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Characterization of Microbiota Isolated from Traditional Honduran Cheese

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Abstract

This study aimed to describe the microbial populations involved in the production of traditional raw milk, semi-dry cheese from Honduras, Central America, particularly the indigenous lactic acid bacteria (LAB) responsible for the organoleptic characteristics of this product. A total of 48 cheese samples were collected from seven of the 18 country's Departments. Phenotypic methods were used to count, identify and compare the composition of bacteria and yeasts populations in the samples. Twenty LAB isolates were randomly selected to analyse its 16S-23S rRNA spacer region. This method was also applied to understand the diversity of bacterial communities present in the samples. LAB colonies reached average counts of 4.79 lg CFU g^{-1}. Twelve LAB species were identified based on a conventional phenotypic approach. *Lactococcus lactis* and *Enterococcus faecium* were the most frequently identified species according to microbiological and molecular methods. No significant correlation was observed among LAB species and geographical distribution. Four genera and five species were identified among the isolated yeasts. This is the first report describing the indigenous biota of traditional raw milk cheeses from Honduras. Our results may provide useful information for valuable strain selection as starter cultures for the industrialization of traditional dairy products in Honduras.

Keywords: Lactic acid bacteria; 16S rRNA gene; Artisanal cheese; Honduras

Introduction

Lactic acid bacteria (LAB) are part of the normal gastrointestinal biota of ruminants. Because they promote biochemical and sensory changes during cheese ripening, these bacteria have great commercial importance as cheese starter cultures [1,2]. They also are known for their role in the inhibition of pathogens [3-6]. Traditional dairy products are common components of the human diet in many regions of the world, and each of these products has particular characteristics [7-15]. Microbial biota of artisanal cheeses is without a doubt the most important factor conferring them unique properties. The description of the adventitious microbiota present in traditional raw milk cheeses is therefore a relevant issue for the cheese industry. Studies from other countries report *Lactococcus* sp., *Enterococcus* sp., *Lactobacillus* sp., and *Leuconostoc* sp. as the most common isolates from artisanal cheeses [7,12,13,16].

In Honduras there are two well-differentiated cheese producers, those who are industrialized and the artisans, who utilize traditional methods learned informally. According to the national agriculture Ministry, there are in the country over 472 manufacturers of artisanal cheese, of which 66% do not meet national food safety requirements [17]. Among several varieties, artisanal semi-dry (semi-hard) cheeses are the most popular among the local consumers because of their unique aroma, flavour and texture. These characteristics are derived from specific tropical environmental conditions and from traditional manufacturing practices. Semi-dry cheeses are made from raw cow´s milk without culture starters so the fermentation takes place through a natural process. More than 600 million litres of milk are used for this purpose each year in Honduras, but despite their commercial importance, little is known of the microbial biota present in these products. Due to the lack of food safety standards in the manufacturing process, and the use of raw milk of unknown microbiological quality, local artisanal cheeses are always a potential risk for the health of consumers [18]. Moreover, they cannot be exported to markets demanding international quality standards. These regulations include standardized manufacturing practices such as the pasteurization of milk and the use of culture starters to ensure the safety of the product [19].

The identification of LAB species in dairy products usually relies on phenotypic methods, mainly based on carbohydrate fermentation patterns. Although such conventional approaches have allowed for a practical LAB classification [14,20], some authors suggest that phenotypic data should be complemented with molecular techniques for a more specific LAB species identification [7,21,22], including 16S rRNA gene sequence analysis and DGGE. Molecular techniques have also led to an improved understanding of bacterial dynamics present in cheese samples. Data on sequence variability in 16S-23S rRNA regions (rRNA gene spacer analysis or RSA) could be valuable for an initial understanding of the microbial population diversity (richness) of artisanal cheese. However, due to its lack of resolution the information produced by such technology has to be complemented by more specific techniques [12,13].

This study describes first the enumeration of LAB and yeasts, and then the isolation and identification of microorganisms from 48 cheese samples obtained from seven regions in Honduras. LAB species were identified on the basis of their phenotypic profile, and complemented with rRNA gene sequence analysis of some bacterial isolates. To understand the composition of bacterial communities, an RSA analysis was also carried out. Since existing microbiota in artisanal cheeses is the best adapted to local conditions, their use ensures the typicality of those traditional products and their preservation. This report will provide

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useful information on the diversity of the microbiota in traditional cheese products from Honduras. Moreover, the obtained LAB pure cultures will be valuable for further selection of starter cultures used in the local dairy industry.

