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Spatiotemporal dynamics of the microbial diversity on salt-preserved goatskins assessed by culturing and 16S rRNA gene amplicon sequencing

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Abstract

Wet-salted skin, as a special artificial high-salt environment, is rich in protein, fat, collagen and other nutrient substrates, and is a rich resource of halotolerant and halophilic microorganisms. However, knowledge gaps regarding the microbial community structure and inter taxa associations of wet-salted skin are large. In this study, the spatiotemporal dynamics and community structure of microorganisms present on wet-salted goatskins were investigated using 16S rRNA gene amplicon sequencing and culturable technique. Alpha diversity analysis based on Sobs, Chao, Ace and Shannon indices revealed that microbial diversity on the wet-salted goatskins exhibited a trend of 'down → up → down → flat' with time. During preservation, genera belonging to the bacteria domain such as *Acinetobacter*, *Weissella* and *Streptococcus* were slowly dying out, whereas those belonging to halophilic archaea such as *Natrialba* and *Haloterrigena* were gradually flourishing. Moreover, to resist high-salt stress, microorganisms on the wet-salted goatskin gradually migrated from the outside to the inside, eventually leading to the microbial diversity inside the skin being the same as or even higher than that on the skin surface. Venn diagram analysis revealed that the strains of some genera, including *Psychrobacter*, *Salimicrobium*, *Salinicola*, *Ornithinibacillus*, *Halomonas*, *Bacillus* and *Chromohalobacter*, were distributed throughout the interior and exterior of the wet-salted goatskin and existed during various periods. Accordingly, 45 protease-producing halophilic or halotolerant microorganisms were isolated and screened from the wet-salted goatskin using the gradient dilution plate method. Importantly, 16S rRNA genes of some bacteria exhibited less than 99.5% similarity to valid published species, indicating that they likely are novel species and have a good potential for application.

Keywords: Wet-salted skin, Spatiotemporal dynamics, Community structure, 16S rRNA gene amplicon sequencing

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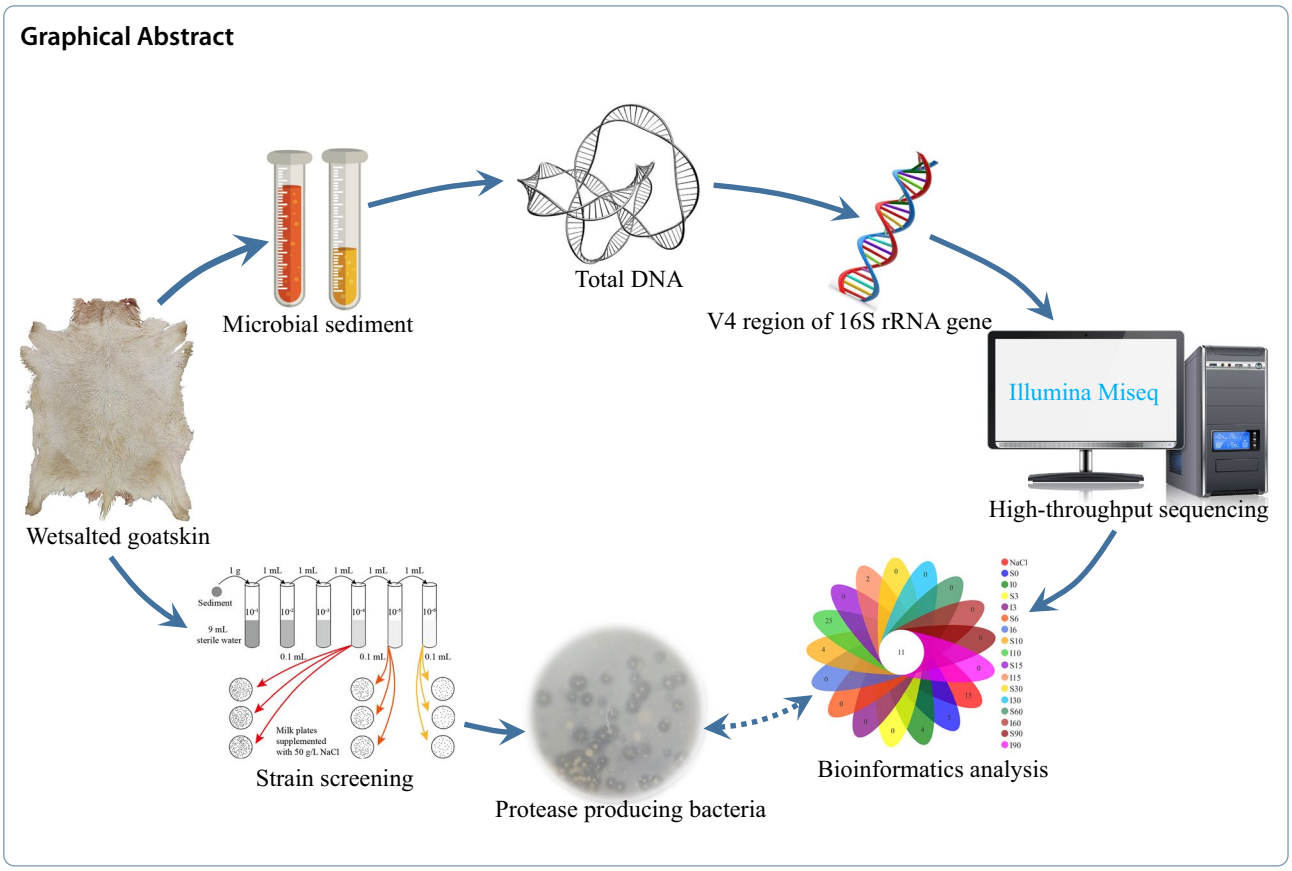

1 Introduction

The leather industry is an age-old industry producing various goods such as leather bags, leather shoes and leather garments, which have always been popular and preferred among consumers globally. The raw hides and skins, coming from the meat industry, are used as raw material for this industry. Animal skin, a fibrous proteinaceous material, contains hair, sweat glands, fat, blood vessels, water and collagen fibers [1]. It is more susceptible to be attacked by microorganisms, including bacteria, fungi and archaea, which derived from various sources such as air, water, soil, manure and extraneous filth [2]. Hence, the raw material starts degenerating if it is not properly treated within a few hours after being stripped from the animal [3]. The spoilt skins will severely affect leather quality, causing economic losses and environmental pollution. To date, various skin preservation methods have been discovered by researchers to increase the shelf lives of skin. These methods include sun drying, freezing, and use of sodium chloride, boric acid and aryl alcohol [4]. NaCl application is the most prevalent technique for hide/skin preservation because of its accessibility, low cost and ease of operation [5]. The generally accepted treatment is to add 30–50% (w/w) NaCl to the inside and outside of the skin according to skin weight, thus reducing the moisture content from 70 to 30% and making the skin unfavorable for the growth of ordinary bacteria [6].

