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The wooden shelf surface and cheese rind mutually exchange microbiota during the traditional ripening process

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ABSTRACT

The rind acts as a protective barrier for internally-bacterial ripened cheeses. Unlike surface-inoculated smear cheeses, centripetal maturation is not assumed to occur in these cheeses. This research was aimed to evaluate the microbial diversity of the wooden shelves used for the ripening of Protected Denomination of Origin (PDO) Pecorino di Filiano and Protected Geographical Indication (PGI) Canestrato di Moliterno cheeses. The microorganisms associated with the rind of these cheeses were also investigated. Both wooden shelf surfaces and cheese rinds were sampled by brushing method to collect their biofilms. Wooden shelves showed levels of total mesophilic microorganisms (TMM) between 5.6 and 7.2 log CFU/cm^2, while cheese rinds between 6.1 and 7.8 log CFU/cm^2. The major dairy pathogens (*Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus*) were never detected, while mesophilic and thermophilic bacteria dominated the surfaces of all wooden shelves and cheese rinds. LAB community was represented by *Enterococcus* spp., *Leuconostoc* spp., and *Marinilactibacillus* spp. Among yeasts, *Debaryomyces* spp., *Candida* spp., were identified, while *Aspergillus* spp., and *Penicillium* spp., dominated the community of filamentous fungi. MiSeq Illumina analysis identified 15 phyla, 13 classes, 28 orders, 54 families, and 56 genera among bacteria. *Staphylococcus* spp. was identified from all wooden surfaces, with a maximum abundance of 71 %. *Brevibacterium*, *Corynebacterium* and halophilic bacteria were detected in almost all samples. Regarding fungi, wooden shelves mainly hosted *Aspergillus*, *Penicillium* and *Debaryomyces hansenii*, while cheese rinds especially *Penicillium* and *D. hansenii*. Alpha diversity confirmed a strict correlation between the microbiota of wooden shelves and that of cheese rinds for the majority of factories. This study confirmed that the wooden shelves used for cheese ripening are microbiologically active and represent safe systems. Furthermore, the results of this work clarified the transfer flow between wooden shelves and PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheese surfaces: smear-active microorganisms are mainly transferred from wooden shelves to cheese rind, which potentially contribute to the development of the final organoleptic characteristics; meanwhile, cheeses transfer LAB that are potentially involved in defining the safety aspects of the shelves.

1. Introduction

Cheese production is a very ancient daily activity in Italy (Gobbetti et al., 2018). This old cheese-making tradition has favored the production of several kinds of cheese, strongly linked to their respective territories of origin and, even today, produced using traditional processes; several traditional and typical southern Italian cheeses are still produced with tools made of wood (Busetta et al., 2023a; Busetta et al., 2023b; Settanni and Moschetti, 2014). For several traditional cheese productions, the ripening process occurs onto wooden shelves (Settanni et al., 2021; Wadhawan et al., 2021).

Thanks to its durability, low cost, and local availability, wood has been one of the most commonly used materials for dairy tools for centuries (Aviat et al., 2016). In the last years, the safety of wooden tools

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used in cheese making has been discussed at the European level, because the porous structure of the wooden surfaces makes their sanitization very difficult. However, according to several studies, wooden tools do not pose any hygiene issues and do not represent any microbiological risks for the human health (Busetta et al., 2023a; Cruciata et al., 2018; Licitra et al., 2007; Lortal et al., 2014; Scatassa et al., 2015; Sun and D'Amico, 2023a; Sun and D'Amico, 2023b). In light of the European Regulation (EC) no. 2074/2005, which derogates from EC no. 852/2004 for food with traditional characteristics (Commission Regulation, 2005), wood is currently used in cheese making in France and Italy. In these countries, wood is in contact with the product throughout the entire transformation process until the final ripening stage. It is worth noting that after the US Food and Drugs Administration advice against Italian and French cheeses ripened on wooden shelves, there has been an American re-discovery of traditional cheeses ripened on wooden shelves in the very last years (Sun and D'Amico, 2021; Sun and D'Amico, 2023a; Sun and D'Amico, 2023b; Wadhawan et al., 2021).

The use of wood during the ripening phase encourages the development of the rind and enhances the organoleptic qualities and typical features of cheeses (Richard, 1997). Mariani et al. (2007) demonstrated the suitability of wooden shelves for cheese ripening and reported that the microbial community of the shelves in contact with French Reblochon de Savoie smear cheeses is composed of micrococci-corynebacteria, yeasts, and molds, as well as LAB, *Staphylococcus*, and *Pseudomonas*. The authors monitored the microbial composition over time stating that it is quite stable and very similar to that of the cheese surface. Another work of the same research group (Mariani et al., 2011) verified the anti-*Listeria* activity of the resident biofilms present onto the wooden shelves used for cheese ripening, supporting the hypothesis that the wooden shelves regulate the development of microflora during ripening, and represent an essential source for the development of the microbial ecosystem expected on the cheese rind. Besides the protection effects against undesired pathogenic and/or spoilage microorganisms, Irlinger and Mounier (2009) demonstrated also the role of rind microbiota in the development of the sensory characteristics. Settanni et al. (2021) analyzed the microbial composition of the wooden shelves applied for ripening traditional cheeses produced in Sicily (southern Italy) focusing on the bacterial community, but there is still very little information on the microbiological traits and function of wooden shelves used to ripen traditional cheeses made in Italy. Information on the microbial composition of the wooden shelves and their transfer to cheese rind are necessary to better qualify traditional cheeses made with raw milk. These data are of considerable value also in view of territory valorization and, especially, to arrest land abandonment phenomenon of rural areas (Fontefrancesco et al., 2023).

Based on the above considerations, the present work aimed to deeply investigate and characterize bacterial and fungal communities associated with wooden shelves and rind of two typical hard cheeses of Basilicata region (southern continental Italy), Protected Denomination of Origin (PDO) Pecorino di Filiano and Protected Geographical Indication (PGI) Canestrato di Moliterno cheeses, through a culture-dependent and independent approach. Microbial biofilms from wooden shelves and cheese rinds were characterized using MiSeq Illumina technology and subjected to plate counts to detect the levels of the main dairy wanted and undesired microorganisms. Furthermore, bacteria, yeasts, and molds from wooden shelves and cheese rinds were genetically characterized.

2. Materials and methods

2.1. Collection of wooden shelf and cheese rind biofilms

Biofilms formed onto the surface of the wooden shelves used to ripen traditional cheeses and those developed onto the rind of PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheeses were collected from six dairies (A–F) of Basilicata region, all located in the inland of Potenza province. In particular, the dairy factories A and B produced PGI Canestrato di Moliterno cheeses, while factories C–F PDO Pecorino di Filiano cheeses. In each dairy factory investigated, samples were collected from the cheese rinds (CR-A–CR-F) and the wooden shelves (WS-A–WS-F) at the cheese rind/wooden shelf contact interface; the contact between shelves and cheese rinds lasted 3–4 months for PGI Canestrato di Moliterno cheeses and 1–2 months for PDO Pecorino di Filiano cheeses. The characteristics of the wooden shelves used for cheese ripening are reported in Table 1.

Biofilm collection from both cheese rinds and wooden shelves occurred by a non-destructive method through 100 cm^2 area delimiters (Area Space 100, VWR International PBI s.r.l., Milan, Italy). The method described by Settanni et al. (2021) was applied for sampling the surfaces of the wooden shelves and was also adapted to sample cheese rinds (Fig. 1). Cheese rind was sampled from a given cheese from both top and bottom dishes, because cheeses were turned upside down weekly. Briefly, the delimited area was brushed with a sterile toothbrush and, then, a sterile gauze, previously wet in Ringer's solution (Sigma-Aldrich, Milan, Italy) was put on the brushed area to collect the microorganisms. The toothbrush was energetically shaken and washed in 100 mL of Ringer's solution contained into a 200 mL-volume Anicrin liquid container (Anicrin, Scorzé, Italy) and the contaminated gauze was transferred in this container. All containers with the biofilms from the wooden shelves or cheese rinds were kept under refrigeration by means of a portable fridge with reusable ice packs and transported to the Laboratory of Agricultural Microbiology of the University of Palermo (Italy). Both wooden shelves and cheese rinds were sampled in triplicate from each dairy factory: three 100 cm^2 areas from three distinct wooden shelves as well as top and bottom dishes from three cheeses (one per wooden shelf) were sampled.

2.2. Microbiological analysis

All samples were subjected to decimal serial dilution in Ringer's solution. In particular, the first dilution of these samples was obtained from 1 mL of the vigorously manually shaken cell suspension of toothbrush and gauze from each wooden shelf or cheese rind added with 9 mL Ringer's solution. All other dilutions were performed at 1:10 ratio. All

Table 1
Characteristics of the wooden shelves used for cheese ripening.

Wooden shelf[a]	Cheese typology	City	Age (years)	Wood type[b]	Cleaning procedure
WS-A	PGI Canestrato di Moliterno	Moliterno	2	Chestnut	Surface scraping – water pushing broom
WS-B	PGI Canestrato di Moliterno	Moliterno	8	Silver fir	24 h sun exposure – surface scraping – pressure washer
WS-C	PDO Pecorino di Filiano	Filiano	5	Stone pine	Pressure washer – 8 h sun exposure
WS-D	PDO Pecorino di Filiano	Filiano	15	Silver fir	Water pushing broom
WS-E	PDO Pecorino di Filiano	Filiano	2	Silver fir	Water pushing broom
WS-F	PDO Pecorino di Filiano	Filiano	10	Silver fir	10 % NaOH pushing broom – pressure washer

[a] WS-A to -F, wooden shelves from factories A to F.
[b] Tree species: chestnut, *Castanea sativa* Miller; silver fir, *Abies alba* L.; stone pine, *Pinus pinea* L.

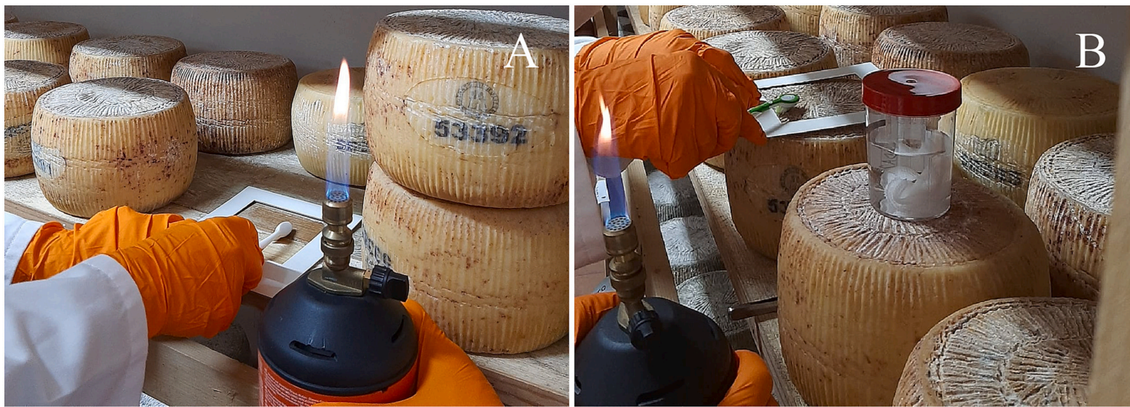

Fig. 1. Biofilm collection from wooden shelves surfaces (A) and cheese rind surfaces (B) with a sterile toothbrush.