Materials and Methods

Cheese samples

A total of 48 artisanal semi-dry cheese samples were collected from the same number of processing plants from March to July 2015. Samples originated in 7 of the 18 Departments (Honduras's sub-national political divisions) as follows: Yoro (n=19); Olancho (n=10); El Paraíso (n=7); Atlántida (n=5); Ocotepeque (n=4); Valle (n=2); and Colón (n=1) (Figure 1). None of the manufacturers used commercial lactic acid starters. Samples of cheese ripened approximately for 30 days at 25ºC and weighed around 400 g. All samples were transported under refrigeration (4°C) to the laboratory in Honduras's capital, Tegucigalpa, for further analysis.

Enumeration and identification of lactic acid bacteria (LAB) and yeasts

Samples were analysed by conventional microbiological methods [23]. Aliquots of 25 g of each sample were diluted and homogenized in 225 mL of saline peptone water (Acumedia, Neogen Corporation, Lansing, MI). For total aerobic bacterial colony counts, ten 10-fold dilutions were plated onto MRS agar (Criterion™, Hardy Diagnostics, CA, USA) and incubated at 30°C for 24 to 48 hours. MRS agar was used to perform LAB counts after incubation at 25°C for 24 to 48 hours under an atmosphere of 5-7% CO_2. For each sample, 5 to 7 colonies with typical morphological characteristics (colour, shape and size) were selected and pure colonies isolated by streaking on the same MRS medium. Yeasts were enumerated on PDA agar plates (Hardy Diagnostics) acidified to pH 3.5 with sterilized tartaric acid (Merck, Darmstadt, Germany) according to methodology described elsewhere [24]. Yeast cultures were incubated at 25°C for 3 to 5 days. For each sample, all different colonies were selected and pure colonies isolated by streaking on PDA plates. All cultures were done in duplicate. Total aerobic bacteria, total LAB colony counts, and total yeast colony counts were determined, and the average number of colonies calculated for both duplicates. Results were expressed as the logarithm of colony forming units (CFU) per gram of cheese. LAB isolates were maintained at -20°C in 10% skim milk (Hardy Diagnostics) and 3% L-glutamine (Sigma, St. Louis, MO, USA) until further phenotypic identification and molecular analysis. Yeast isolates were maintained at room temperature in sterile distilled water [25].

Figure 1: Map showing the distribution of sampling sites of artisanal cheese in seven Departments of Honduras.

Some isolated LAB strains were assigned to genus level according to the Bergey´s Manual of Bacteriology, based on their phenotype, including cell morphology, Gram stain, and catalase reaction as presumptive evidence [26]. Standard biochemical methods were used to identify the following lactic acid bacteria at the genus level: *Lactobacillus*, *Lactococcus* and *Leuconostoc*. Catalase-negative colonies of Gram-positive cocci and bacilli were identified by the API 20 Strep and API CHL50 systems, respectively (BioMérieux, Marcy l'Etoile, France) according to manufacturer instructions. All the isolates were identified twice by the API systems in order to confirm reproducibility of this method. Yeasts were identified by the RapID™ Yeast Plus System (Remel Inc., Lenexa, KS, USA) according to panel's instructions.

DNA extraction from bacterial cultures

Bacterial DNA was extracted from 24-hr cultures, following a modified CTAB (cetyltrimethylammonium bromide) based method [27]. Briefly, the bacterial sediment was treated with 400 µl of 2% CTAB and incubated for 45 min at 65°C, while mixing every 5 minutes. Then one volume of chloroform was added to the solution, vortexed vigorously and separated by centrifugation at 13,000 rpm for 2 minutes. The aqueous phase was then transferred to a new vial and DNA was precipitated with two volumes of cold absolute ethanol, with gentle shaking. After a three-minute centrifugation step, the supernatant was removed and the dry pellet was resuspended in sterile Milli-Q water.