Nevertheless, a special type of organisms, named extremophiles, can reproduce freely in extreme environments, such as high temperature, hypoxia, high pressure, low temperature and high salt [7]. Halophilic extremophiles, or simply halophiles, are microorganisms that can grow and often thrive in high salt (NaCl) concentration areas. Therefore, NaCl curing cannot inhibit the reproduction of halophiles but creates an improved breeding environment for them by inhibiting the growth of other non-halophiles. Moreover, halophiles are a potential source of novel extremozymes such as amylase, protease, collagenase, cellulase, xylanase, esterase and lipase [8]. These extremozymes could dehair the hair, damage the collagen dermal fibers and reduce leather quality [9–12]. The extracellular halo-alkali-thermophilic protease from *Halobacterium* sp. strain HP25, an obligate halophile that grew optimally at 25% (w/v) NaCl, was purified by Elbanna et al. [13]. Ali et al. [14] also noted the highest protease yield at 7% (w/v) NaCl from *Bacillus subtilis*

strain BLK-1.5, which was isolated from the salt mines of Karak, Pakistan.

Until now, various halophiles have been isolated from wet-salted animal skins, including *Haloarcula salaria* AT1, *Halobacterium salinarum* 22T6 [15], *Halomonas halmophila* HS2e and *Oceanobacillus picturae* HS4a [16]. However, unlike the increasing knowledge garnered from cultivable microorganisms isolated from wet-salted skins, considerably less is known about the spatiotemporal dynamics of microbial community distribution and their movement trail in NaCl-cured skins. However, this information is crucial to gain an improved understanding of microbial ecology and to reduce damage caused to the original material. Afterward, high throughput DNA sequencing, a culture-independent molecular screening technique, including 16S rRNA gene amplicon sequencing and metagenomics, has allowed the examination of a facet of microbial diversity that is not necessarily reflected by culturing study results [17]. Compared with other non-culture-based methods, the amplicon sequencing methodology with a higher sensitivity and detection ability can comprehensively identify microbial populations present in the sample. Moreover, it offers the advantage of detecting microbes that existing in a viable but nonculturable state [18]. We here first investigated the microbial diversity on wet-salted ovine skins from five different world regions, namely Australia, Spain, Inner Mongolia (China), Zhejiang (China) and Hebei (China). We then reported the spatiotemporal dynamics of the microbial diversity on NaCl-fixed fresh goatskin by using 16S rDNA amplicon sequencing. These results will be useful for further understanding the reasons for the spoilage of wet-salted skins and provide additional knowledge to obtain high-quality leather.

2 Materials and methods

2.1 Site description and sample collection

Wet-salted ovine skins without no apparent damage were randomly collected from Australia, Spain, Inner Mongolia (China), Zhejiang Province (China) and Hebei Province (China). Each sample was placed in a sterile plastic bag and transported to the lab, and processed within 24 h. First, the surface of the skins was cleaned several times with sterile water and the filtrate was collected. After several long high-speed (12,000 rpm in 50 mL EP tubes) centrifugation steps, the microorganism containing sediment was obtained. Second, the surface-treated samples were shredded, beaten and washed with sterile water. The microbial deposits were collected through filtration and centrifugation at 10,000 × g for 10 min. The same methods were used to dispose the five different sample sources, and accordingly, 10 microbial sediments were obtained.

In addition, a fresh goatskin was obtained from a farm on the outskirts of Chengdu (30°67′ N, 104°07′ E), Sichuan Province, south-west China. After sampling (goatskins without salt curing treatment were marked as sample 0), the remaining skin was smeared with 40% (w/w) NaCl, and then placed at room temperature. Then, the microbial communities were harvested at 7 time points: 3, 6, 10, 15, 30, 60 and 90 days by using the aforementioned operation.

2.2 Total genomic DNA extraction

Each sediment sample was ground with liquid nitrogen, and subjected to genomic DNA extraction by using a specific DNA extraction kit (MOBIO Laboratories, Inc., USA) according to the manufacturer's protocols. The genomic DNA quality was determined through 1% (w/v) agarose gel electrophoresis in 1 × Tris–acetate-EDTA buffer, and the A260/A280 absorbance ratio was calculated to estimate DNA concentration and purity by using a Nanodrop Life Spectrophotometer (Thermo Fisher Scientific, USA).

2.3 16S rDNA V4 region amplification and sequencing

Qualified DNA samples were used as templates for polymerase chain reaction (PCR). The V4 hypervariable region of the microbial (for both bacteria and archaea) 16S ribosomal RNA gene was amplified using the specific primers 515FmodF (5'-GTGYCAGCMGCCGCGGTAA-3') [19] and 806RmodR (5'-GGACTACNVGGGTWT CTAAT-3') [20]. The PCR mixture (20 μL) contained FastPfu buffer, dNTPs, primers, TransStart FastPfu DNA polymerase (TransGen Biotech, China), bovine serum albumin, template DNA and ddH_2O. All PCR reactions were conducted in an ABI GeneAmp®9700 thermocycler by using the following procedure: initial denaturation at 95 °C for 3 min; 28 cycles of denaturation 95 °C for 30 s, annealing at 53 °C for 30 s and extension at 72 °C for 45 s; and a final extension at 72 °C for 10 min. The PCR products were detected through 2% (w/v) agarose gel electrophoresis and purified using a QIAquick Gel Extraction Kit (QIAGEN, Germany). The collected DNA fragments were analyzed using high throughput sequencing on an Illumina Miseq platform at Majorbio Bio-Pharm Technology Co., Ltd (Shanghai, China).

