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Microbial biogeography of Spanish-style green olive fermentations in the province of Seville, Spain

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ABSTRACT

The aim of this study is to examine the biogeography of the microbial communities associated to the Spanish-style green olive fermentations in the province of Seville (Andalucía, south-western Spain). Also, to understand how microorganisms colonize and persist in non-sterile food fermentations across a specific table olive producing area, i.e. a specific "agroecosystem". The microbial diversity, bacteria and yeast, in 30 ten-ton fermenters of three different fermentations yards (*patios*) along the olive fermentation was studied. A total of 951 microbial isolates were obtained which were clustered according to their RAPD profile. A total of 376 distinct genotypes were identified, belonging to 57 different microbial species, 41 bacterial and 16 yeast species. Up to 16 bacterial species had not been described before in table olives. Only the species *Lactobacillus pentosus* showed a ubiquitous presence in all 30 fermenters. *Pediococcus parvulus, Lactobacillus collinoides/paracollinoides, Lactobacillus coryniformis, Lactobacillus plantarum, Pichia manshurica* and *Candida thaimueangensis* were found in every *patio*. Cosmopolitan strains, up to 15, were shared by the three *patios* and belonged to the species *L. pentosus* (12 strains), *P. parvulus* (1), *L. collinoides/paracollinoides* (1) and *P. manshurica* (1). To expand our biodiversity analyses to the "regional" level, we have compared our results with those obtained from two previously studied *patios* of similar characteristics and in the same geographical area. PERMANOVA analysis of the microbial community composition revealed significant differences among different *patios* in their structure at every fermentation stage. In contrast, SIMPER analyses showed that, as fermentation progressed, the overall dissimilarities among *patios* were reduced. Discriminant species were identified for each fermentation stage. Among these, *L. pentosus* and *P. parvulus* were "eu-constant" species, while *L. collinides/paracollinoides* and *Marinilactibacillus psychrotolerans* group were "constant" species that could be considered microbial key taxa based on the occurrence stability index. The characteristic and, presumably, well adapted microbiota associated to the Spanish-style olive fermentations at the specific geographic area described here is a valuable natural resource which should be preserved conveniently. To our knowledge, this is the first study on the microbial biogeography of table olive fermentations, both at the species and strain levels.

1. Introduction

Fermentation of table olives has been traditionally present in the Mediterranean countries for centuries as a way to make these fruits edible as well as to preserve them for a long period of time. In Spain, the main world producer of table olives, Spanish-style is the most popular preparation. This preparation is actually one of the three most commercially important worldwide, representing up to 60% of the world table olive production (Botta and Cocolin, 2012). The procedure is defined by an initial alkali treatment (1.8–3.5% [w/v] NaOH) of the green fruits followed by a washing step and covering of the treated fruits with brine (10–12% NaCl) (Rejano et al., 2010). Such a processing is aimed at neutralizing inhibitory phenolic compounds while removing bitterness and allowing subsequent natural lactic acid fermentation in the cover brine.

This lactic acid fermentation normally takes place spontaneously, leaded by the indigenous microbiota from the raw materials (olives, salt, water) or that acquired during the processing at the factory's facilities (fermenters, tanks, pipelines, pumps) and fermenters yards (*patios*) (De Castro et al., 2002; Garrido-Fernández et al., 1997; Lanza, 2013; Lucena-Padrós et al., 2014a). Being open to that many variable sources of contaminant microorganisms from the environment, this is a

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not-fully-predictable, microbiologically complex fermentation. However, it is more than probable that at least part of the microbial diversity associated to this fermentation at a specific *patio,* or even defined in a specific geographical region or ecosystem, has been "domesticated" by the continuous replicate of peculiar processing conditions and know-how. This might represent a significant contribution to "unique" qualities or even "terroir" aspects of this product as it has been shown for other fermented foods like wine (Tofalo et al., 2014) or cheese (Capozzi and Spano, 2011).