Ribosomal DNA sequence analysis of LAB isolates

Twenty random LAB isolates were analysed by amplification and sequencing of the 16S-23S rRNA spacer region (RSA), according to Dolci et al. 2008 [12]. This ribosomal region is highly polymorphic and allows obtaining amplicons with different molecular weights in order to assign each isolate to a hypothetical genus [28]. PCR reactions were performed in a volume of 25 µL, containing 12.5 µL of Master Mix (Promega Corporation, Madison, WI, USA), and 1.25 pmol of each primer (G1: 5´-GAAGTCGTAACAAGG-3´ and L1: 5´-CAAGGCATCCACCGT-3´) [11,29]. One µL of DNA was added to the mixture. The PCR conditions were as follows: one step of 95°C for 5 min, 40 cycles of 2 min at 95°C, 1 min at 55°C, 2 min at 72°C, and a final extension step for 10 min at 72°C. Electrophoresis of products was carried out in 2% agarose. PCR products were purified and sequenced in both directions in Macrogen Inc. (Maryland, USA). Nucleotide sequences were manually corrected using the Geneious® 9.1.2 software (Biomatters Ltd.) and queried against the GenBank database using the BLAST tool.

DNA extraction from cheese

DNA was also purified from one gram of cheese as previously described [30,31]. Briefly, each sample was suspended in 12 mL of 2% sodium citrate and incubated 30 min at 45ºC. After shaking for 5 min, samples were centrifuged for 10 min at 6000 rpm, and the pellet resuspended in 5 mL of TE buffer. Further centrifugation at 3,500 rpm was required to obtain a pellet that was diluted in 300 µl of TE. This suspension was centrifuged for 5 min at 14,000 rpm. The pellet was resuspended in 100 µL of a proteinase K buffer (50 mM Tris-HCl, 10 mM EDTA, 0.5% SDS, pH 7.5). This was transferred to a new vial containing 0.3 g of 0.1 mm glass beads, 20 µl of proteinase K (25 mg/ml)

and 20 µl of lysozyme (25 mg/ml). After 1-hour incubation at 50°C, 150 µl of breaking buffer (4% Triton X-100, 2% SDS, 20 mM Tris pH 8, 200 mM NaCl and 2 mM EDTA pH 8) were added. For DNA extraction, 300 µl of phenol-chloroform-isoamyl alcohol, pH 6.7, in a proportion of 25:24:1 were added. Three 2-min bead beater (Bio Spec Products Inc., USA) treatments were performed, at maximum speed. The aqueous phase was recovered into a new 1.5 ml tube. A second extraction step was performed by addition of 300 µl of phenol-chloroform-isoamyl alcohol and 300 µl of TE buffer. After 10 min of centrifugation at 12,000 rpm, the aqueous phase was recovered and precipitated with 1 mL of cold absolute ethanol. The DNA pellet was obtained by centrifugation at 14,000 rpm for 10 min. Each pellet was air-dried and 50 µl of sterile Milli-Q water was added. The purity and concentration of the DNA from the cheese samples and microorganisms cultures was evaluated using a NanoDrop ND-1000 (Thermo Fisher Scientific Inc.). All DNA samples were diluted to a concentration of 40 ng/µL until further use.

Amplification of a ribosomal spacer from bacterial communities

To study the diversity (richness) and relative abundance (evenness) of bacterial communities on cheese samples, the same ribosomal spacer used for bacterial isolates was amplified using total DNA obtained from each cheese as template. Twenty µl of PCR products were analysed on 2% agarose gels running at 70 V for 2.5 hours. Three replicates from each cheese sample were used to assess the consistency of band profiles and only the repetitive bands were scored. Each band was scored for the presence (coded as 1) or absence (coded as 0) of homologous bands (bands with the same size) for all samples. Based on the band profile for each cheese sample, a dendrogram was constructed by UPGMA cluster analysis using the Pearson (r) coefficient and the DendroUPGMA web-based utility [32]. Four parameters were computed based on the band profiles: total number of bands (TNB), number of polymorphic bands (NPB), number of community profiles (NCP), and the number of exclusive bands (NEB). TNB was calculated by the sum of different bands for all samples. NPB represent the bands that are not always present in every sample. NCP was calculated based on the number of different electrophoretic profiles; and NEB was scored as those bands present in only one sample.