2.4 Sequence denoising

The original image data file generated through high-throughput sequencing was converted into the raw sequence after base recognition by using CASAVA software, which was called raw data or raw read and stored in Fastq format. The raw reads were trimmed for barcodes and primers by using Trimmomatic [21], and chimeric sequences were removed using the Uchime algorithm

[22]. Using PEAR [23], the effective reads were merged and grouped into operational taxonomic units (OTUs) in UPARSE [24] at a 97% similarity. The raw reads have been deposited into the NCBI Sequence Read Archive database under the accession numbers PRJNA755827 and PRJNA755859.

2.5 OTU clustering, taxonomic diversity and bioinformatics analysis

To validate the microbial diversity on wet-salted skins, α-diversity indices, including Sobs, Chao, Simpson and Shannon indices were quantified in terms of OTU richness. Moreover, rarefaction curves of the observed numbers of OTUs were plotted to evaluate sample adequacy. To remove the bias triggered by different sequencing depths, the OTU table was rarefied and an even sampling depth was established by randomly subsampling the same number of sequences from each sample. OTU taxonomy was annotated using the SILVA database [25] with the Ribosomal Database Project classifier at a 70% confidence threshold (version 2.11) [26]. Meanwhile, Veen analysis was conducted to address the commonalities and differences between samples, and to identify the species with the greatest impact on the wet-salted skin. These analyses were conducted using the 'vegan' package in R [27].

2.6 Culturable bacteria isolation and identification

Based on the results of bioinformatics analysis and the fact that wet-salted skins lose hair during preservation, the sediments collected from wet-salted goatskins were selected and spread on Luria–Bertani (LB) agar medium supplemented with 5% (w/v) NaCl and 2% (w/v) milk by using the standard dilution plating technique. This lead to the isolation and screening of protease-producing bacteria. The plates were incubated at 30 °C and examined daily, and a single colony was transferred to fresh high salinity LB plates. The bacteria were identified on the basis of morphological characteristics and results of 16S rRNA gene analysis, and the sequences obtained were uploaded to the GenBank database and their similarity with published sequences in the EzBioCloud database was determined using BLAST. The protease activity was measured using the Li protocol [10] with casein as a substrate.

3 Results and discussion

3.1 Diversity and abundance of microorganisms in wet-salted ovine skins from five different areas

Microorganisms from the inside and outside of the wet-salted ovine skins from Australia, Spain, Inner Mongolia, Zhejiang Province and Hebei Province were collected and named as A (_SB and _IB), S (_SB and _IB), I (_SB and _IB), Z (_SB and _IB) and H (_SB and _IB), respectively. SB and IB indicate the surface and inside of the wet-salted ovine skins, respectively. The genomic DNA of the 10 examined samples was extracted and used as a template to amplify the V4 region of the bacterial 16S rDNA gene. Following pyrosequencing run in Illumina HiSeq, 656,506 original sequences were generated, including A_SB, A_IB, S_SB, S_IB, I_SB, I_IB, Z_SB, Z_IB, H_SB and H_IB with 53,755, 73,805, 77,594, 53,614, 54,572, 77,844, 60,841, 59,930, 69,768 and 74,783 sequence tags, respectively. In total, 607,934 sequences were obtained after the quality control, filtration, and optimization of the original sequence, including 51,406, 71,055, 71,159, 50,207, 46,308, 69,785, 54,263, 55,686, 67,046 and 71,019 sequences from A_SB, A_IB, S_SB, S_IB, I_SB, I_IB, Z_SB, Z_IB, H_SB and H_IB, respectively. The corresponding high-quality pyrotag sequences were clustered with a 97% similarity cut-off into 1365, 1399, 1813, 2216, 2559, 2691, 2294, 2367, 2083 and 2514 OTUs after singleton removal.

Some of the sequences were randomly selected from the samples, and the corresponding number of OTUs was counted. The results of multiple random samplings were used to plot dilution curves, presenting the number of sequences drawn and the corresponding number of OTUs. Under the Shannon and Sobs algorithms, the dilution curves of the 10 samples tended to be flat, thereby indicating that the sample size and sequencing data were reasonable and that the resulting sequence could reflect the actual bacterial community structure (Additional file 1: Fig. S1). Furthermore, alpha diversity analysis under the Sobs, Chao, Ace and Shannon indices showed that the microbial diversity inside the wet-salted ovine skins was usually higher than that on the skin surface (Additional file 1: Fig. S2). Because the interior of the wet-salted skin is rich in fat, protein and other nutrients, and is less affected by high NaCl concentrations, microorganisms tend to grow on the inside under salt stress, displaying signs of migration from outside to inside the skin. Therefore, microbial diversity inside the wet-salted skin is equal to or often higher than that outside the skin.

3.2 Temporal profiles of the microbial community

3.2.1 Sequence processing and assigning taxonomic identity

To profile the microbial community composition of the wet-salted goatskin, we performed 454 pyrotag sequencing of the V4 region of the 16S rRNA gene on wet-salted hide samples from 8 different times. In total, 3,381,689 high-quality sequences (~253 bp on average) were obtained from the 51 samples in the pyrosequencing run, with coverage ranging from 32,381 to 74,992 sequences per sample (Table 1). All effective sequences were clustered on the basis of 97% sequence identity cut-off, and

Table 1 Sequencing data of microbial samples of wet-salted goatskin at different time points

Time	Groups	Samples	Reads	OTUs	Time	Groups	Samples	Reads	OTUs
NaCl		NaCl_1	38,527	174					
		NaCl_2	65,723	199					
		NaCl_3	32,381	171					
T0	S0	0_SB_1	71,169	708	T15	S15	15_SB_1	74,596	472
		0_SB_2	69,628	654			15_SB_2	73,047	479
		0_SB_3	74,992	703			15_SB_3	69,781	392
	I0	0_IB_1	69,952	387		I15	15_IB_1	49,980	510
		0_IB_2	72,474	487			15_IB_2	70,452	688
		0_IB_3	72,437	311			15_IB_3	69,486	537
T3	S3	3_SB_1	71,859	612	T30	S30	30_SB_1	71,657	96
		3_SB_2	72,800	350			30_SB_2	61,744	64
		3_SB_3	69,287	390			30_SB_3	69,685	106
	I3	3_IB_1	71,708	204		I30	30_IB_1	73,626	113
		3_IB_2	72,368	196			30_IB_2	59,688	125
		3_IB_3	71,150	292			30_IB_3	70,507	162
T6	S6	6_SB_1	43,945	644	T60	S60	60_SB_1	42,699	50
		6_SB_2	50,828	612			60_SB_2	55,747	46
		6_SB_3	70,214	684			60_SB_3	73,349	46
	I6	6_IB_1	60,637	621		I60	60_IB_1	70,046	46
		6_IB_2	74,267	563			60_IB_2	72,962	63
		6_IB_3	73,751	746			60_IB_3	70,413	46
T10	S10	10_SB_1	73,135	894	T90	S90	90_SB_1	72,949	48
		10_SB_2	73,158	922			90_SB_2	73,515	50
		10_SB_3	72,889	916			90_SB_3	62,207	36
	I10	10_IB_1	56,624	961		I90	90_IB_1	46,969	41
		10_IB_2	68,164	952			90_IB_2	73,350	56
		10_IB_3	72,033	995			90_IB_3	67,134	67