cell suspensions were plated on agar media to allow the development of different microbial groups: total mesophilic microorganisms (TMM) on plate count agar (PCA) supplemented with 1 g/L skimmed milk (SM), typically used for dairy samples, under aerobic incubation at 30 °C for 72 h; total psychrotrophic microorganisms (TPM) on SM-PCA under aerobic incubation at 7 °C for 7 d; thermophilic and mesophilic LAB cocci on M17 agar under anaerobic incubation, occurred into hermetically sealed jars containing the AnaeroGen AN25 sachets (Oxoid), at 44 and 30 °C, respectively, for 48 h; thermophilic and mesophilic LAB rods on de Man-Rogosa-Sharpe (MRS) agar, acidified to pH 5.4 with lactic acid (5 M), under anaerobic incubation at 44 and 30 °C, respectively, for 48 h; enterococci on kanamycin esculin azide (KAA) agar under aerobic incubation at 37 °C for 24 h; coagulase-positive staphylococci (CPS) on Baird-Parker (BP) agar supplemented with rabbit plasma fibrinogen under aerobic incubation at 37 °C for 48 h; members of *Enterobacteriaceae* family on violet red bile glucose agar (VRGBA) under microaerobic incubation, obtained by pouring a top VRBGA layer onto the surface of the bottom VRBGA layer inoculated by pour plate method, at 37 °C for 24 h; total coliforms on violet red bile agar (VRBA) under microaerobic incubation at 37 °C for 24 h; pseudomonads on *Pseudomonas* agar base (PAB) supplemented with cephaloridine sodium fusidate cetrimide under aerobic incubation at 25 °C for 48 h; yeasts on yeast peptone dextrose (YPD) under aerobic incubation at 28 °C for 48 h; molds on potato dextrose agar (PDA) supplemented with 0.1 g/L chloramphenicol to avoid bacterial growth under aerobic incubation at 25 °C for 7 d; *Escherichia coli* and *Salmonella* spp. on Hektoen enteric agar (HEA) under aerobic incubation at 37 °C for 24 h; *Listeria monocytogenes* on *Listeria* selective agar base (LSAB) added with SR0140E supplement under aerobic incubation at 37 °C for 48 h. All media and supplements were purchased from Oxoid (Milan, Italy). Plate counts were performed in duplicate.

2.3. Isolation and identification of LAB

Presumptive LAB were randomly picked up from the highest dilutions plated on agar media. At least five colonies characterized by the same shape, size, width, elevation, thickness, uniformity, color and surface opacity were picked up from MRS and M17 agar plates. The colonies were then transferred into the corresponding broth media. After overnight growth at the optimal incubation conditions, all isolates were purified by streaking technique until obtaining colonies with identical appearance. All presumptive LAB were preliminarily tested for their general characteristics: Gram type was determined after exposure to a 3 % (w/v) KOH solution (Gregersen, 1978); catalase test was carried out with 3 % (v/v) H_2O_2 (Koneman et al., 1997). Presumptive LAB cultures were stocked into 1.5 mL vials containing 20 % (v/v) glycerol at −80 °C. As described by Barbaccia et al. (2021), all pure cultures were subjected to a grouping based on cell morphology and arrangement of cells. Furthermore, all isolates were tested for growth at 15 and 45 °C, heat resistance (60 °C for 30 min), hydrolysis of arginine and aesculin, acid production from carbohydrates, and CO_2 production from glucose as described by Gaglio et al. (2014). Presumptive LAB cocci were also analyzed for their growth at pH 9.2 and in the presence of NaCl (6.5 g/L) because enterococci are positive to these tests.

All pure cultures were then subjected to DNA extraction using a DNA-SORB-B kit (Sacace Biotechnologies Srl, Como, Italy) following the protocol provided by the manufacturer. The differentiation of the isolates was obtained through random amplification of polymorphic DNA (RAPD)-PCR analysis, that is commonly used to fingerprint bacteria; to this purpose, bacterial DNAs were individually amplified by single primers M13, AB111, and AB106 (Gaglio et al., 2017). The resulting polymorphic profiles were analyzed by GelCompare II software version 6.5 (Applied-Maths, Saint-Marten-Latem, Belgium). This program allows to generate a dendrogram to evaluate the similarity among PCR pattern products. The isolates sharing a very high similarity are considered to represent the same strain. All different strains were identified by sequencing the 16S rRNA gene and the sequences were compared with those available in GenBank/EMBL/DDBJ and EzTaxon database (http://eztaxon-e.ezbiocloud.net/taxonomy).

2.4. Isolation and identification of fungi

2.4.1. Yeasts

At least five colonies with the same morphology were also isolated from YPD to analyze the composition of unicellular fungi. Fungal colonies were purified to homogeneity by streaking onto Malt Extract Agar (MEA). This operation was repeated for all different yeast colony morphologies. Yeast isolates were then subjected to genetic characterization. First discrimination of the yeasts was performed by restriction fragment length polymorphism (RFLP) of the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8 S rRNA gene. DNA amplification occurred with the primer pair ITS1/ITS4 as described by Esteve-Zarzoso et al. (1999). The generated amplicons were digested with the endonucleases *Cfo*I, *Hae*III and *Hinf*I (MBI Fermentas, St. Leon-Rot, Germany) at 37 °C for 8 h. ITS amplicons as well as their restriction fragments were run on 1.5 % (w/v) agarose gel in 1× TBE (89 mM Tris–borate, 2 mM EDTA, pH 8) buffer. The isolates were further processed by sequencing the D1/D2 region of the 26S rRNA gene to confirm the preliminary RFLP identification. The identities of the sequences were determined by BLASTN search (http://www.ncbi.nlm.nih.gov).

2.4.2. Filamentous fungi

Fungi were collected from PDA and transferred to malt extract agar (MEA) (Oxoid, Milan, Italy). Petri dishes were incubated at 25 °C and observed every 24 h until a consistent development of mycelium was observed (1–2 weeks). All different fungal colonies were picked up from

agar plates and purified after consecutive sub-culturing steps onto MEA. After incubation, fungi were subjected to morphological characterization including color (on both sides of the plate), diffusible pigments, exudates, texture, growth zones, aerial and submerged hyphae, growth rate, and topography (White et al., 1990). According to the standard protocol described by O'Donnell et al. (1999), the genomic DNA was extracted from single-spore cultures. Fungi were analyzed by RLFP of the region spanning the internal transcribed spacers (ITS1 and ITS2) and the 5.8S rRNA gene. DNA fragments were amplified with the primer pair ITS1F (Gardes and Bruns, 1993)/ITS4 (White et al., 1990a) and the resulting amplicons were treated with the endonucleases *Cfo*I and *Hae*III as above reported. The isolates were finally processed by sequencing the 5.8S-ITS rRNA region and the sequences were identified by BLASTN search.

2.5. Culture-independent analysis of total bacterial community

2.5.1. DNA extraction, MiSeq library preparation and Illumina sequencing

Amplicon library preparation, quality and quantification of pooled libraries, and pair-end sequencing using the Illumina MiSeq system (Illumina, USA) were performed at the Sequencing Platform, Fondazione Edmund Mach (FEM, San Michele a/Adige, Italy). Briefly, for each sample, a 464-nucleotide sequence of the V3-V4 region (Baker et al., 2003; Claesson et al., 2010), of the 16S rRNA gene (*E. coli* positions 341 to 805) and ITS3/ITS4 specific for the ITS2 fungi region (Mbareche et al., 2021) were amplified for bacteria and yeasts, respectively. Unique barcodes were attached before the forward primers to facilitate the pooling and subsequent differentiation of samples. To prevent preferential sequencing of the smaller amplicons, the amplicons were cleaned using the Agencourt AMPure kit (Beckman coulter) according to the manufacturer's instructions; subsequently, DNA concentrations of the amplicons were determined using the Quant-iT PicoGreen dsDNA kit (Invitrogen) following the manufacturer's instructions. In order to ensure the absence of primer dimers and to assay the purity, the generated amplicon libraries quality was evaluated by a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) using the High Sensitivity DNA Kit (Agilent). Following the quantitation, cleaned amplicons were mixed and combined in equimolar ratios.

2.5.2. Illumina data analysis and sequences identification by QIIME2

Raw paired-end FASTQ files were demultiplexed using idemp (https://github.com/yhwu/idemp/blob/master/idemp.cpp) and imported into Quantitative Insights Into Microbial Ecology (Qiime2, version 2018.2). Sequences were quality filtered, trimmed, de-noised, and merged using DADA2 (Callahan et al., 2016). Chimeric sequences were identified and removed via the consensus method in DADA2. Representative bacterial sequences were aligned with MAFFT and used for phylogenetic reconstruction in FastTree using plugins alignment and phylogeny (Katoh and Standley, 2013; Price et al., 2009). For bacteria, taxonomic and compositional analyses were conducted by using plugins feature-classifier (https://github.com/qiime2/q2-feature-classifier). A pre-trained Naive Bayes classifier based on the Greengenes 13_8 99 % Operational Taxonomic Units (OTUs) database which had been previously trimmed to the V4 region of 16S rDNA, bound by the 341F/805R primer pair, was applied to paired-end sequence reads to generate taxonomy tables. For fungi, sequences were classified to the species-level using a 97 or 99 % threshold dynamic classifier created using UNITE software version 8.0 (Kõljalg et al., 2013; UNITE Community, 2019). The data generated by MiSeq Illumina sequencing were deposited in the NCBI Sequence Read Archive (SRA) and are available under Ac. No. PRJNA996329.

2.6. Statistical analysis

Data on plate count were statistically analyzed by one-way variance analysis (ANOVA) using the XLStat software version 2020.3.1 for Microsoft Excel (Addinsoft, New York, NY, USA) and growth medium was the variable included in the model. The Tukey's test for $p < 0.05$ was applied to determine the difference between means.

Regarding Miseq Illumina data: Alpha-diversity was performed with observed OTUs number, Evenness and Shannon diversity index; Beta-diversity was calculated using weighted Unifrac distance matrix and Jaccard distance matrix for bacteria and fungi respectively. Both alpha-diversity and beta-diversity were constructed for all samples using the plug-in in QIIME2 package. The similarity matrices were visualized using TREX webserver for visualization of phylogenetic Tree (http://www.trex.uqam.ca/index.php?action=trex, Boc et al., 2012).

3. Results

3.1. Microbiological analysis by culture-dependent approach

The levels of the microbial groups constituting the biofilms of the wooden shelves and cheese rinds sampled in this study are shown in Table 2. Wooden shelves hosted TMM in the range 5.6–7.2 log CFU/cm^2 with the lowest values showed by sample WS-D and the highest by WS-A. Except samples WS-E showing TPM (6.7 log CFU/cm^2) at higher levels than TMM, in general psychrotrophic microorganisms were counted at lower numbers than mesophilic ones. The presence of LAB was revealed in all samples of wooden shelf biofilms. However, the four groups investigated (mesophilic and thermophilic rods and cocci) were not always detected altogether. Although LAB cocci were detected at consistent levels (3.9–6.4 and 2.8–5.3 log CFU/cm^2 for mesophilic and thermophilic, respectively) in all wooden shelves, only sample WS-C showed detectable levels of mesophilic rods (3.45 log CFU/cm^2). Except WS-A and WS-B that did not host thermophilic rod LAB, the other wooden shelves showed different cell densities (2.6–3.4 log CFU/cm^2) within this group. Enterococci were detected in all wooden shelf associated biofilms and their levels were 0.8–1.7 log cycles lower than those registered on M17 incubated at 30 °C. Filamentous and unicellular fungi were also investigated. Yeasts were consistently found on the examined wooden shelves. In particular, yeast loads of the wooden shelves WS-C and WS-D (4.1 and 4.2 log CFU/cm^2, respectively) were significantly lower than those developed on the other wooden shelf surfaces (on average, 5.9 log CFU/cm^2). The wooden shelves used for ripening PGI Canestrato di Moliterno cheeses (WS-A and WS-B) were characterized by the highest levels of molds (5.2–5.4 log CFU/cm^2), significantly different from the number of colonies (4.1–5.1 log CFU/cm^2) displayed by the wooden shelves used for ripening PDO Pecorino di Filiano cheeses (WS-C–WS-F). Members of Enterobacteriaceae and coliforms were not found, *E. coli*, *L. monocytogenes*, *Salmonella* spp. and CPS were below their detection limit (1 log CFU/cm^2) in all wooden shelf biofilms collected, while pseudomonads were found only in biofilms collected from samples WS-D–WS-F (2.0–2.9 log CFU/cm^2).