Results and Discussion

Microbial counts

The lactic acid bacteria (LAB) counts, and total bacterial counts, in all cheese samples are presented in Table 1. Culture procedures were performed only under mesophilic conditions since the artisanal cheese production in Honduras is carried out at room temperature. LAB counts ranged from 1.00 to 8.95 lg CFU g^{-1}, with an overall average of 4.76 ± 1.99. Samples with the highest LAB counts were found in the Departments of Olancho and Atlántida, while the lowest counts were reported in El Paraíso. As also shown in Table 1, the total bacterial count was significantly higher (approximately *c*. 2 logs) for the samples from the seven Departments, compared to the LAB count, with a wider range of 1.00 to 10.60 lg CFU g^{-1}, and an average of 6.18 ± 2.36. Elevated values of viable LAB counts are similar to the average range reported by other authors for traditional dairy products [7,12,33].

Isolation and identification of microorganisms

A total of 103 LAB strains were selected for further identification from 48 semi-dry cheese samples. One to three colonies were selected from each cheese sample based on morphological characteristics, Gram reaction, non-motility, absence of catalase activity and spores. Most of them were cocci and coccobacilli (92.23%) while the rest were rods (7.77%). Other authors have also reported a higher proportion of cocci from artisanal cheeses [7,13,34]. This finding could be consistent with high levels of observed bovine faecal contamination in the milking process in rural areas of Honduras, [35] and a greater resistance of enterococci to adverse conditions during the maturation of cheese [36].

The API systems, based on carbohydrate fermentation patterns assigned the isolates to 12 different species belonging to the following six genera: *Lactococcus, Enterococcus, Aerococcus, Leuconostoc, Lactobacillus, Streptococcus*. The two more frequently isolated species were *Lactococcus lactis (L. lactis* subsp. *lactis and L. lactis* subsp. *cremoris),* and *Enterococcus faecium*, isolated from 16 (30.2%) and 8 (15.1%) cheese samples, respectively (Table 2). Among the *Enterococcus* genus, 7 (13.21%) cheese samples showed *E. faecalis*, 2 (3.8%) *E. durans*, and 1 (1.9%) *E. casseliflavus*. Among the *Lactobacillus* genus, 4 (7.6%) samples revealed *L. acidophilus*, and 1 (1.9%) *L. plantarum*. *Aerococcus viridans* was isolated from 8 (15.1%) samples, and *Leuconostoc* sp. from 4 (7.6%) samples. Finally, only two samples revealed the presence of *Streptococci* (*S. mitis* and *S. bovis*). Our results are consistent with those reported in the literature, where the composition of bacterial species in several artisanal cheeses varies greatly depending on the region where they are produced. However, in general terms, the dominant biota is composed of lactobacilli [15,30], enterococci [7,34], and lactococci [11,37], among other less represented species.

By analysing the distribution of LAB species isolated according to geographical provenance, it seems clear that there is a homogeneous distribution of LAB species throughout the country and, as shown in Table 3, it was not possible to establish any major correlation among LAB species and their geographical distribution. A similar result was reported by Kongo et al. when identifying dominant lactic acid bacteria from seven dairies located in Azores, a small group of Portuguese islands [15]. The small size of the Honduran territory and the similarity in the agro-ecological characteristics surrounding the cheese processing plants in Honduras could partially explain this phenomenon. However, the most significant reason for this bacterial population similarity could be the use of similar production processes at the manufacturing

Sampling location (province)	No of samples/province	LAB count [lg CFU g-1 ± SD (range)]	Total bacterial count [lg CFU g^{-1} ± SD (range)]
Olancho	10	5.89 ± 1.55 (3.00-8.30)	7.38 ± 1.15 (5.30-9.00)
Atlantida	5	5.87 ± 0.98 (4.78-7.11)	7.52 ± 0.74 (7.00-8.60)
Colon	1	7.30	8.90
Yoro	19	4.50 ± 2.18 (1.00-8.95)	5.89 ± 2.68 (1.00-10.60)
El Paraiso	7	2.95 ± 1.90 (1.00-4.90)	3.76 ± 2.60 (1.00-6.30)
Ocotepeque	4	4.17 ± 0.90 (3.30-5.30)	6.17 ± 1.15 (4.69-7.48)
Vallc	2	4.97 ± 0.71 (4.47-5.47)	6.75 ± 0.21 (6.60-6.90)
Total	48	4.76 ± 1.99	6.18 ± 2.36

Table 1: LAB count and total bacterial count in 48 cheese samples and sampling location in Honduras.