NaCl represents sodium chloride pellets; T represents time

each sample had 36–995 OTUs (Table 1). In addition, as shown in Additional file 1: Fig. S3, the rarefaction curves of all samples under Chao and Shannon indices tended to approach the saturation plateau. Thus, the sequencing depth basically covered all species in the samples, and the existing data could truly reflect the microbial community structure of the wet-salted skin, meeting the requirements of subsequent bioinformatics analysis.

3.2.2 Analysis of microbial diversity of complex bacterial communities

As observed in Fig. 1, the microbial community diversity inside and on the surface of the wet-salted goatskins changed significantly with an increase in salting time after industrial NaCl treatment, and the alpha diversity at Sobs, Chao, Ace and Shannon indexes showed an overall trend of 'down → up → down → flat'. Initially, animal skins, as an ideal habitat, gathered numerous microorganisms, such as *Halomonas*, *Staphylococcus*, *Vibrio* and *Weissella* species (Fig. 2). In the absence of environmental stress, microbes more easily accumulated on animal skin surface, resulting in a considerably higher microbial diversity outside the skins than on the inside. The high osmotic pressure formed by uniform application of large amounts of NaCl on goatskins creates a special high-salt environment, which often causes water loss from the cells of common microorganisms. This leads to cell wall separation and affects the metabolic reproduction of these microorganisms, but has no effect on halotolerant and halophilic bacteria. This results in the gradual extinction of common microorganisms such as *Acinetobacter*, *Weissella*, *Streptococcus*, *Kocuria* and *Macrococcus* species (Fig. 3a), and the reduction in microbial communities and diversity on the surface and inside of the skin. With the removal of salt-intolerant microorganisms, *Staphylococcus*, *Halomonas*, *Salinivibrio*, *Salimicrobium*, *Chromohalobacter* and other salt-loving and salt-tolerant microorganisms received a wider space for growth and more nutrient substrates (Fig. 3b). They

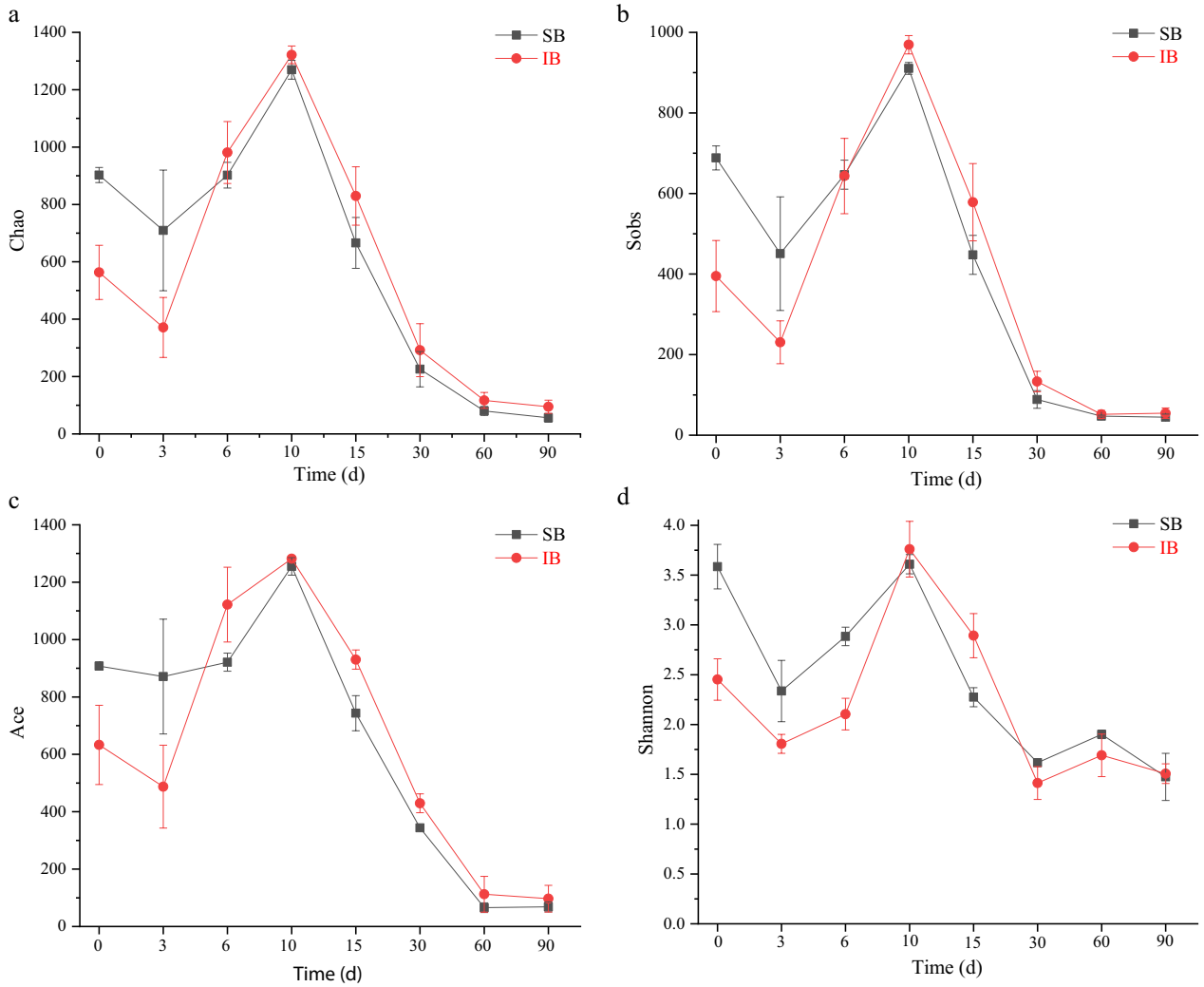

Fig. 1 Alpha diversity of wet-salted goatskin microorganisms at different time under Chao (**a**), Sobs (**b**), Ace (**c**) and Shannon (**d**) indices