Although biogeography of plants and animals has been studied for a long time, the biogeography of microbial communities has only been addressed recently (Martiny et al., 2006). The aim of this study is to examine the biogeography and biodiversity of the microbial communities associated to Spanish-style green olive fermentations in the province of Seville (Andalucía, south-west Spain). This agroecosystem concentrates up to 62% of the national table olive production (AICA, 2017) so that, actually, the Spanish-style preparation is more known in Spain as the "Sevillian" style. Besides, we chose the Manzanilla variety as it has been traditionally considered the most popular olive variety used for the Spanish-style preparation (Garrido-Fernández et al., 1997). In fact, the growing area of this olive tree variety is centered in the province of Seville (ca. 60%) and there is currently a debate on its admittance as a new Protected Geographical Indication (PGI) in Europe. The results of this study show the diversity of the microbial communities at a specific olive tree growing region, where climatic and geographical factors are expected to be similar. These factors are undoubtedly influencing fitness and competitiveness of microbial taxa and therefore most probably selecting a common indigenous microbiota in the fermentation yards of this geographical area. For this work, we selected three table olive manufacturing companies located in the province of Seville. In the fermenters of these companies we studied the microbial diversity along Spanish-style green olive fermentations by culture dependent techniques. In addition, to expand our biodiversity analyses to the "regional" or "ecosystem" level, we have compared our results with those obtained from two previously studied yards of similar characteristics and in the same geographical area (Lucena-Padrós et al., 2014a and 2014b; Lucena-Padrós and Ruiz-Barba, 2016). Therefore, a total of 50 ten-ton fermenters at five different locations of the same region were considered at the time of extracting conclusions on how relevant microorganisms are distributed and have an impact on green olive fermentations into a defined producing area. In summary, the objective of this study is to understand how microorganisms colonize and persist in non-sterile food fermentations, such as table olive fermentations, across a specific producing area. In particular, we want to know if there are microbial key taxa associated to the whole producing area or if they are specific of a particular *patio* or even of a particular fermentation stage.

2. Materials and methods

2.1. Origin of the samples and sampling strategy

Samples of Spanish-style green-olive fermenting brines were taken during the 2012–2013 season from the fermenters located at the *patios* of three table-olive producing companies in the province of Seville. The selected *patios* have small-medium dimensions with different processing capabilities: *patio* A, with 171 fermenters, located in the town of Almensilla; *patio* B, with 96 fermenters, in the town of Pilas; and *patio* C, with 49 fermenters, also in Pilas. They are able to handle 2500, 1000 and 500 tonnes of olives per season, respectively. At each *patio*, fermentation was followed in ten fermenters of a capacity of 10 tonnes of olives and 5500–6000 L of brine each, made in polyester and glass fibre. All of them were located outdoor, buried in the ground of their respective *patio.* With few modifications, the traditional Spanish-style (or Sevillian) procedure to prepare green olives was followed (Rejano et al., 2010). Briefly, green olives were submitted to a storage step of 24 h in water previous to an alkali treatment with a solution of NaOH (2–3% [w/v]) with the addition of NaCl (10 g/L) for 6–7 h. The treated olives were then washed with water to remove the excess of alkali, covered with brine (10–11% [w/v] NaCl) and used to fill the fermenters up. Brines were then acidified by the addition of 5 L food-grade HCl plus 5 L food-grade lactic acid in *patio* A, and with 15 L of food-grade HCl in *patios* B and C, although in patio C acidification took place after the first week of fermentation. The Manzanilla olive variety was used in all cases and no starter culture was applied. Fermentations were set up during October 2012 and monitored fermenters had similar fermenting starting dates. Fifty-ml samples were taken from each of the ten fermenters at each of the three *patios* studied, in coincidence with the middle and final stages of green-olive fermentations. Thus, a total of 60 samples were taken in this way. In addition, the ten fermenters at *patio* A were also sampled at the initial fermentation stage. In summary, a total of 70 samples were taken across the season. Regarding timing, brine samples were collected in a range of time after brining of 6–8 (1st week), 23–42 (3rd- 6th week), and 92–177 (13th - 25th week) fermentation days, for the initial, middle and final stage sampling points, respectively. Samples were added glycerol (20% [v/v]) and stored at $-80\,^{\circ}C$ until analysis.

2.2. Isolation and enumeration of microorganisms

Stored samples were defrosted at room temperature, serially diluted in peptone water (0.1% w/v) and spread in duplicates onto agar plates of a battery of six different culture media as follows: i) Brain Heart Infusion (BHI; Biokar Diagnostics, Beauvais, France) supplemented with 0.05% L-cysteine (AppliChem, Darmstadt, Germany); ii) de Man-Rogosa-Sharpe (MRS; Biokar Diagnostics) supplemented with 0.02 g/L bromophenol blue (AppliChem) and L-cysteine (MRS-BPB; Lee and Lee, 2008); iii) Reinforced Clostridial Medium (RCM; Biokar Diagnostics); iv) Violet Red Bile with Glucose Agar (VRBG; CONDA, Madrid, Spain); v) GYECN medium based on GYEC medium (Ntougias and Russell, 2001) composed of 1% (w/v) glucose (Sigma), 0.5% (w/v) yeast extract (Oxoid) 0.1% (w/v) L-cysteine (AppliChem, Darmstadt, Germany) and 100 mM $NaHCO_3$/1 mM K_2HPO_4 buffer (pH 9), containing 0.1% (w/v) NH_4SO_4 plus 0.1 mM $MgSO_4{*}7H_2O$ and supplemented with 2.5% NaCl (w/v); and vi) Glucose -Yeast Extract Agar supplemented with oxytetracycline (0.1 g/L) (OGYE; Mossel et al., 1962). BHI, MRS-BPB, GYECN and RCM plates were incubated under anaerobic conditions at 30 °C for three days, except for RCM, where seven-day incubations were used. For anaerobic incubations a DG250 Anaerobic Workstation (Don Whitley Scientific Ltd., Shipley, West Yorkshire, UK) was used, with a gas mixture consisting of 10% H_2–10% CO_2-80% N_2. OGYE plates were incubated aerobically at 30 °C for 2 days, while VRBG ones were incubated at 37 °C for 24 h. Agar was added to the respective broth medium at 1.5% (w/v). Prior to spreading onto RCM agar plates, samples were pasteurized at 75 °C for 15 min in a water bath. GYECN and VRBG media were used only with samples taken at the initial fermentation stage. For further studies, a single colony of each different morphotype detected in each culture medium and at every sampling point was selected from plates with low counts. They were further purified by repeated subculturing and examined under a phase-contrast microscope (Olympus Optical Co., Tokyo, Japan) to distinguish their cell morphology. Purified isolates were preserved frozen at $-80\,^{\circ}C$ in their culture medium containing glycerol (20% v/v). All isolates were subjected to genotyping as described below.