N	LAB	Identification by API	Observed amplification products bp	Identification by DNA sequencing according to BLAST analysis
1	*Aeroccoccus viridans I*	97.20	300	*Lactococcus lactis*
2	*Aeroccoccus viridans I*	56.00	280/320	*Enterococcus faecium*
3	*Aeroccoccus viridans II*	97.80	300	*Lactococcus lactis*
4	*Enterococcus durans*	95.00	320/400	*Enterococcus faecium*
5	*Enterococcus durans*	86.00	300	*Lactococcus lactis*
6	*Enterococcus faecalis*	99.90	300	*Lactococcus lactis*
7	*Enterococcus faecium*	99.80	320/400	*Enterococcus faecium*
8	*Enterococcus faecium*	58.00	320/400	*Enterococcus faecium*
9	*Lactococcus lactis sub cremoris*	86.00	300	*Lactococcus lactis*
10	*Lactococcus lactis sub cremoris*	79.00	300	*Lactococcus lactis*
11	*Lactococcus lactis sub lactis*	88.00	320	-
12	*Lactococcus lactis sub lactis*	57.60	300	*Lactococcus lactis*
13	*Leuconostoc sp*	99.80	300/320	*Lactococcus lactis*
14	*Lactobacillus acidophilus I*	99.80	300	*Lactococcus lactis*
15	*Leuconostoc mesenteroides sub cremoris*	97.00	300	*Lactococcus lactis*
16	*Lactobacillus plantarum I*	97.20	320/400	*Enterococcus faecium*
17	*Lactobacillus plantarum II*	68.00	320/400	*Enterococcus faecium*
18	*Lactobacillus acidophilus I*	90.20	300	-
19	*Enterococcus casseliflavus*	56.00	280/320	-
20	*Streptococcus bovis II*	67.00	320/380	*Enterococcus faecium*

Table 2: Identity of isolated LAB species by conventional methods and by BLAST comparison of the products obtained by PCR of the 16S-23S rRNA spacer (RSA).

LAB species	AT n=5	CO n=1	EP n=7	OC n=4	OL n=10	VA n=2	YO n=19	Number of isolates	%
Lactococcus lactis subsp.lactis	3	1	2	-	4	-	3	13	24.53
Enterococcus faecium	2	-	-	-	2	2	2	8	15.09
Aerococcus viridans	1	1	-	-	4	-	2	8	15.09
Enterococcus faecium	-2	-	2	3	-	-	2	7	13.21
Leuconostoc sp.	-	-	-	-	2	-	-	4	7.55
Lactobacillus acidophilus	1	1	-	-	3	-	-	4	7.55
L.lactis subsp.cremoris	-	-	-	-	2	-	-	3	5.66
Enterococcus durans	-	-	1	-	1	-	-	2	3.77
Streptococcus bovis	-	-	1	-	-	-	-	1	1.89
Enterococcus casseliflavus	-	-	-	-	1	-	-	1	1.89
Streptococcus mitis	-	-	-	-	-	-	1	1	1.89
Lactobacillus plantarum	1	-	-	-	-	-	-	1	1.89

Table 3: Frequency of LAB species isolated from cheese samples (n) and distribution according to geographic origin. (Departments: AT: Atlántida, CO: Colón, EP: El Paraíso, OL: Olancho, VA: Valle, YO: Yoro).

plants. A different scenario arises when analysis is done on the same type of dairy product from geographically distant regions, as it can be shown when predominant LAB species are described for yak milk from very distant Asian regions [9,10,20,38,39].

The probability of species identification with the API systems ranged from 35% to 99.9%. This system classified 66 (64%) isolates with an excellent probability (>90%); 21 (20.3%) isolates with probabilities between 80 and 89.9%; 12 (11.6%) isolates with 60 to 79.9%. The remaining four isolates (3.88%) were poorly identified with <59% probability. To confirm the phenotypic identification results, only 20 of 103 isolates were selected for further DNA sequence analysis because of limited funding. That selection was based on two criteria: (a) species representativeness (belonging to one of the 12 identified species), and (b) an excellent or poor percentage of ID level according to the API approach (Tables 2 and 3). Given the low number of LAB isolates selected for further genotype-based identification by 16S-23S rRNA gene is not possible to make any consideration about the biodiversity of cheeses collected within the geographical area of interest through this approach.