thus proliferated and the diversity of microbial communities on the wet-salted skins rapidly increased from the 3rd day of storage and peaked on the 10th day, with the detection of over 900 species. From the 10th day, after the the salt curing time was extended, water loss on the wet-salted skin was considerable, and the salt concentration further increased beyond the tolerance range of slight halophile, moderate halophile and halotolerant bacteria. This thus inhibited the reproduction of all microorganisms except extreme halophile, and gradually decreased the microbial community diversity both inside and outside the wet-salted skin. Ultimately, the wet-salted skin with high salt, little water and many nutrient substrates became an excellent habitat for extremophilic prokaryotic microorganisms such as *Alkalibacillus*, *Halovibrio*, *Natrialba* and *Haloterrigena* strains (Fig. 3c). On the other hand, they maintained a low microbial community diversity on the inside and outside. Furthermore, from the 10th day of salt curing, the microbial diversity (Fig. 1) and the content of *Alkalibacillus*, *Halovibrio* and *Natrialba* strains were higher inside the skin than on the skin surface (Fig. 3c). This indicated that some wet-salted skin microorganisms would migrate from the skin surface to the interior under salt stress. This is consistent with the results of research about microorganisms on wet-salted ovine skins from five different regions (Additional file 1: Fig. S2). More importantly, the surface and inside of the wet-salted skin was almost devoid of archaea in the early salting stage. However, when the salting time was extended, the percentage of archaea on the inside and outside of the skins increased, and gradually, they became the dominant prokaryotic microorganisms. The relative abundance of archaea on the inside and outside

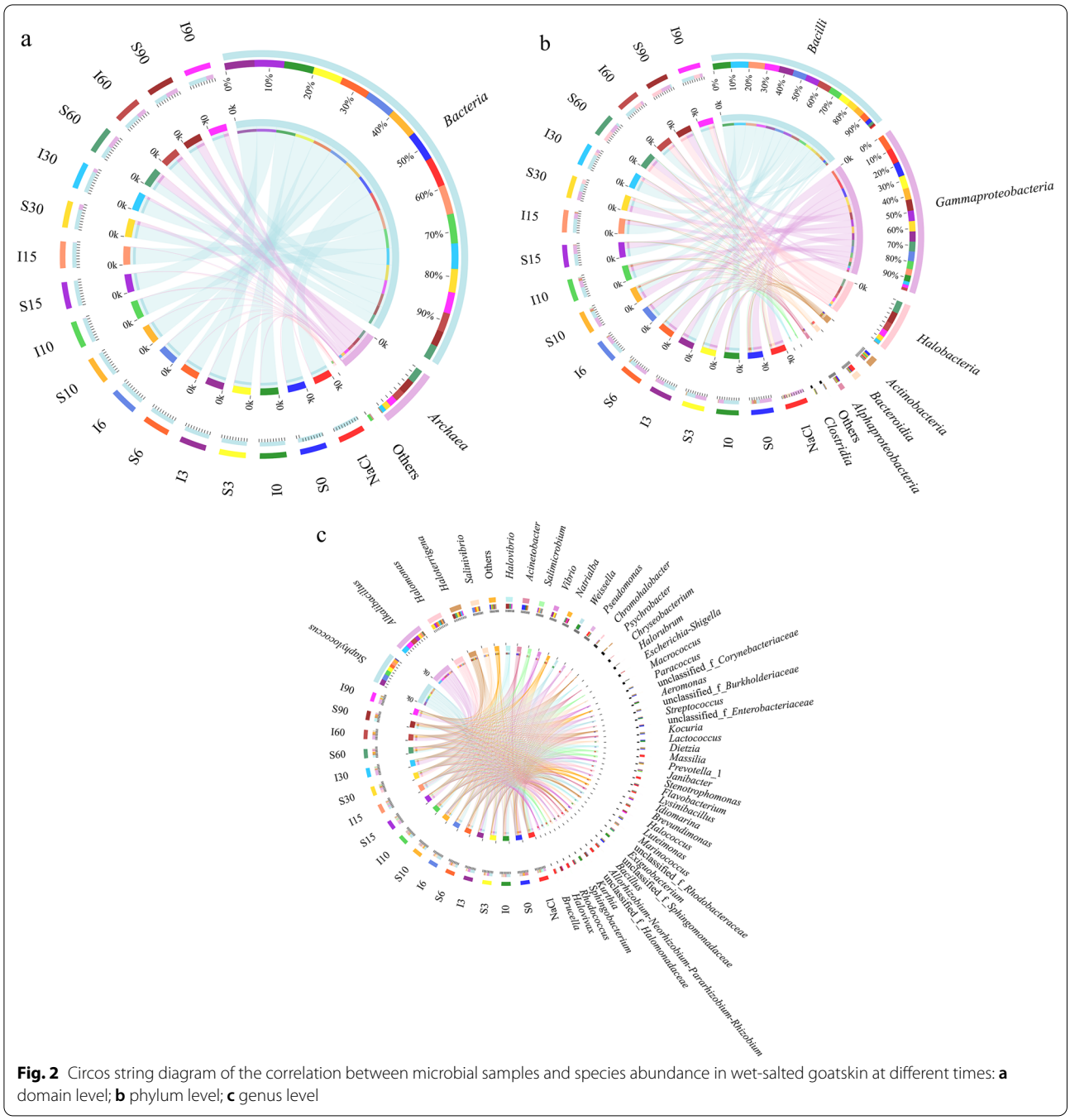

Fig. 2 Circos string diagram of the correlation between microbial samples and species abundance in wet-salted goatskin at different times: **a** domain level; **b** phylum level; **c** genus level

of the skins was as high as 28.58 and 48.50% respectively on the 90th day of salting (Fig. 2). This indicated the gradual transition from bacteria to archaea on the wet-salted skins with the extended salt curing time.