TMM of the cheese rind biofilms ranged between 6.1 and 7.8 log CFU/cm^2 and lower values were registered for TPM. The rind of the PDO Pecorino di Filiano cheeses showed the lowest loads of pseudomonads (5.4–5.6 log CFU/cm^2), whereas PGI Canestrato di Moliterno cheese rinds showed the highest loads of TMM (7.5 log CFU/cm^2, on average) and yeasts (6.6 log CFU/cm^2, on average). The cheese rinds of PGI Canestrato di Moliterno cheeses showed also the highest loads for mesophilic and thermophilic LAB cocci. Only sample CR-A hosted detectable levels of mesophilic LAB rods and the levels registered were quite consistent (5.1 log CFU/cm^2), while CR-C sample was characterized by undetectable levels of all LAB groups investigated; in sample CR-C not even enterococci were detected, even though they ranged between 2.0 and 3.6 log CFU/cm^2 in the other cheese rind samples. However, cheese rind from Factory C was characterized by very high levels of molds (7.2 log CFU/cm^2). Like wooden shelves, none of the cheese rind samples investigated showed detectable levels of members of Enterobacteriaceae, coliforms, *Salmonella* spp., *L. monocytogenes*, *E. coli* and CPS. The levels of pseudomonads (3.4–3.7 Log CFU/ cm^2) were similar

Table 2
Microbial loads in wooden shelves and cheese rinds.

Sample	Bacterial count													
	LSAB	KAA	HEA	BP	VRBA	VRBGA	PCA 30 °C	PCA 7 °C	MRS 30 °C	MRS 44 °C	M17 30 °C	M17 44 °C	PAB	YPD	PDA
WS-A	<1	4.3 ± 0.5 ab	<1	<1	0	0	7.2 ± 0.1 a	6.1 ± 0.1 b	0 b	0 d	5.7 ± 0.2 b	3.6 ± 0.1 c	<1 c	5.8 ± 0.2 ab	5.2 ± 0.1 a
WS-B	<1	4.7 ± 0.2 a	<1	<1	0	0	7.2 ± 0.1 a	6.0 ± 0.2 b	0 b	0 d	5.6 ± 0.2 b	3.8 ± 0.2 c	<1 c	5.6 ± 0.2 b	5.4 ± 0.1 a
WS-C	<1	3.2 ± 0.4 bc	<1	<1	0	0	6.4 ± 0.1 b	4.1 ± 0.1 d	3.45 ± 0.2 a	2.6 ± 0.1 b	4.6 ± 0.2 c	4.3 ± 0.2 b	<1 c	4.2 ± 0.1 c	4.1 ± 0.2 b
WS-D	<1	3.1 ± 0.4 c	<1	<1	0	0	5.6 ± 0.2 c	3.2 ± 0.1 e	0 b	3.4 ± 0.2 a	3.9 ± 0.1 d	5.3 ± 0.2 a	2.9 ± 0.2 a	4.1 ± 0.1 c	4.5 ± 0.1 b
WS-E	<1	4.8 ± 0.6 a	<1	<1	0	0	6.4 ± 0.1 b	6.7 ± 0.1 a	0 b	3.4 ± 0.2 a	6.4 ± 0.1 a	4.3 ± 0.2 b	2.0 ± 0.1 b	6.1 ± 0.3 a	5.0 ± 0.1 a
WS-F	<1	3.8 ± 0.2 abc	<1	<1	0	0	6.7 ± 0.3 b	5.6 ± 0.2 c	0 b	2.1 ± 0.2 c	5.5 ± 0.1 b	2.8 ± 0.1 d	2.7 ± 0.2 a	6.2 ± 0.1 a	5.1 ± 0.2 a
p-Value	n.e.	<0.001	n.e.	n.e.	n.e.	n.e.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CR-A	<1	2.0 ± 0.3 c	<1	<1	0	0	7.8 ± 0.1 a	6.2 ± 0.1 ab	5.1 ± 0.1 a	2.2 ± 0.1 d	7.2 ± 0.1 a	5.3 ± 0.1 b	3.7 ± 0.1 a	6.7 ± 0.2 a	2.5 ± 0.1 d
CR-B	<1	3.5 ± 0.1 a	<1	<1	0	0	7.2 ± 0.3 b	6.5 ± 0.1 a	0 b	3.2 ± 0.1 b	7.0 ± 0.1 ab	5.7 ± 0.1 a	3.5 ± 0.1 a	6.5 ± 0.1 a	4.2 ± 0.1 c
CR-C	<1	<1 d	<1	<1	0	0	6.8 ± 0.1 bc	5.9 ± 0.1 b	0 b	0 e	0 d	0 f	3.5 ± 0.1 a	5.1 ± 0.2 c	7.2 ± 0.1 a
CR-D	<1	2.4 ± 0.2 bc	<1	<1	0	0	6.1 ± 0.1 d	5.9 ± 0.2 b	0 b	2.8 ± 0.1 c	6.3 ± 0.2 c	2.9 ± 0.1 e	3.4 ± 0.2 a	6.4 ± 0.1 ab	4.7 ± 0.1 b
CR-E	<1	3.6 ± 0.3 a	<1	<1	0	0	6.7 ± 0.1 c	6.2 ± 0.1 ab	0 b	4.1 ± 0.1 a	6.8 ± 0.1 b	4.9 ± 0.1 c	3.4 ± 0.2 a	6.0 ± 0.1 b	4.3 ± 0.2 c
CR-F	<1	2.8 ± 0.1 b	<1	<1	0	0	7.2 ± 0.1 b	5.3 ± 0.1 c	0 b	3.3 ± 0.2 b	6.1 ± 0.1 c	4.3 ± 0.1 d	3.6 ± 0.1 a	5.3 ± 0.1 c	4.5 ± 0.1 bc
p-Value	n.e.	<0.0001	n.e.	n.e.	n.e.	n.e.	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.137	<0.0001	<0.0001

Results indicate mean values ± S.D. of four plate counts (carried out in duplicates for two independent samplings). Units are Log CFU/cm^2 for wooden shelves and cheese rinds. Data within a column followed by different letters are significantly different according to Tukey's test ($p < 0.05$).

Abbreviations: LSAB, *Listeria* selective agar base for L. *monocytogenes*; KAA, kanamycin aesculinazide agar for enterococci; HEA, hektoen enteric agar for *E. coli* (red colonies) and *Salmonella* spp. (black colonies); BP, baird-parker agar for CPS, coagulase-positive staphylococci; VRBA, violet red bile agar for coliforms; VRBGA, violet red bile glucose agar for *Enterobacteriaceae*; PCA-SkM 30 °C, plate count agar added with skimmed milk incubated at 30 °C for total mesophilic microorganisms; PCA-SkM 7 °C, plate count agar added with skimmed milk incubated at 7 °C for total psychrotrophic microorganisms; MRS 30 °C, de Man-Rogosa-Sharpe agar for mesophilic rod LAB; MRS 44 °C, de Man-Rogosa-Sharpe agar medium for thermophilic rod LAB; M17 30 °C, medium 17 agar incubated at 30 °C for mesophilic coccus LAB; M17 44 °C, medium 17 agar incubated at 44 °C for thermophilic coccus LAB; PAB, *Pseudomonas* agar base for pseudomonads; YPD, yeast peptone dextrose agar for yeasts; PDA, potato dextrose agar for molds; WS, wooden shelves; CR, cheese rind; A–F, dairy factory; dairy factory A to B produce PGI Canestrato di Moliterno, and dairy factory C to F produce PDO Pecorino di Filiano.

among cheese rinds.

3.2. LAB differentiation and identification

Four hundred and eighty colonies of presumptive LAB were isolated from the biofilms of wooden shelves and cheese rinds. Only 442 isolates were still considered putative LAB as being Gram-positive and catalase-negative. After cell morphology determination by microscopic inspection, the vast majority of these isolates (n = 383) were coccus shaped, while barely 59 isolates were rods. The microscopic inspection also allowed to determine cell arrangement. Morphological data were then combined with the behavior of the cultures in different conditions and four phenotypic groups were obtained (Table 3). LAB cocci constituted three groups (I, II, and III); all of them were arranged in short-chain. Among these, only members of Group III showed an obligate hetero-fermentative metabolism, as being able to produce CO_2 from glucose. LAB rods were all members of Group IV characterized by a hetero-fermentative behavior.

Approximately 25 % of the isolates within each phenotypic group was subjected to randomly amplified polymorphic DNA (RAPD) analysis to perform a LAB typing. RAPD patterns were used to construct two different dendrograms, one for the isolates of wooden shelf origin (Fig. 2) and another one for the isolates collected from cheese rinds. This approach identified 29 distinct RAPD profiles, 20 for cheese rind LAB and nine for wooden shelf LAB. All 29 LAB characterized by diverse RAPD patterns were considered different strains and were then processed by 16S rRNA gene sequencing for species allotting. These 29 strains were confirmed to be representative of the LAB group (Ac. No OR337840-OR337868) and belonged to three genera: *Enterococcus*, *Leuconostoc* and *Marinilactibacillus*. LAB identified from the biofilms of wooden shelves were all *Enterococcus faecium* (Group I). This species was predominant also in cheese rinds. However, cheese rinds hosted other enterococci (Group II), *Leuconostoc mesenteroides* (Group III) and *Marinilactibacillus psychrotolerans* (Group IV).

3.3. Identification and distribution of fungi

A total of 120 yeast colonies were isolated from the wooden shelves and the cheese rinds analyzed. Microscopic analysis did not succeed in differentiating yeasts. Thus, all isolates were subjected to molecular identification. The results of RFLP of the 5.8S-ITS region recognized two

Table 3
Phenotypic grouping of LAB isolated from wooden shelves and cheese rinds.

Characters	Clusters[a]			
	I (n = 329)	II (n = 28)	III (n = 26)	IV (n = 59)
Morphology	C	C	C	R
Cell disposition	sc	sc	sc	sc
Growth:				
15 °C	−	−	+	+
45 °C	+	+	−	−
pH 9.6	+	+	−	nd
6.5 % NaCl	+	+	+	nd
Resistance to 60 °C	−	−	−	+
Hydrolysis of:				
Arginine	+	+	−	−
Aesculin	+	+	+	+
Acid production from:				
Arabinose	+	−	+	+
Ribose	+	+	+	+
Xylose	+	+	+	+
Fructose	+	+	+	+
Galactose	+	+	+	+
Lactose	+	+	+	+
Sucrose	+	−	+	+
Glycerol	+	+	+	+
CO_2 from glucose	−	−	+	+

[a] Abbreviations: C, coccus; R, rods; sc, short chain; n.d., not determined.