As shown in Figure 2 and Table 2, 11 isolates produced a 300 bp band when the 16S-23S ribosomal spacer region was amplified, while seven isolates produced a 320 bp amplicon. The remaining 2 isolates revealed size bands of 280 bp. Interestingly, a BLAST analysis of the 20 PCR products revealed that nine of them, with a size of 300 bp, corresponded to *Lactococcus lactis* with a similarity of 100%, and six products of 320 bp corresponded to *Enterococcus faecium* with a 99% of similarity. Five isolates could not be identified through this molecular approach. Among those 20 isolates, only the identification of four was consistent among API systems and the DNA sequence. Some reports also describe inconsistencies among phenotypic and genotypic approaches for the identification of some LAB species [7,15,21,22,40], suggesting that carbohydrate fermentation-based assays are not sufficiently reliable and therefore they should be complemented with a molecular technique. On the other hand, other authors indicate that similarities in the ribosomal sequence of lactic acid bacteria are too high, and in consequence some isolates would be misidentified through the exclusive analysis of this genic region [10,41]. The implementation of further molecular technique such as DGGE, PFGE, or pyrosequencing would seem to be a good solution to increase the accuracy of LAB

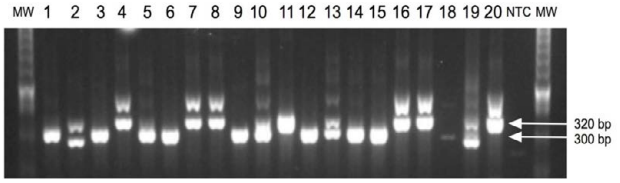

Figure 2: Amplification products of the internal transcribed 16S-23S rRNA spacer of 20 bacterial isolates.

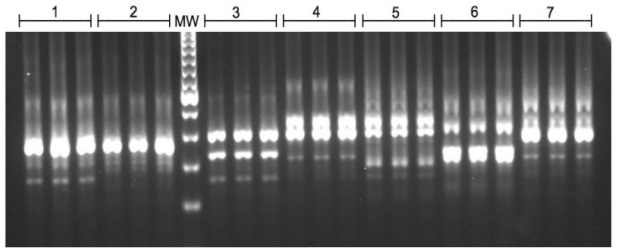

Figure 3: RSA profiles of seven cheese samples run by triplicate.

species identification [10,14,30]. In any case, both approaches agree that the two most frequently LAB species present in the samples are *Lactococcus lactis* and *Enterococcus faecium*.

Yeast counts were determined for only 24 (50%) samples because of methodological limitations. Eleven of these samples revealed colonies with morphological yeast appearance. Yeast viable counts ranged from 3.48 to 8.00 lg CFU g^{-1}, with a mean value of 5.14 ± 1.26. As for LAB count, yeast count seems to fall within the range reported for other artisanal cheeses [12,13,34]. Twelve representative colonies were selected based on macroscopic appearance for further identification by a phenotypic approach. Four of the isolates were identified as genus *Rhodotorula* (3 *R. rubra* and 1 *R. glutinis*), 2 were identified as *Cryptococcus albidus*, 2 as *Candida stellatoidea*, 2 as *Trichosporon beigelii*, and 1 as *Kluyveromyces* sp. *Kluyveromyces* and *Candida* species have been reported as dominant in similar cases [30,42,43]. These genera have shown metabolic activities that contribute to the development of aroma and flavour of cheeses [44,45], and in some cases they have been described as contaminants of the environment, particularly *Rhodotorula* species [46].

Molecular analysis of bacterial communities

The diversity and relative abundance of bacterial communities were analysed for 48 cheese samples through an RSA approach. The selected primers (G1 and L1) have targets on ribosomal conserved regions in order to extend the possibility of amplifying the greatest number of bacterial groups present in the cheese samples. All samples amplified with very high reproducibility when 3 replicates were run simultaneously (Figure 3). The total number of scored bands (TNB) and the number of polymorphic bands (NPB) was 11; there was only one exclusive band (NEB) with a molecular weight of 480 bp, while the number of community profiles (NCP) was 15. The maximum number of bands (n=3) was present in 12 (25%) samples. Twelve samples showed the presence of 2 bands (25%), while most of samples (n=22; 45.83%) showed one amplification band. The minimum number of bands was 0 in the remaining 2 samples (4.17%).