3.2.3 Correlation analysis of microorganisms in wet-salted skin

Many environmental factors affect the microbial community composition of wet-salted skins, but the most influential environmental factor is time (T). Therefore, in terms of relative abundance, the top 50 OTUs were selected at the genus taxonomic level and correlation network plots were drawn using the correlation coefficient type Spearman with the environmental factor T. As shown in Fig. 4a, extremophilic prokaryotic microorganisms, represented by species of the genera *Alkalibacillus*, *Natrialba* and *Haloterrigena*, showed a better positive correlation with time, the longer the salt curing time, the

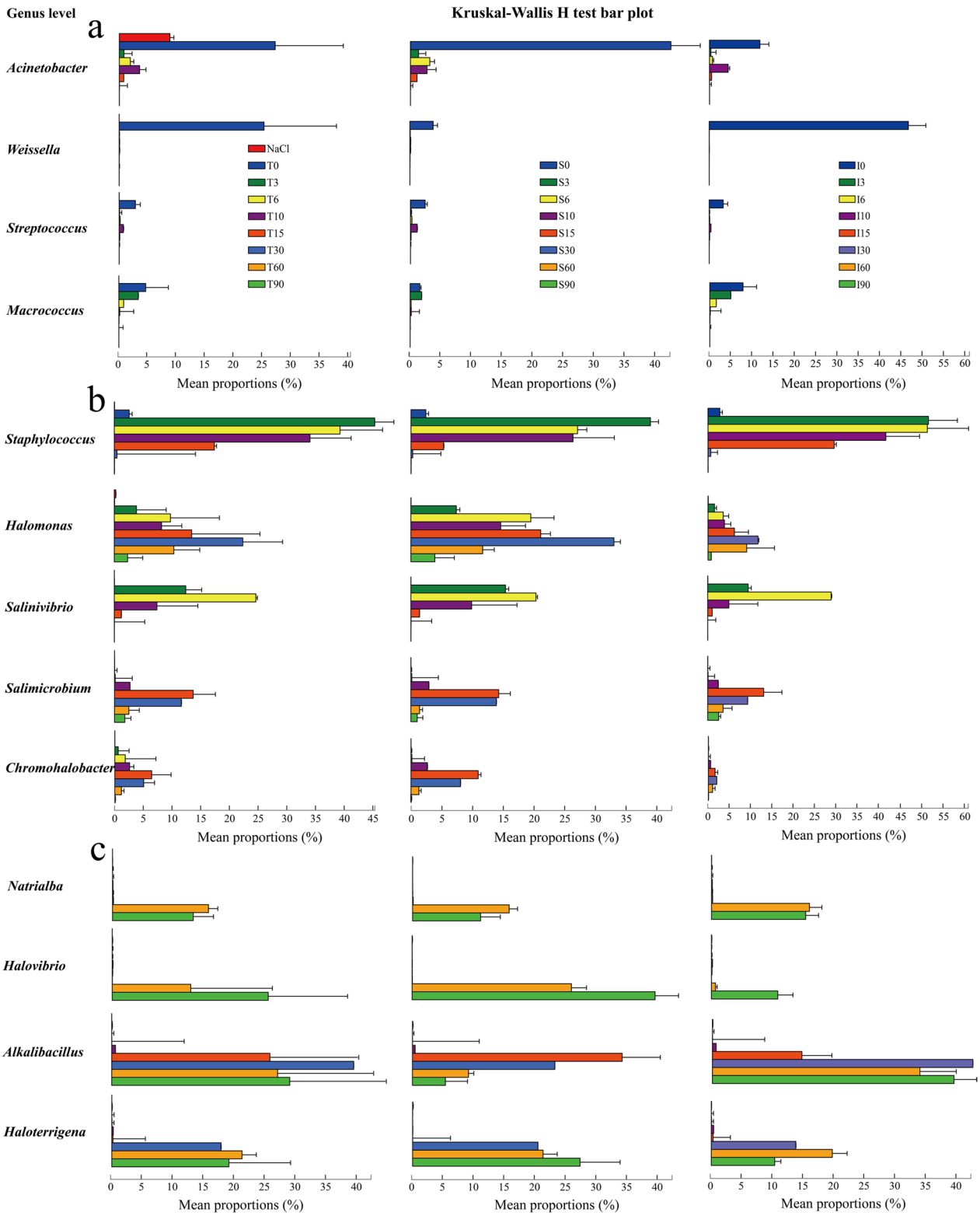

Fig. 3 Changes in the relative abundance of typical common microorganisms (**a**), moderate halophiles (**b**) and extreme halophiles (**c**) on wet-salted goatskin. NaCl indicates the negative control derived from sodium chloride particles; S indicates microbial samples derived from the animal skin surface; I represents microbial samples derived from the interior of animal skins; and the numbers indicate time