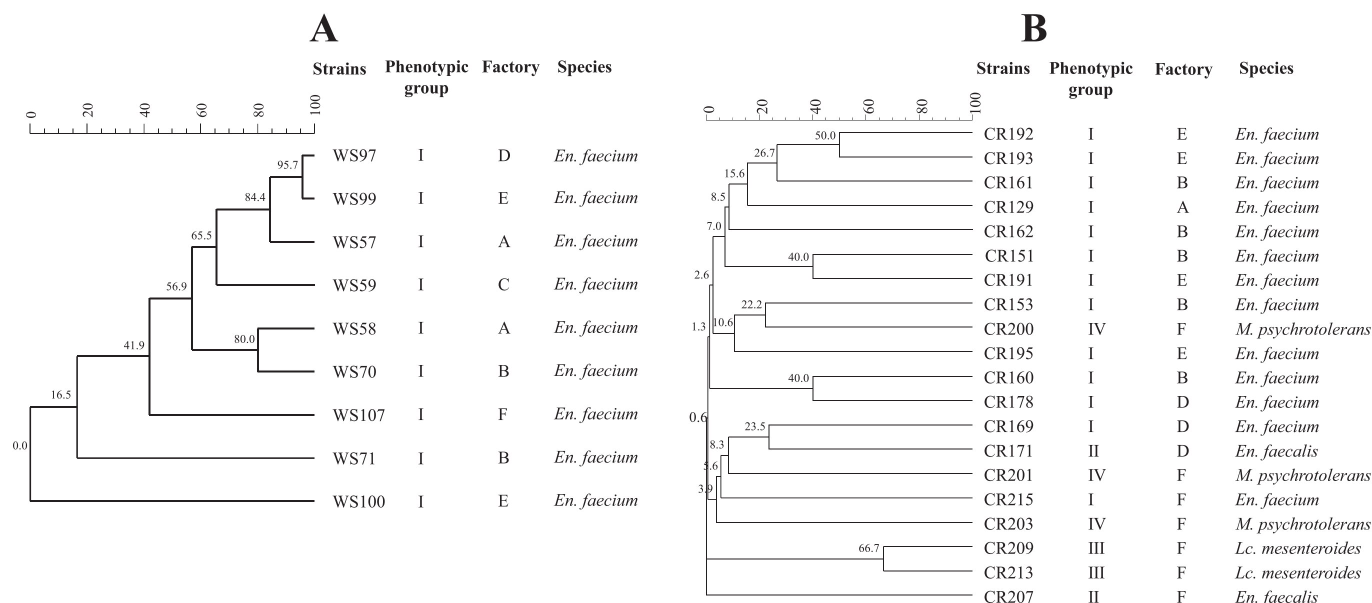

Fig. 2. Dendrogram obtained from combined RAPD-PCR patterns of LAB strains from wooden shelves (A) and cheese rind (B) from two different cheeses (PGI Canestrato di Moliterno; PDO Pecorino di Filiano) generated with the primers M13, AB106 and AB111. Abbreviations: *En.*, *Enterococcus*; *M.*, *Marinilactibacillus*; *Lc.*, *Leuconostoc*.

yeast groups (Table 4): *Candida parapsilosis* (Group I) and *Debaryomyces hansenii* (Group II). The genotypic identification of yeasts was completed by pairwise alignment of D1/D2 region of the 26S rRNA gene that confirmed species allotting.

The same wooden shelves and cheese rinds allowed to isolate 170 filamentous fungi. The molds were divided into 10 groups after microscopic inspection (Table 5). The results of the RLFP for the 5.8S-ITS region using the endonucleases *Cfo*I and *Hae*III confirmed microscopic grouping. All isolates were also processed by sequencing of the 5.8S-ITS rRNA gene that unequivocally identified the following species: *Aspergillus* spp., *Aspergillus creber*, *Aspergillus versicolor*, *Penicillium* spp., *Penicillium chrysogenum*, *Penicillium commune*, *Penicillium crustosum*, *Penicillium solitum*, *Penicillium verrucosum*, and *Talaromyces rugulosus*. The species most frequently isolated were *Penicillium chrysogenum*, *Penicillium commune*, *Aspergillus creber*, and *Aspergillus versicolor*. In terms of mold diversity, the most diverse samples were wooden shelf from Factory F and cheese rinds from Factories A and D.

3.4. Comparison of polymorphic profiles of bacteria and fungi isolated from wooden shelves and cheese rinds

The possible transfer of microorganisms from the wooden shelves to cheese rinds and vice versa was estimated through comparison of the RAPD profiles of the strains identified from both sources of isolation. Regarding LAB, the nine polymorphic profiles characterizing the nine strains from wooden shelves were compared to the 20 profiles of cheese rind strains and only two strains shared the same RAPD patterns (WS58 and WS97 from the wooden shelves with CR129 and CR169 of cheese rind origin, respectively).

RAPD-PCR analysis was also applied on yeast strains (Fig. 3) and the comparison of the resulting patterns confirmed that several *D. hansenii* strains from wooden shelves and cheese rinds shared the same polymorphic profile. As an example, the wooden shelf strain WS3 from factory A shares the same RAPD profile with the strain CR34 detected on the cheese rind analyzed in this factory.

In case of molds, RAPD analysis did not succeed in finding homology between the strains isolated from wooden shelves and cheese rinds (data not shown).

3.5. Characterization of cheese microbiota by Illumina analysis

DNA-based Illumina technology was successfully applied to deeply investigate on the composition of total bacterial and fungal communities of the samples object of this study. MiSeq Illumina analysis identified 15 phyla, 13 classes, 28 orders, 54 families, and 56 genera within bacteria. The sequences were grouped into operational taxonomic units (OTUs). The relative abundances (RA) % of the OTUs identified from both wooden shelves and cheese rinds are reported in Fig. 4. As suggested by Logares et al. (2014) the abundant communities considered were those with an individual RA ≥ 0.1 %. All wooden shelves were characterized by the presence of staphylococci. RA of bacterial group ranged from 3.01 to 74.21 %. In particular, the wooden shelves WS-A–WS-D showed an absolute dominance of staphylococci, with the species *Staphylococcus equorum* being mostly represented among this group (27.71–46.88 %). These four samples displayed also a massive presence of unspeciated staphylococci (14.70–42.86 %) and a minor presence of *Staphylococcus sciuri*. The wooden shelves analyzed hosted also consistent RA % of *Brevibacterium*, until 25.04 % in WS-B, and *Corynebacterium*, which was

Table 4
Molecular identification of yeast species.

Species	Restriction profile	5.8S-ITS PCR	Size of restriction fragment			No. of isolates[a]	Accession number[b]
			*Cfo*I	*Hae*III	*Hinf*I		
Candida parapsilosis	I	540	300 + 235	400 + 110	290 + 255	13 (2)	OR337917-OR337918
Debaryomyces hansenii	II	507–554	300 + 300 + 300	420 + 150 + 90	320 + 320	107 (16)	OR337919-OR337934

[a] Number of isolates per each yeast species.
[b] Accession number of D1/D2 region of the 26S rRna gene of isolates deposited into Genbank database.

Table 5
Molecular identification of filamentous fungi.

Specie (% identity[a])	Group	5.8S-ITS PCR	Size of restriction fragments		Accession number	Source of isolation	No. of isolates
			*Cfo*I	*Hae*III			
Aspergillus creber (99 %)	I	746–747	264 + 247 + 177 + 58	401 + 164 + 70	OR342688-OR342689	WS-D, WS-E	2
Aspergillus spp. (99 %)	II		247 + 177 + 90	358 + 97 + 70	OR342690	WS-E	1
Aspergillus versicolor (100 %)	III	520	247 + 177 + 90	349 + 97 + 70	OR342691-OR342692	WS-F, CR-B	2
Penicillium chrysogenum (99 %)	IV	470–557	170 + 92	255 + 97 + 68	OR342693-OR342700	WS-A, WS-F, CR-A, CR-C, CR-E	8
Penicillium commune (99 %)	V	532–554	180 + 89 + 84	257 + 95 + 69	OR342701-OR342702	WS-C, CR-D	2
Penicillium crustosum (99 %)	VI	616	179 + 149 + 109	255 + 155 + 69+ 64	OR342703	WS-B	1
Penicillium solitum (100 %)	VII	562	180 + 89 + 77	258 + 95 + 68	OR342704	WS-C	1
Penicillium spp. (100 %)	VIII		180 + 88 + 78	257 + 94 + 68	OR342705	WS-D	1
Penicillium verrucosum (99 %)	IX	556	179 + 112 + 88	257 + 118 + 67	OR342706	CR-D	1
Talaromyces rugulosus (99 %)	X	506	166+ 144 + 111 + 60	399+ 92	OR342707	WS-F	1

[a] Based on an NCBI database BlastN search of the 5.8S-ITS1 rRNA gene sequences.

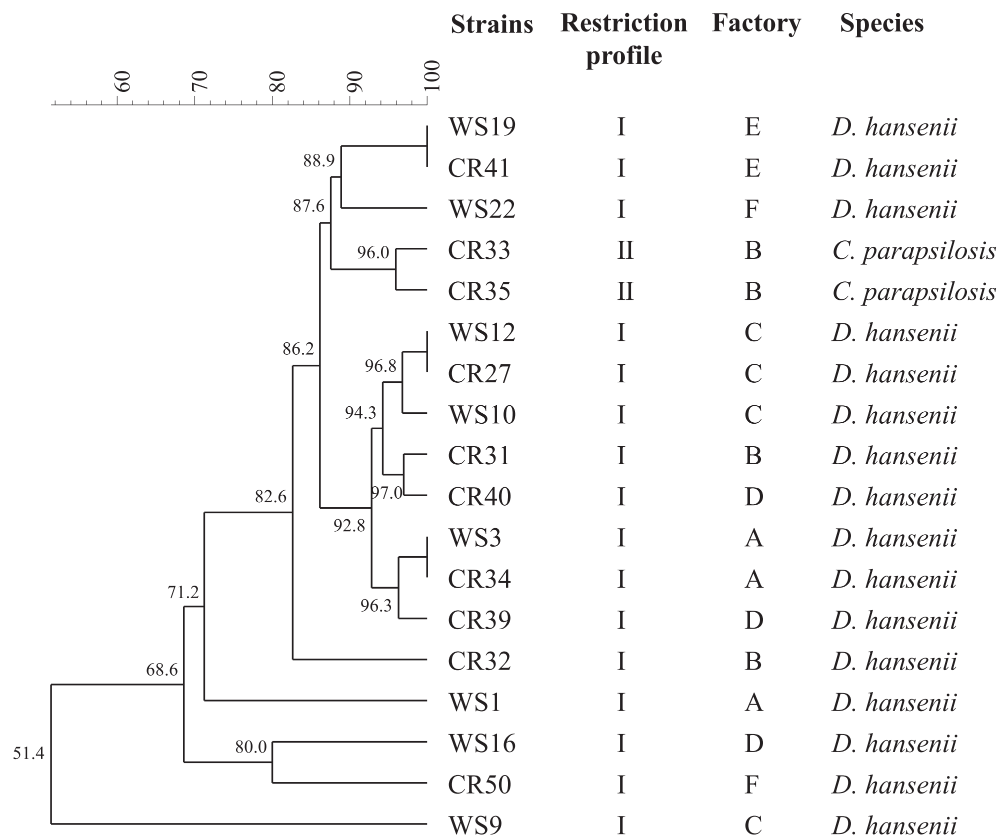

Fig. 3. Dendrogram obtained from combined RAPD-PCR profiles of yeast strains from wooden shelves (A) and cheese rind (B) from two different cheeses (PGI Canestrato di Moliterno; PDO Pecorino di Filiano) generated with the primers M13. Abbreviations: C., *Candida*; D., *Debaryomyces*.

absent in WS-E biofilm, but accounted for 70.99 % in WS-F. Except for 1.28 % in WS-C sample, Micrococcaceae were present in wooden shelf biofilms at a RA <1 %. Among Actinobacteria, *Brachybacterium* was identified in all wooden shelves, even though the RA was barely 0.34 % in WS-D. LAB populations were poorly present on the wooden shelves and were only represented by *Streptococcus* genus (0.41–1.79 %) in five out of six biofilms analyzed. Halophilic and/or halotolerant bacteria were found at consistent levels only in WS-E sample. In this biofilm *Chromohalobacter* accounted for a very high RA (51.55 %), but *Halomonas* and *Salinisphaera* were detected at relevant levels (10.23 and 10.19 %, respectively).