The molecular weights of the 11 amplicons ranged from 250 bp to 500 bp. If each band with a particular size represents an operational taxonomic unit (OTU), the total number of OTU´s would be consistent with the total number of isolated LAB species (n=11). However, is well known that the RSA method is able to amplify bacterial taxa other than LAB, therefore some of those bands would represent species commonly found in artisanal cheeses, such as coliforms and staphylococci [12]. Implementing more accurate molecular techniques, such as DGGE, PFGE or next generation sequencing, could be a useful approach to describe the bacterial diversity within the artisanal cheeses of Honduras in the future [30,47].

A binary matrix was used for building a dendrogram (Figure 4) that shows a similarity analysis among bacterial communities within the cheese samples. No clear clustering pattern was observed among samples, except for 22 of them clustering together and characterized by a unique amplification band migrating approximately at 380 bp. Despite the lack of clustering, there were 8 samples that formed 3 clusters, each with the same band profile: Cluster I, composed by 4 samples showing 3 migrating bands at 280, 320 and 380 bp; cluster II with 2 samples and 2 bands, at 250 and 380 bp; and the third cluster also with 2 samples, and 2 bands at 280 and 380 bp. The three most common bands were those migrating at 380 bp, 280 bp, and 320 bp, present in 38 (79.2%), 10 (20.8%), and 8 (16.7%) of the samples respectively. The dendrogram pattern was compared with the geographical origin of the samples but no clear correlation was observed. According to these findings, it is not possible to establish a unique or characteristic molecular profile of bands based on samples' provenance, which is consistent with the results found through the API-based systems. As also shown in Figure 4, the number of cultured LAB isolates and the number of amplification

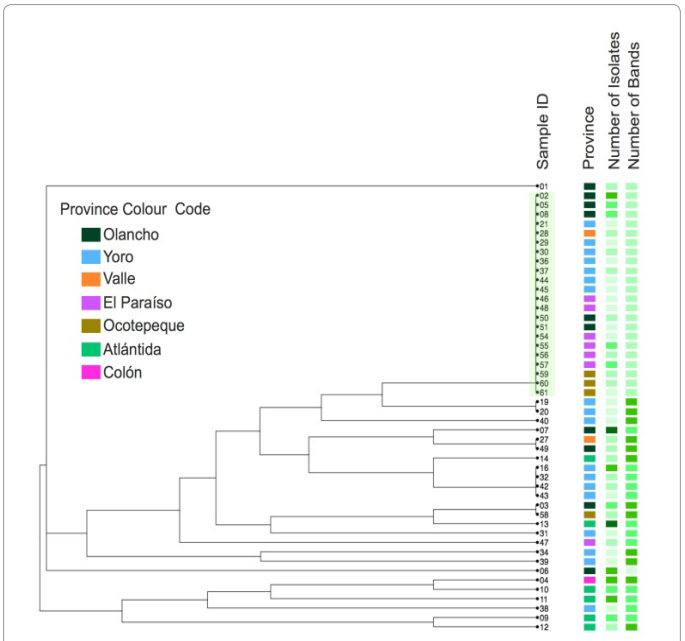

Figure 4: Dendrogram of cluster analysis generated by means of the UPGMA method. The diagram illustrates the similarity among bacterial communities within 48 cheese samples, based on the band profile (molecular weight and number of bands) of each sample. The band profile was obtained after amplification of the 16S-23S ribosomal spacer region (RSA). The column at the left side of the figure represents the Department of origin for each sample, while the second and third columns at the right indicate the number of LAB species isolated from each sample, and the number of amplification bands in the agarose gel, respectively. The colours of the boxes in the bars at the right denote the number of individuals. The darker the green colour in the box the higher the number of bacterial species and the number of bands respectively.

bands obtained from each cheese sample reveal low consistency. This could mean that the culturing process was not able to recover the entire diversity of LAB species in the cheese samples, or that many of the amplification bands observed in the gels belong to other bacterial taxa.

Conclusion

In conclusion, this study aimed to count, isolate and to identify some of the LAB species in artisanal Honduran semi-dry cheese. Among the identified isolates, the more common species were *Lactococcus lactis* and *Enterococcus faecium*. To our knowledge, this is the first report of the microbial diversity responsible for the organoleptic characteristics of artisanal cheeses in Honduras. A potential use of these isolates as starters on pasteurized milk would be desirable to improve the product quality as well as consumer safety.

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