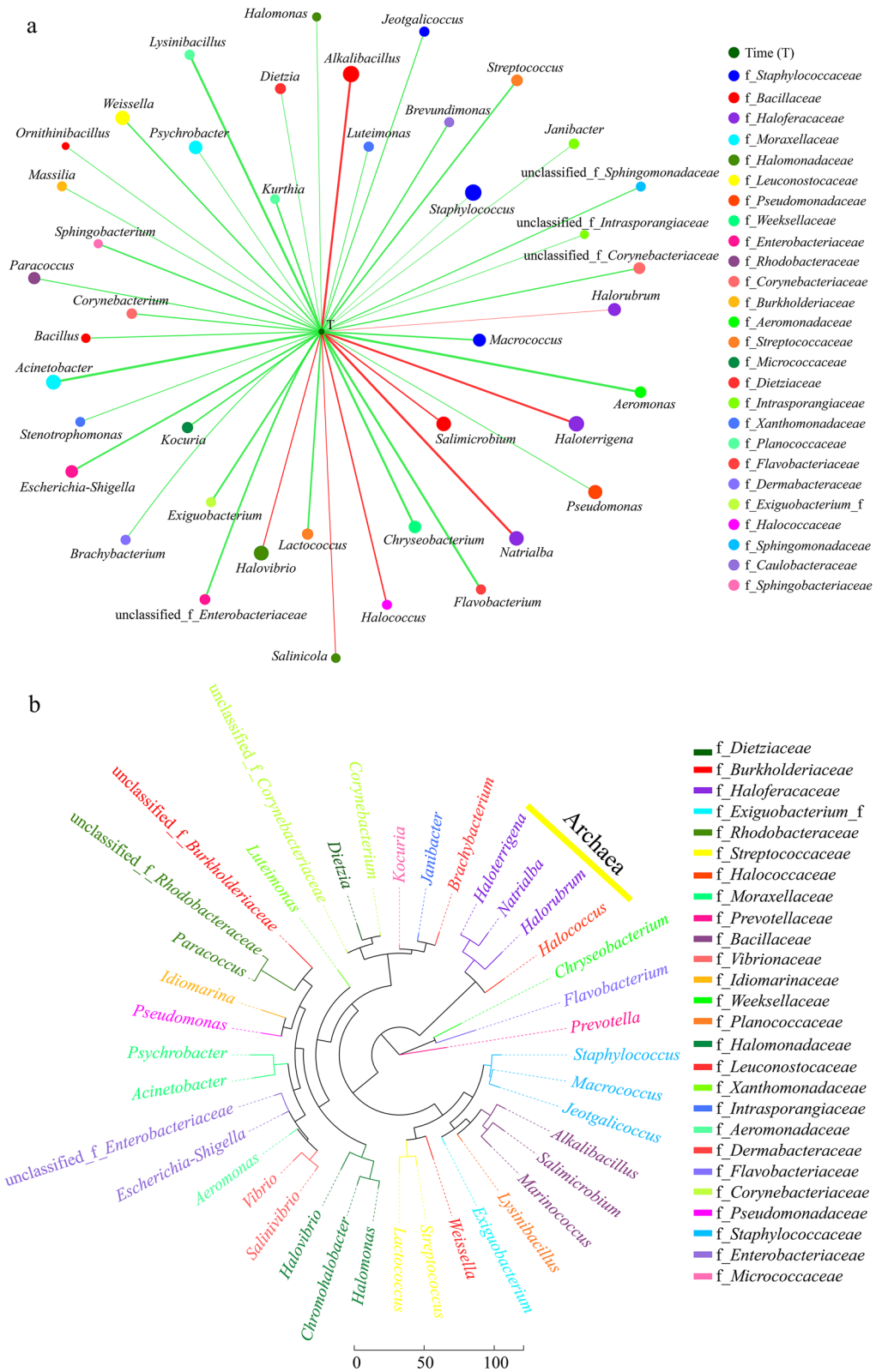

Fig. 4 Correlation network among microorganisms in wet-salted goatskin. **a** network diagram of the correlation between wet-salted skin microorganisms and time. Red lines indicate a positive correlation, green lines indicate a negative correlation; thicker lines indicate a higher correlation between the two, and more lines indicate a stronger connection with the node. **b** phylogenetic relationships among microorganisms on wet-salted goatskin. **c** composition of unique and shared bacteria in 17 microbial samples groups

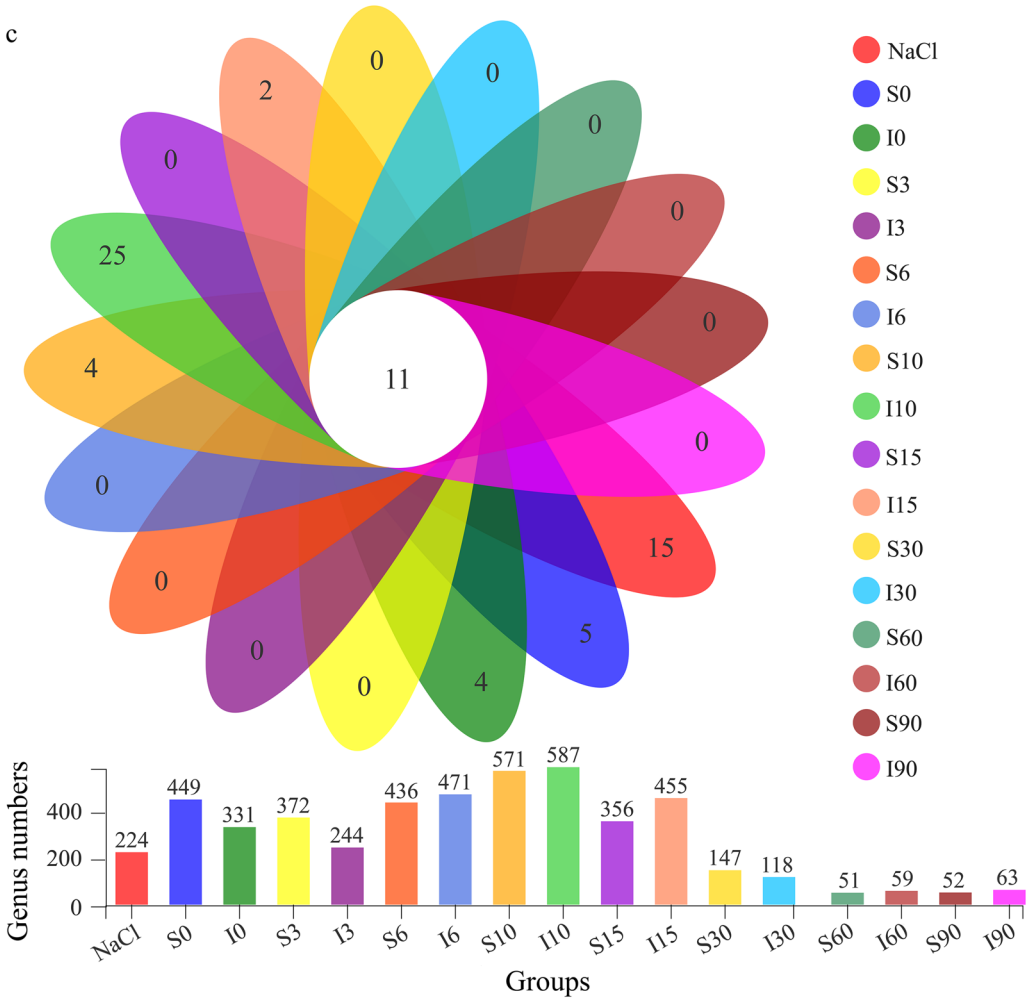

Fig. 4 continued

more ideal conditions for their survival and the higher their abundance. By contrast, non-halophile represented by members of the genera *Lysinibacillus*, *Acinetobacter* and *Streptococcus* displayed a negative correlation with time; the longer the salting time, the more severely they were inhibited and the lower their abundance on the wet-salted skin.