The bacterial community of the cheese rinds object of investigation showed a high biodiversity, but also these samples analyzed were greatly dominated by staphylococci (12.18–91.43 %). In particular, *S. equorum* represented 78.00 % of total biodiversity of cheese rind from Factory D. Samples CR-A, CR-B and CR-E displayed unspeciated *Staphylococcus* at 48.43, 64.93 and 60.53 %, respectively. *Brevibacterium* was not detected in CR-C and CR-D, but ranged from 0.91 until 27.74 % in the other samples. *Corynebacterium*, absent in CR-C, was detected at low levels in CR-A–CR-D samples (0.38–1.65 %) and accounted for 46.78 % of total biodiversity in CR-F. Micrococcaceae and *Brachybacterium* were basically present in CR-A and at a low RA % in a few other samples.

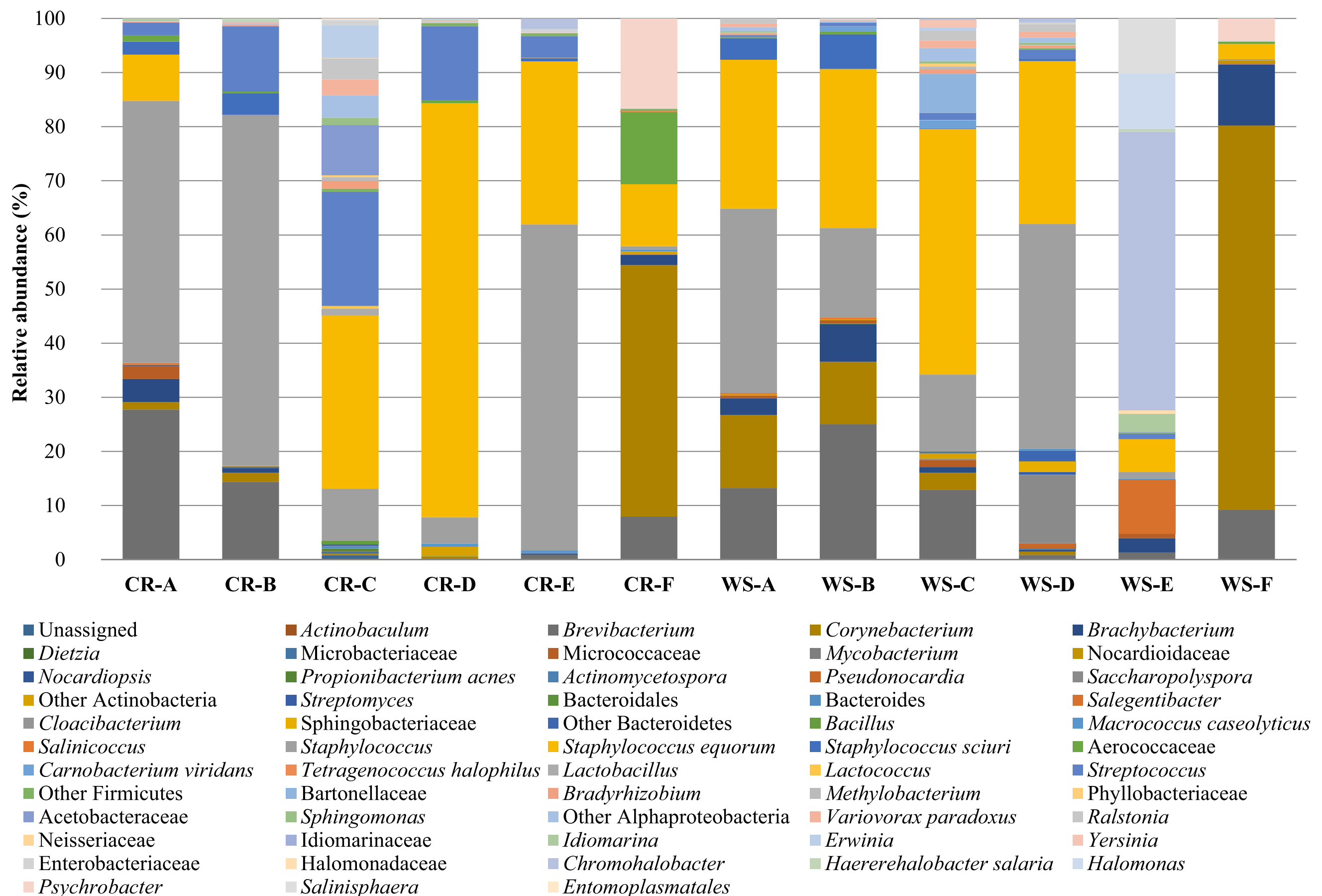

Fig. 4. Relative abundances (%) of bacterial genera identified by MiSeq Illumina in wooden shelves and cheese rind from two different cheeses. Abbreviations: WS, wooden shelves; RC, rind cheese; A–B, factories A to B produced PGI Canestrato di Moliterno; C–F, factories C to F produced PDO Pecorino di Filiano.

Except *Lactobacillus* and *Lactococcus* (1.13 and 0.57 %, respectively) in CR-C, LAB community of cheese rinds of the factories A–E was mainly represented by *Streptococcus* genus (2.35–21.57). Regarding halophilic/halotolerant bacteria, apart 1.99 % for *Chromohalobacter* in CR-E and 16.68 % for *Psychrobacter* in CR-F, other genera within this group were not significant enough to be mentioned on cheese rinds.

Regarding fungal microbiota, Fig. 5 shows RA % and distribution of the groups identified within wooden shelves and cheese rinds of the six factories object of study. Illumina technology identified 3 phyla, 12 classes, 18 orders, 32 families, and 35 genera within fungi. All wooden shelves mainly hosted *Aspergillus*, *Penicillium* and *D. hansenii*. Among aspergilli, the species detected at the highest RA % (3.60–33.54 %) was *Aspergillus sydowii*. The genus *Penicillium* covered 41.07 % of total fungal OTUs in sample WS-C. *Debaryomyces hansenii* found on the wooden shelves used to ripen PDO Pecorino di Filiano of the factories E and F accounted for very high RA % (91.91 and 89.94 %, respectively). *Eremascus fertilis* was detected only in WS-A, but its presence was particularly consistent (30.61 %). WS-A samples also showed the presence of *Priceomyces melissophilus*, *Basidiomycota* and *Moniliella*. *Candida* and *Acremonium*, in particular *Acremonium charticola* and *Acremonium alternatum* characterized sample WS-*B. Candida zeylanoides* (3.41 %) was only detected on WS-F surface. WS-A and WS-D were the only two wooden shelves to host Ascomycota (4.83–28.01, respectively).

Cheese rind fungal community diversity was almost similar to that registered on the wooden shelves, even though the main group identified were *Penicillium* and *D. hansenii*. *Penicillium* ranged from 3.62 % in sample CR-E to 90.34 % in sample CR-C; on the contrary, *D. hansenii* from 1.89 % in sample CR-C to 95.07 in sample CR-*E. Candida* at a consistent RA (41.47 %), *Acremonium* (36.47 %), particularly with the species *A. charticola* (22.01 %) and *C. parapsilosis*, characterized cheese rind of Factory B. Capnodiales (1.55 %), *Cladosporium* (0.78 %), Aspergillaceae, *Penicillium catenatum* (1.76 %), *Candida friedrichii* (0.54 %) and *Blastobotrys nivea* (0.36 %) were also detected in sample CR-C. Low RA % of *Cladopsorium* were also found in CR-A, CR-D and CR-F. Regarding *A. sydowii*, found at consistent RA % onto the wooden shelves, it was not detected in sample CR-C and registered at low levels in the other cheese rind samples, except CR-F characterized by 3.34 % for this species.

Alpha diversity analysis resulting from OTUs data of bacteria and fungi are graphically presented in Fig. 6. The weighted unifrac dist matrix for bacteria (data not shown) generated a tree (Fig. 6A) that showed a few correlations among cheese crusts and wooden shelves. In particular, two mega-clusters were obtained: the upper cluster included both cheese rinds and wooden shelves of Factories A and F, while the downer cluster, showed the presence of cheese rinds CR-D and CR-C and the corresponding wooden shelf samples WS-D and WS-C. Regarding fungi, the Fig. 6B displays a higher level of similarity calculated by Jaccard's distance matrix (data not shown). As a matter of fact, fungal diversity of cheese rinds mirrored strongly that of the wooden shelves for all factories except Factory D. For the Factories A, B, C, E and F, each sub-cluster included the wooden shelf and the corresponding cheese rind.

4. Discussion

The present study was mainly aimed to characterize the microbial diversity of the wooden shelves of PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheeses. Unlike previous works focusing exclusively of the bacterial communities, this work also took into account yeasts and filamentous fungi. Fungi are key elements of cheese

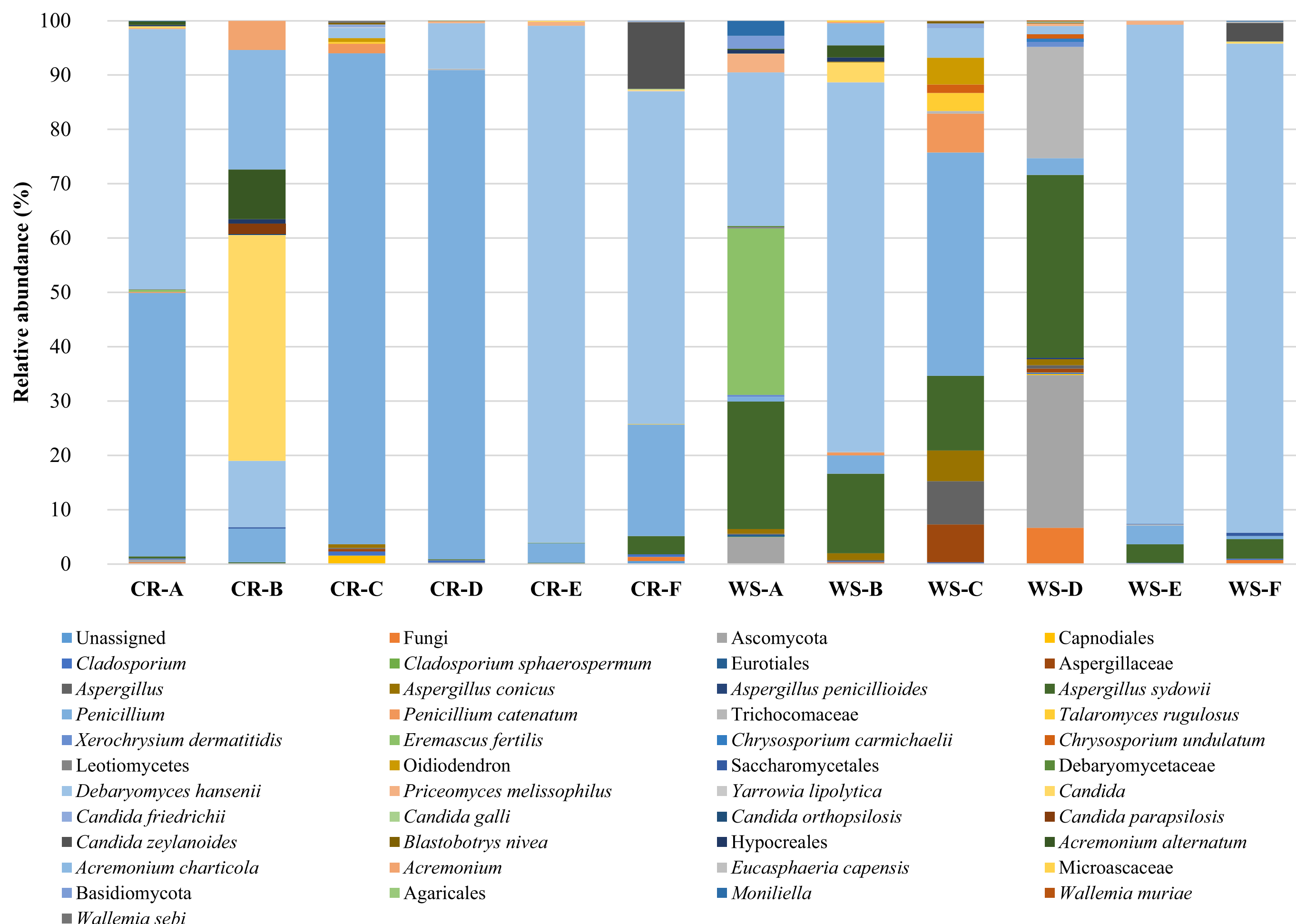

Fig. 5. Relative abundances (%) of fungi genera identified by MiSEq Illumina in wooden shelves and cheese rind from two different cheeses. Abbreviations: WS, wooden shelves; RC, rind cheese; A–B, factories A to B produced PGI Canestrato di Moliterno; C–F, factories C to F produced PDO Pecorino di Filiano.

ripening environment; indeed, they play important roles in shaping the communities of microbes in aging facilities. In view of a better understanding of the influence of these ripening tools on the microbiota of cheese rinds during ripening, the crusts of the cheeses were also object of the microbiological investigation performed in this study. A culture-dependent approach was applied to estimate the levels of viable microorganisms present on wooden shelves and cheese rinds.

