	0,00	0,00