To reveal the affinities of microorganisms on wet-salted skins at the genus level from the perspective of molecular evolution, the OTU data corresponding to the top 40 genera in terms of relative abundance were subjected to random sampling and multiple sequence comparisons. The phylogenetic evolutionary tree was constructed using the maximum parsimony (MP) method [22]. As seen in Fig. 4b, 40 genera distributed in 26 families formed a good topology in the MP evolutionary tree. The non-halophile genera *Acinetobacter* and *Psychrobacter* are grouped together in the family *Moraxellaceae*, the moderate halophile genera *Halomonas*, *Chromohalobacter* and *Halovibrio* are grouped together in the family *Halomonadaceae*, and the archaeal genera *Halococcus*, *Halorubrum*, *Natrialba* and *Haloterrigena* form a large independent branch. These facts suggest that certain affinities exist among microorganisms on wet-salted skins and the species belonging to the same family taxonomic level may have similar salt tolerance and halophilicity.

To visualize the similarity and overlap of microbial species composition of wet-salted skins at different time, a petal map of all samples was constructed using R language based on the species classification at the genus level. Figure 4c reveals that the wet-salted goatskin contained the most species on the 10th day of storage, with 587 and 571 genera on the inside and outside of the hide, respectively. In addition, 17 groups of microbial samples shared 11 genera, including *Salinicola*, *Staphylococcus*, *Luteimonas*, *Psychrobacter*, *Salimicrobium*,

Ornithinibacillus, *Halomonas*, *Bacillus*, *Brevundimonas*, *Chromohalobacter*, and unclassified genera of the family *Burkholderiaceae*. This indicated that strains belonging to these genera were present throughout wet-salted skin preservation and distributed throughout the interior and exterior of the skin. Some species of these genera have been reported to produce pigments, lipases, proteases and collagenases [10, 29–32]. Thus, their growth and metabolism have been hypothesized to lead to erythema, desquamation and rotting of wet-salted skins.

3.3 Screening and preliminary identification of protease-producing bacteria

The aforementioned results of bioinformatics analysis revealed that numerous microorganisms were accumulated on the wet-salted goatskin, especially the halophilic bacteria belonging to genera such as *Salinicola*, *Ornithinibacillus*, *Alkalibacillus*, *Chromohalobacter*, *Halomonas* and *Natrialba.* They resulted in a very high diversity of wet-salted skin microorganisms. In high-salt environments, halophiles have evolved unique physiological structures and metabolic pathways to synthesize active substances such as tetrahydropyrimidines, polysaccharides and proteases. Of them, proteases are the main substances responsible for localized hair loss or hair loosening in wet-salted goatskins (Additional file 1: Fig. S4a). Accordingly, 45 strains of protease-producing bacteria were screened and isolated from the microbial samples of the wet-salted goatskin (Additional file 1: Fig. S4b), some of which could also produce white, yellow, orange and red pigments on modified LB plates (Additional file 1: Fig. S4c).

The 16S rRNA genes of protease-producing bacteria were amplified, sequenced, registered in the NCBI database and searched in the EzBioCloud database. As listed in Additional file 1: Table S1, the 45 strains were distributed in the genera *Bacillus*, *Arthrobacter*, *Comamonas*, *Chryseobacterium*, *Erwinia*, *Aeromonas*, *Halobacillus*, *Halomonas*, *Oceanobacillus*, *Ornithinibacillus*, *Staphylococcus*, *Piscibacillus*, *Salinicola*, *Salinicoccus*, *Virgibacillus*, *Nocardiopsis*, *Citricoccus* and *Kocuria*, which basically corroborated the results of the bioinformatics analysis. Most importantly, the 16S rRNA genes of some strains, including L3, L5 and L9, showed 98.22–99.50% similarity to known species, with 30–80% probability of being new species and having greater potential for application.

4 Conclusions

16S amplicon sequencing and bioinformatics analysis revealed that microbial diversity on the wet-salted goatskin showed a trend of 'down → up → down → flat' over time. To resist high salt stress, the microbial community changed from bacteria to archaea, and migrated from outside to inside the skin. Moreover, traces of species belonging to the genera *Staphylococcus*, *Luteimonas*, *Psychrobacter*, *Salimicrobium*, *Salinicola*, *Halomonas*, *Bacillus*, and *Ornithinibacillus* were found at all times during wet-salted goatskin preservation. Based on the results of bioinformatics analysis of wet-salted goatskin microorganisms and the fact that the goatskin showed local hair loss during storage, 45 strains of halophilic or halotolerant microorganisms secreting extracellular active proteases were isolated and screened, among which strains L3, L5 and L9 were suspected to be new species. These results fill the gap in the study of the microbial community structure of wet-salted skins and provide a theoretical basis for the further improvement and development of methods for high-quality preservation of animal skins.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42825-022-00107-1.

Additional file 1. **Table S1.** Comparison results of 45 protease-producing strains in EzBioCloud database. **Fig. S1.** Rarefaction curves of 16S rRNA gene sequencing of the wet-salted ovine skins from five areas. a: the rarefaction curve was assembled using Shannon index; b: the rarefaction curve was executed using Sobs algorithm. A, S, I, Z and H represent Australia, Spain, Inner Mongolia (China), Zhejiang Province (China) and Hebei Province (China), respectively. SB and IB indicate the surface and the inside of the wet-salted ovine skins, respectively. **Fig. S2.** Alpha diversity analysis of microorganisms present in the interior and exterior of wet-salted ovine skins from five different areas at indices Sobs, Shannon, Chao and Simpson. A, S, I, Z and H represent Australia, Spain, Inner Mongolia (China), Zhejiang Province (China) and Hebei Province (China), respectively. SB and IB indicate the surface and the inside of the wet-salted ovine skins, respectively. **Fig. S3.** Dilution curves of Chao (a) and Shannon (b) indices for microbial communities in homemade salt-preserved goatskin. **Fig. S4.** Screening and characterization of protease-producing bacteria: a, localized dehairing of wet-salted goatskin; b, milk plate hydrolysis circle screening bacteria; c, characterization of protease-producing bacteria on the modified LB plates.

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Author contributions

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Availability of data and materials

All data from this study are presented in the paper.

Declarations

Competing interests

The authors declare that they have no competing interests.

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