The levels of TMM characterizing the six wooden shelves under investigation are comparable to those found for the aging of French Reblochon cheese (Mariani et al., 2007). In particular, TMM data registered for three of the four wooden shelves used for ripening Pecorino di Filiano cheeses are highly similar to those reported by Guzzon et al. (2017) who explored the microbiota of the red-brown defect in smear-ripened cheeses, starting from the wooden shelves. In the present study, LAB levels of the wooden shelves are comparable to those reported by Settanni et al. (2021), who found thermophilic lactococci in the range of 1.3 to 6.0 log CFU/cm^2 for the wooden shelves used for aging some typical Sicilian cheeses. However, mesophilic lactobacilli were found at lower levels than those found by Galinari et al. (2014) with regards to the wooden shelves used for the ripening of Brazilian cheeses. The levels of *Pseudomonas* estimated for the six wooden shelves of the present study are similar to those reported by Mariani et al. (2007).

The levels of TMM of the cheese rinds were very similar to those registered for Herve cheese, a soft cheese with a washed rind produced in Belgium (Delcenserie et al., 2014). Almost all cheese rinds showed high levels of LAB, specifically mesophilic cocci. These levels were superimposable to those of TMM, indicating that this group dominated the bacterial community of the cheese rinds. The dominance of cocci over rods was previously reported in Danish cheese surfaces (Gori et al., 2013). Molds and yeasts developed on all cheese rinds. PDO Pecorino di Filiano is known to host yeasts, in particular *D. hansenii*, on its crust (Capece and Romano, 2009). Although yeasts are in some cases responsible for cheese spoilage (Fleet, 1990), on the other hand, they can contribute to the centripetal ripening of cheeses by degrading lactate and causing rind deacidification. According to Parente et al. (2022), the increase in pH in the crust during ripening could facilitate growth and survival of pathogenic microorganisms such as *Salmonella* spp., *L. monocytogenes*, *E. coli*, and CPS. However, these microorganisms, generally associated with the criteria of poor hygiene and safety of dairy production, were never detected on the rinds of PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheeses. Plate count method gave a general overview of the viable microbial groups present on both wooden shelves and cheese rinds of these two typical cheeses.

To provide a comprehensive overview on the populations inhabiting wooden shelves and cheese rinds and to retrieve also the level of interaction among them, LAB, yeasts, and molds were isolated, differentiated and identified. With regards to the LAB community, three out of the four viable groups detected were cocci and a total of 29 strains, belonging to three genera (*Enterococcus*, *Leuconostoc* and *Marinilactibacillus*), were identified. The great majority of the LAB species detected on wooden shelves and cheese rinds are typically associated with dairy environments, such as raw milk (Franciosi et al., 2011), cheeses (Di Grigoli et al., 2015; Gaglio et al., 2014), wooden vats

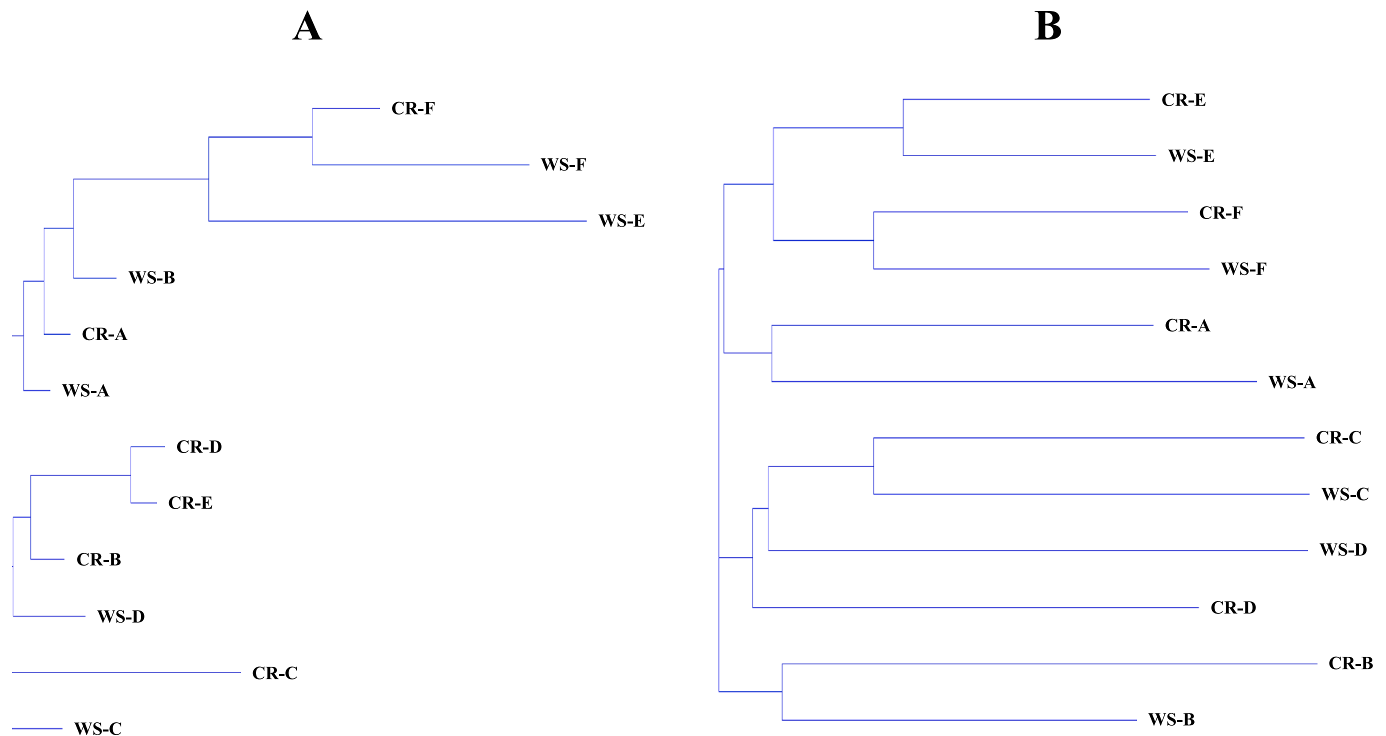

Fig. 6. Alpha diversity distribution: A, bacteria; B, fungi. Abbreviations: WS, wooden shelves; RC, rind cheese; A–B, factories A to B produced PGI Canestrato di Moliterno; C–F, factories C to F produced PDO Pecorino di Filiano.

(Scatassa et al., 2015) and animal rennets (Cruciata et al., 2014), but the LAB communities associated with the samples analyzed from the two Lucanian cheese productions were dominated by enterococci. Regarding wooden shelves, these results are not surprising and confirmed what reported for traditional cheeses produced in Sicily (Settanni et al., 2021). The comparison of the RAPD profiles of the enterococci from wooden shelves with those from cheese rinds indicated that the surfaces of the ripening shelves and cheese crusts might exchange these bacteria. This phenomenon can be highly positive because enterococci contribute to the development of the typical flavor during cheese ripening (Giraffa, 2003). Also *M. psychrotolerans*, a halophilic and alkalophilic LAB species of marine origin, is strongly associated with the dairy environment, but is specifically isolated from the rinds of soft and semi-hard cheeses and derives from the sea salt added to the brines used for salting the cheeses (Vermote et al., 2018). This marine species plays an important role in cheese ripening (Delcenserie et al., 2014; Ishikawa et al., 2007; Ishikawa et al., 2003; Suzuki et al., 2021) and has been detected also in French and German cheeses (Feurer et al., 2004; Maoz et al., 2003).

After applying RLFP of 5.8S-ITS rRNA region and its sequencing, yeast isolates were differentiated into two groups represented by *Debaryomyces* and *Candida*. The results unequivocally revealed that *D. hansenii* dominated in both PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheese productions, because they were detected on the wooden shelves as well as on the cheese rind surfaces. Low pH, high salt content and low water activity characterize the environment of this yeast species and, for this reason, it has been isolated from artisanal cheeses (Cosentino et al., 2001; Fadda et al., 2004), including PDO Pecorino di Filiano (Capece and Romano, 2009). Although *C. parapsilosis* is widely considered as an opportunistic human pathogen capable of causing invasive candidiasis (Banjara et al., 2015; Lima et al., 2019; Trofa et al., 2008), its presence on cheese rinds has never been linked to human outbreaks. Furthermore, Geronikou et al. (2022) confirmed its positive impact on the organoleptic characteristics of cheeses. Concerning molds, *Penicillum* and *Aspergillus* were most frequently found in both samples object of study (wooden shelves and cheese rinds). Several species detected in this study, such as *P. crustosum*, *P. solitum*, and *P. verrucosum* were previously reported as environmental contaminants of cheese and dairy productions (Kure et al., 2001; Serra et al., 2003). In general, conidia of molds are airborne transmittable and they can easily dominate cheese ripening environments (De Santi et al., 2010; Decontardi et al., 2017).

Next generation sequence analysis showed that all samples were characterized by the presence of *S. equorum*. Bacteria of this species are generally found in cheese rinds and this is due to their high salt tolerance (Jeong et al., 2014; Jeong et al., 2017). The presence of *S. equorum* onto the surface of wooden shelves used for cheese ripening is also very common (Settanni et al., 2021; Wadhawan et al., 2021) and plays an important role in the inhibition of *L. monocytogenes* (Wadhawan et al., 2023). The presence of *Corynebacterium* in the biofilms collected from wooden shelves and cheese rinds is not negative, since these bacteria are commonly found onto the surface of cheeses (Bockelmann et al., 2005; Fontana et al., 2010; Rea et al., 2007) and are considered important for the ripening of smear cheeses (Brennan et al., 2004; Guzzon et al., 2017; Mariani et al., 2007). The presence of *Corynebacterium*, *Brevibacterium*, and *Brachybacterium* is not surprising on the samples analyzed in this study, since they are part of the bacterial community of smear cheese rind (Schornsteiner et al., 2014). The presence of halophilic genera such as *Chromohalobacter*, *Halomonas* and *Salinisphaera* on wooden shelves could be related to the sea salt used for preparing the brines (Vermote et al., 2018). LAB community of the cheese rinds was mainly dominated by *Streptococcus*, confirming previous results showed for cheese crusts (Sant'Anna et al., 2019). Even though at very low percentages, LAB presence onto the wooden shelves is imputable to cheese rinds. These low percentages were also found by Settanni et al. (2021) who studied 18 wooden shelves for the bacterial communities.

Illumina technology was also applied to study yeast and mold microbiota of wooden shelves and cheese rinds. This approach confirmed what found with the culture-dependent tools: *D. hanseni* dominated cheese rinds of both cheeses object of investigation, providing further insights to the results showed by Capece and Romano

(2009) of PDO Pecorino di Filiano cheese; *Aspergillus* and *Penicillum*, generally characterizing cheese aging processes (Moubasher et al., 2018), were mainly associated to PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheeses.

5. Conclusions

The combined (culture-dependent and -independent) approach applied in this study clearly showed that the bacterial and fungal groups identified from the wooden shelves used for ripening PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheeses are those typically associated with smear cheese rinds. The microbiotas found onto the rinds of both cheeses were confirmed to be those characterizing smear cheeses. Even though PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheeses are not inoculated with smear microorganisms, the contact with the wooden shelves determines a surface ripening. Thus, the rind of the cheeses in contact with these shelves take advantages from the wooden biofilms and are actively involved in generating a protective barrier against the major dairy pathogens. Some bacteria, specifically enterococci, were mostly isolated from cheese rinds, but also found on the wooden shelves, suggesting a mutual microbial transfer between wooden shelves and cheese rinds. In light of the results of the present study, PDO Pecorino di Filiano and PGI Canestrato di Moliterno cheeses are no more considered cheeses with an exclusive internal bacterial ripening, but have to be inserted in the smear cheese typology since bacterial and yeast microbiota of the rinds clearly indicated the occurrence of a centripetal maturation.

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Declaration of competing interest

No conflict of interest exists.

Data availability

Data will be made available on request.

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References

Aviat, F., Gerhards, C., Rodriguez-Jerez, J.J., Michel, V., Bayon, I.L., Ismail, R., Federighi, M., 2016. Microbial safety of wood in contact with food: a review. Compr. Rev. Food Sci. Food Saf. 15 (3), 491–505. https://doi.org/10.1111/1541-4337.12199.

Baker, G.C., Smith, J.J., Cowan, D.A., 2003. Review and re-analysis of domain-specific 16S primers. J. Microbiol. Methods 55, 541–555. https://doi.org/10.1016/j.mimet.2003.08.009.

Banjara, N., Suhr, M.J., Hallen-Adams, H.E., 2015. Diversity of yeast and mold species from a variety of cheese types. Curr. Microbiol. 70, 792–800. https://doi.org/10.1007/s00284-015-0790-1.

Barbaccia, P., Busetta, G., Matraxia, M., Sutera, A.M., Craparo, V., Moschetti, G., Francesca, N., Settanni, L., Gaglio, R., 2021. Monitoring commercial starter culture development in presence of red grape pomace powder to produce polyphenol-enriched fresh ovine cheeses at industrial scale level. Ferment 7, 35. https://doi.org/10.3390/fermentation7010035.

Boc, A., Diallo, Alpha B., Makarenkov, V., 2012. T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. Nucleic Acids Res. 40 (W1), W573–W579. https://doi.org/10.1093/nar/gks485.

Bockelmann, W., Willems, K.P., Neve, H., Heller, K.H., 2005. Cultures for the ripening of smear cheeses. Int. Dairy J. 15, 719–773. https://doi.org/10.1016/j.idairyj.2004.08.022.

Brennan, N.M., Cogan, T.M., Loessner, M., Scherer, S., 2004. Bacterial surface-ripened cheeses. In: Fox, P.F., McSweeney, P.L.H., Cogan, T.M., Guinee, T.P. (Eds.), Cheese: Chemistry, Physics and Microbiology. Elsevier, New York, pp. 199–225.

Busetta, G., Gaglio, R., Mangione, G., Garofalo, G., Franciosi, E., Gannuscio, R., Caccamo, M., Todaro, M., Di Gerlando, R., Settanni, L., Licitra, G., 2023a. Effect of commission implementing regulation (EU) 2020/1319 on the bacterial composition of PDO Provola dei Nebrodi cheese. Int. J. Food Microbiol. 394, 110188 https://doi.org/10.1016/j.ijfoodmicro.2023.110188.

Busetta, G., Garofalo, G., Barbera, M., Di Trana, A., Claps, S., Lovallo, C., Franciosi, E., Gaglio, R., Settanni, L., 2023b. Metagenomic, microbiological, chemical and sensory profiling of Caciocavallo Podolico Lucano cheese. Food Res. Int. 169, 112926 https://doi.org/10.1016/j.foodres.2023.112926.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Capece, A., Romano, P., 2009. "Pecorino di Filiano" cheese as a selective habitat for the yeast species, *Debaryomyces hansenii*. Int. J. Food Microbiol. 132 (2–3), 180–184. https://doi.org/10.1016/j.ijfoodmicro.2009.04.007.

Claesson, M.J., Wang, Q., O'Sullivan, O., Greene-Diniz, R., Cole, J.R., Ross, R.P., O'Toole, P.W., 2010. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. Nucleic Acids Res. 38, e200 https://doi.org/10.1093/nar/gkq873.

Commission Regulation (EC) No 2074/2005 Laying down implementing measures for certain products under regulation (EC) No. 853/2004 of the European Parliament and of the Council and for the organisation of official controls under regulation (EC) No. 854/2004 of the European Parliament and of the Council and regulation (EC) No. 882/2004 of the European Parliament and of the Council, derogating from regulation (EC) No. 852/2004 of the European Parliament and of the Council and amending regulations (EC) No. 853/2004 and (EC) No. 854/2004. Off. J. Eur. Union 338:27–59.

Cosentino, S., Fadda, M.E., Deplano, M., Mulargia, A.F., Palmas, F., 2001. Yeasts associated with Sardinian ewe's dairy products. Int. J. Food Microbiol. 69 (1–2), 53–58. https://doi.org/10.1016/S0168-1605(01)00572-4.

Cruciata, M., Sannino, C., Ercolini, D., Scatassa, M.L., De Filippis, F., Mancuso, I., La Storia, A., Moschetti, G., Settanni, L., 2014. Animal rennets as sources of dairy lactic acid bacteria. Appl. Environ. Microbiol. 80, 2050–2061. https://doi.org/10.1128/AEM.03837-13.

Cruciata, M., Gaglio, R., Scatassa, M.L., Sala, G., Cardamone, C., Palmeri, M., Moschetti, G., La Mantia, T., Settanni, L., 2018. Formation and characterization of early bacterial biofilms on different wood typologies applied in dairy production. Appl. Environ. Microbiol. 84, e02107-17 https://doi.org/10.1128/AEM.02107-17.

De Santi, M., Sisti, M., Barbieri, E., Piccoli, G., Brandi, G., Stocchi, V., 2010. A combined morphologic and molecular approach for characterizing fungal microflora from a traditional Italian cheese (Fossa cheese). Int. Dairy J. 20 (7), 465–471. https://doi.org/10.1016/j.idairyj.2010.02.004.

Decontardi, S., Mauro, A., Lima, N., Battilani, P., 2017. Survey of Penicillia associated with Italian grana cheese. Int. J. Food Microbiol. 246, 25–31. https://doi.org/10.1016/j.ijfoodmicro.2017.01.019.

Delcenserie, V., Taminiau, B., Delhalle, L., Nezer, C., Doyen, P., Crevecoeur, S., Roussey, D., Korsak, N., Daube, G., 2014. Microbiota characterization of a Belgian protected designation of origin cheese, Herve cheese, using metagenomic analysis. J. Dairy Sci. 97 (10), 6046–6056.

Di Grigoli, A., Francesca, N., Gaglio, R., Guarrasi, V., Moschetti, M., Scatassa, M.L., Settanni, L., Bonanno, A., 2015. The influence of the wooden equipment employed for cheese manufacture on the characteristics of a traditional stretched cheese during ripening. Food Microbiol. 46, 81–91. https://doi.org/10.1016/j.fm.2014.07.008.

Esteve-Zarzoso, B., Belloch, C., Uruburu, F., Querol, A., 1999. Identification of yeasts by RFLP analysis of the 5.8 S rRNA gene and the two ribosomal internal transcribed spacers. Int. J. Syst. Evol. 49 (1), 329–337. https://doi.org/10.1099/00207713-49-1-329.

Fadda, M.E., Mossa, V., Pisano, M.B., Deplano, M., Cosentino, S., 2004. Occurrence and characterization of yeasts isolated from artisanal Fiore Sardo cheese. Int. J. Food Microbiol. 95 (1), 51–59. https://doi.org/10.1016/j.ijfoodmicro.2004.02.001.

Feurer, C., Irlinger, F., Spinnler, H.E., Glaser, P., Vallaeys, T., 2004. Assessment of the rind microbial diversity in a farmhouse-produced vs a pasteurized industrially produced soft red-smear cheese using both cultivation and rDNA-based methods. J. Appl. Microbiol. 97 (3), 546–556. https://doi.org/10.1111/j.1365-2672.2004.02333.x.

Fleet, G.H., 1990. Yeasts in dairy products. J. Appl. Bacteriol. 68, 199–211.

Fontana, C., Cappa, F., Rebecchi, A., Cocconcelli, P.S., 2010. Surface microbiota analysis of taleggio, gorgonzola, casera, scimudin and formaggio di fossa Italian cheeses. Int. J. Food Microbiol. 138 (3), 205–211. https://doi.org/10.1016/j.ijfoodmicro.2010.01.017.

Fontefrancesco, M.F., Zocchi, D.M., Pieroni, A., 2023. The intersections between food and cultural landscape: insights from three mountain case studies. Land 12, 676. https://doi.org/10.3390/land12030676.

Franciosi, E., Settanni, L., Cologna, N., Cavazza, A., Poznanski, E., 2011. Microbial analysis of raw cows' milk used for cheese-making: influence of storage treatments on microbial composition and other technological traits. World J. Microbiol. Biotechnol. 27, 171–180. https://doi.org/10.1007/s11274-010-0443-2.

Gaglio, R., Francesca, N., Di Gerlando, R., Cruciata, M., Guarcello, R., Portolano, B., Moschetti, G., Settanni, L., 2014. Identification, typing and investigation of the dairy characteristics of lactic acid bacteria isolated from "Vastedda della valle del Belìce" cheeses. Dairy Sci. Technol. 94, 157–180. https://doi.org/10.1007/s13594-013-0150-5.

Gaglio, R., Francesca, N., Di Gerlando, R., Mahony, J., De Martino, S., Stucchi, C., Moschetti, G., Settanni, L., 2017. Enteric bacteria of food ice and their survival in alcoholic beverages and soft drinks. Food Microbiol. 67, 17–22. https://doi.org/10.1016/j.fm.2017.04.020.

Galinari, É., Nóbrega, J.E.D., Andrade, N.J.D., Ferreira, C.L.D.L.F., 2014. Microbiological aspects of the biofilm on wooden utensils used to make a Brazilian artisanal cheese. Braz. J. Microbiol. 45, 713–720. https://doi.org/10.1590/s1517-83822014000200047.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2 (2), 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.

Geronikou, A., Larsen, N., Lillevang, S.K., Jespersen, L., 2022. Occurrence and identification of yeasts in production of white-brined cheese. Microorganisms 10 (6), 1079. https://doi.org/10.3390/microorganisms10061079.

Giraffa, G., 2003. Functionality of enterococci in dairy products. Int. J. Food Microbiol. 88 (2–3), 215–222. https://doi.org/10.1016/S0168-1605(03)00183-1.

Gobbetti, M., Neviani, E., Fox, P., 2018. The Cheeses of Italy, Science and Technology, first ed. Springer, Switzerland.

Gori, K., Ryssel, M., Arneborg, N., Jespersen, L., 2013. Isolation and identification of the microbiota of Danish farmhouse and industrially produced surface-ripened cheeses. Microb. Ecol. 65, 602–615. https://doi.org/10.1007/s00248-012-0138-3.

Gregersen, T., 1978. Rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur. J. Appl. Microbiol. Biotechnol. 5, 123–127. https://doi.org/10.1007/BF00498806.

Guzzon, R., Carafa, I., Tuohy, K., Cervantes, G., Vernetti, L., Barmaz, A., Larcher, R., Franciosi, E., 2017. Exploring the microbiota of the red-brown defect in smear-ripened cheese by 454-pyrosequencing and its prevention using different cleaning systems. Food Microbiol. 62, 160–168. https://doi.org/10.1016/j.fm.2016.10.018.

Irlinger, F., Mounier, J., 2009. Microbial interactions in cheese: implications for cheese quality and safety. Curr. Opin. Biotechnol. 20 (2), 142–148. https://doi.org/10.1016/j.copbio.2009.02.016.

Ishikawa, M., Nakajima, K., Yanagi, M., Yamamoto, Y., Yamasato, K., 2003. *Marinilactibacillus psychrotolerans* gen. nov., sp. nov., a halophilic and alkaliphilic marine lactic acid bacterium isolated from marine organisms in temperate and subtropical areas of Japan. Int. J. Syst. Evol. Microbiol. 53 (3), 711–720. https://doi.org/10.1099/ijs.0.02446-0.

Ishikawa, M., Kodama, K., Yasuda, H., Okamoto-Kainuma, A., Koizumi, K., Yamasato, K., 2007. Presence of halophilic and alkaliphilic lactic acid bacteria in various cheeses. Lett. Appl. Microbiol. 44 (3), 308–313. https://doi.org/10.1111/j.1472-765X.2006.02073.x.

Jeong, D.W., Han, S., Lee, J.H., 2014. Safety and technological characterization of *Staphylococcus equorum* isolates from jeotgal, a Korean high-salt-fermented seafood, for starter development. Int. J. Food Microbiol. 188, 108–115. https://doi.org/10.1016/j.ijfoodmicro.2014.07.022.

Jeong, D.W., Heo, S., Ryu, S., Blom, J., Lee, J.H., 2017. Genomic insights into the virulence and salt tolerance of *Staphylococcus equorum*. Sci. Rep. 7 (1), 1–11. https://doi.org/10.1038/s41598-017-05918-5.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277. https://doi.org/10.1111/mec.12481.

Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., Winn, W.C., 1997. The nonfermentative gram-negative bacilli. In: Koneman, E.W., Allen, S.D., Janda, W.M., Schreckenberger, P.C., Winn, W.C. (Eds.), Color Atlas and Textbook of Diagnostic Microbiology, seventh ed. Lippincott, Philadelphia, pp. 253–320.

Kure, C.F., Wasteson, Y., Brendehaug, J., Skaar, I., 2001. Mould contaminants on Jarlsberg and Norvegia cheese blocks from four factories. Int. J. Food Microbiol. 70 (1–2), 21–27. https://doi.org/10.1016/S0168-1605(01)00520-7.

Licitra, G., Ogier, J.C., Parayre, S., Pediliggieri, C., Carnemolla, T.M., Falentin, H., Madec, M.N., Carpino, S., Lortal, S., 2007. Variability of the bacterial biofilms of the "tina" wood vat used in the Ragusano cheese making process. Appl. Environ. Microbiol. 73, 6980–6987. https://doi.org/10.1128/AEM.00835-07.

Lima, G.B.L., Rosa, C.A., Johann, S., Vieira, M.D.L.A., Gomes, F.D.C.O., 2019. Yeasts isolated from tropical fruit ice creams: diversity, antifungal susceptibility and adherence to buccal epithelial cells. Brazilian J. Food Technol. 22 https://doi.org/10.1590/1981-6723.19718.

Logares, R., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R., Claverle, J.M., Decelle, J., Dolan, J.R., Dunthorn, M., Edvardsen, B., Gobet, A., Kooistra, W.H.C.F., Mahé, F., Not, F., Ogata, H., Pawlowsky, J., Pernice, M.C., Romac, S., Shalchian-Tabrizi, K., Simon, N., Stoeck, T., Santini, S., Siano, R., Wincker, P., Zingone, A., Richiards, T.A., de Vergas, C., Massana, R., 2014. Patterns of rare and abundant marine microbial eukaryotes. Curr. Biol. 24 (8), 813–821. https://doi.org/10.1016/j.cub.2014.02.050.

Lortal, S., Licitra, G., Valence, F., 2014. Wooden tools: reservoirs of microbial biodiversity in traditional cheesemaking. Microbiol. Spectr. 2 (1) https://doi.org/10.1128/microbiolspec.cm-0008-2012 (2–1).

Maoz, A., Mayr, R., Scherer, S., 2003. Temporal stability and biodiversity of two complex antilisterial cheese-ripening microbial consortia. Appl. Environ. Microbiol. 69 (7), 4012–4018. https://doi.org/10.1128/AEM.69.7.4012-4018.2003.

Mariani, C., Briandet, R., Chamba, J.F., Notz, E., Carnet-Pantiez, A., Eyoug, R.N., Oulahal, N., 2007. Biofilm ecology of wooden shelves used in ripening the French raw milk smear cheese Reblochon de Savoie. J. Dairy Sci. 90, 1653–1661. https://doi.org/10.3168/jds.2006-190.

Mariani, C., Oulahal, N., Chamba, J.F., Dubois-Brissonnet, F., Notz, E., Briandet, R., 2011. Inhibition of *Listeria monocytogenes* by resident biofilms present on wooden shelves used for cheese ripening. Food Control 22 (8), 1357–1362. https://doi.org/10.1016/j.foodcont.2011.02.012.

Mbareche, H., Veillette, M., Bilodeau, G.J., 2021. In silico study suggesting the bias of primers choice in the molecular identification of fungal aerosols. J. Fungi 7 (2), 99. https://doi.org/10.3390/jof7020099.

Moubasher, A.A., Abdel-Sater, M.A., Soliman, Z.S.M., 2018. Yeasts and filamentous fungi associated with some dairy products in Egypt. J. Mycol. Med. 28 (1), 76–86. https://doi.org/10.1016/j.mycmed.2017.12.003.

O'Donnell, K., Gherbawy, Y., Schweigkofler, W., Adler, A., Prillinger, H., 1999. Phylogenetic analyses of DNA sequence and RAPD data compared in *Fusarium oxysporum* and related species from maize. J. Phytopathol. 147 (7–8), 445–452. https://doi.org/10.1111/j.1439-0434.1999.tb03849.x.

Parente, E., Zotta, T., Ricciardi, A., 2022. A review of methods for the inference and experimental confirmation of microbial association networks in cheese. Int. J. Food Microbiol. 109618 https://doi.org/10.1016/j.ijfoodmicro.2022.109618.

Price, M.N., Dehal, P.S., Arkin, A.P., 2009. FastTree: computing large minimum evolution,trees with profiles instead of a distance matrix. Mol. Biol. Evol. 26, 1641–1650. https://doi.org/10.1093/molbev/msp077.

Rea, M.C., Görges, S., Gelsomino, R., Brennan, N.M., Mounier, J., Vancanneyt, M., Scherer, S., Swings, J., Cogan, T.M., 2007. Stability of the biodiversity of the surface consortia of Gubbeen, a red-smear cheese. J. Dairy Sci. 90 (5), 2200–2210. https://doi.org/10.3168/jds.2006-377.

Richard, J., 1997. Utilisation du bois comme matériau au contact des produits laitiers. Compt. Rend. Acad. Agric. Fr. 83 (5), 27–34.

Sant'Anna, F.M., Wetzels, S.U., Cicco, S.H.S., Figueiredo, R.C., Sales, G.A., Figueiredo, N. C., Nunes, C.A., Schmitz-Esser, S., Mann, E., Wagner, M., Souza, M.R., 2019. Microbial shifts in Minas artisanal cheeses from the Serra do Salitre region of Minas Gerais, Brazil throughout ripening time. Food Microbiol. 82, 349–362. https://doi.org/10.1016/j.fm.2019.02.016.

Scatassa, M.L., Gaglio, R., Macaluso, G., Francesca, N., Randazzo, W., Cardamone, C., Di Grigoli, A., Moschetti, G., Settanni, L., 2015. Transfer, composition and technological characterization of the lactic acid bacterial populations of the wooden vats used to produce traditional stretched cheeses. Food Microbiol. 52, 31–41. https://doi.org/10.1016/j.fm.2015.06.008.

Schornsteiner, E., Mann, E., Bereuter, O., Wagner, M., Schmitz-Esser, S., 2014. Cultivation-independent analysis of microbial communities on Austrian raw milk hard cheese rinds. Int. J. Food Microbiol. 180, 88–97. https://doi.org/10.1016/j.ijfoodmicro.2014.04.010.

Serra, R., Abrunhosa, L., Kozakiewicz, Z., Venâncio, A., Lima, N., 2003. Use of ozone to reduce molds in a cheese ripening room. J. Food Prot. 66 (12), 2355–2358. https://doi.org/10.4315/0362-028X-66.12.2355.

Settanni, L., Moschetti, G., 2014. New trends in technology and identity of traditional dairy and fermented meat production processes: preservation of typicality and hygiene. Trends Food Sci. Technol. 37 (1), 51–58. https://doi.org/10.1016/j.tifs.2014.02.006.

Settanni, L., Busetta, G., Puccio, V., Licitra, G., Franciosi, E., Botta, L., Di Gerlando, R., Todaro, M., Gaglio, R., 2021. In-depth investigation of the safety of wooden shelves used for traditional cheese ripening. Appl. Environ. Microbiol. 87, e01524-21 https://doi.org/10.1128/AEM.01524-2.

Sun, L., D'Amico, D.J., 2021. Composition, succession, and source tracking of microbial communities throughout the traditional production of a farmstead cheese. Msystems 6 (5), e00830-21. https://doi.org/10.1128/mSystems.00830-21.

Sun, L., D'Amico, D.J., 2023a. Characterization of microbial community assembly on new wooden vats for use in cheese production. Food Microbiol. 109, 104154 https://doi.org/10.1016/j.fm.2022.104154.

Sun, L., D'Amico, D.J., 2023b. The impact of environmental conditions and milk type on microbial communities of wooden vats and cheeses produced therein. Food Microbiol. 104319 https://doi.org/10.1016/j.fm.2023.104319.

Suzuki, T., Matsutani, M., Matsuyama, M., Unno, R., Matsushita, H., Sugiyama, M., Yasamoto, K., Koizumi, Y., Ishikawa, M., 2021. Growth and metabolic properties of halophilic and alkaliphilic lactic acid bacterial strains of *Marinilactibacillus psychrotolerans* isolated from surface-ripened soft cheese. Int. Dairy J. 112, 104840 https://doi.org/10.1016/j.idairyj.2020.104840.

Trofa, D., Gácser, A., Nosanchuk, J.D., 2008. *Candida parapsilosis*, an emerging fungal pathogen. Clin. Microbiol. Rev. 21 (4), 606–625. https://doi.org/10.1128/cmr.00013-08.

UNITE Community, 2019. UNITE QIIME Release for Fungi. https://doi.org/10.15156/BIO/786334.

Vermote, L., Verce, M., De Vuyst, L., Weckx, S., 2018. Amplicon and shotgun metagenomic sequencing indicates that microbial ecosystems present in cheese brines reflect environmental inoculation during the cheese production process. Int. Dairy J. 87, 44–53. https://doi.org/10.1016/j.idairyj.2018.07.010.

Wadhawan, K., Steinberger, A.J., Rankin, S.A., Suen, G., Czuprynski, C.J., 2021. Characterizing the microbiota of wooden boards used for cheese ripening. JDS Commun. 2 (4), 171–176. https://doi.org/10.3168/jdsc.2020-0014.

Wadhawan, K., Steinberger, A., Rankin, S., Suen, G., Czuprynski, C., 2023. Inhibition of *Listeria monocytogenes* by broth cultures of surface microbiota of wooden boards used in cheese ripening. Appl. Sci. 13 (10), 5872. https://doi.org/10.3390/app13105872.
White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications, vol. 18 (1), pp. 315–322.
White, T.J., Bruns, T., Lee, S.J.W.T., Taylor, J., 1990a. PCR protocols: a guide to methods and applications. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J. (Eds.), Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. Academic Press, San Diego, pp. 315–322.