2.3. Molecular identification techniques

Total DNA used for further molecular identification was extracted from isolated colonies by the chloroform method described by Ruiz-Barba et al. (2005). Genotyping of the microbial isolates was carried out by Randomly Amplified Polymorphic DNA (RAPD) as previously described in Lucena-Padrós et al. (2014b), using the primer OPL5 (

5′-ACGCAGGCAC-3′). Bacterial isolates were identified to the genus and/or species level by PCR amplification and subsequent sequencing of the 16S *rDNA* gene as previously described in Lucena-Padrós et al. (2014a). Species belonging to the *Lactobacillus plantarum* group were distinguished using a multiplex PCR assay as described by Torriani et al. (2001). Yeast isolates were identified to the genus and/or species level by PCR amplification and further sequencing of the D1/D2 domain of the 26S *rDNA* gene as previously described in Lucena-Padrós et al. (2014a).

2.4. Physico-chemical analyses

Titratable acidity (g/100 mL lactic acid), combined acidity (Eq/100 mL NaOH) and pH were measured using a Metrohm 670 Titroprocessor (Herisau, Switzerland). Salt concentration was determined by titration with $AgNO_3$ and expressed as % (w/v) NaCl.

2.5. Statistical analyses of microbial counts

Counts of microorganisms, based on duplicates, were expressed as the mean values of colony forming units (CFU) per millilitre of brine and transformed to logarithmic values before statistical analyses were performed. Kruskal Wallis tests were applied to determine statistically significant differences between the microbial counts among *patios* at each fermentation stage and for every relevant culture media. The fermentation-time effect on averaged microbial counts recovered from each culture media in *patio* A was tested using Friedman test, while to compare population densities at middle and final fermentation stages in the three *patios*, Wilcoxon's signed-ranks test for two groups was applied. These analyses were performed using the SPSS 23.0 statistical software (SPSS Inc., Chicago, USA).

2.6. Biodiversity analyses

The results of culture-based isolation and subsequent molecular identification of microorganisms obtained in this study were transformed into the ordinal matrix Xc (Mendeley dataset in Lucena-Padrós and Ruiz-Barba, 2019). This matrix was built considering the maximum counts ($\log_{10}$ cfu/ml) reached by each isolated taxon in each brine sample analyzed along the olive fermentations in coincidence with the initial, middle and final fermentation stages. For comparison purposes and to get a better picture at the regional level, the results of two previously obtained sets of data of homologous characteristics were normalized and included in this data matrix (i.e. data for *patios* 1 and 2 described in Lucena-Padrós et al., 2014a, and Lucena-Padrós and Ruiz-Barba, 2016).

2.6.1. Genetic diversity at the local level

Biodiversity of the overall microbial load for each fermenter was evaluated using Margalef's index of genotypes richness (R), ShannonWeaver's index of diversity (H′) and Simpson's index of dominance (D), calculated as described in Ventorino et al. (2007). To compare mean values of biodiversity indexes of *patios*, Kruskal Wallis test was used for each fermentation stage, while to compare differences between middle and final fermentation stages, Wilcoxon's signed-ranks test for two groups was applied for each *patio*. In *patio* A, the fermentation-time effect was tested using the Friedman test. A probability value of $p < 0.05$ was considered statistically significant. These analyses were performed using the SPSS 23.0 statistical software (SPSS Inc., Chicago, USA).

2.6.2. Additive partitioning of species diversity at local and regional levels

The additive partitioning approach with a hierarchical design (Crist and Veech, 2006; Zhang et al., 2014) was used to calculate total species diversity at two different spatial scales: the region (province of Seville) and its local sites (*patios*), since in this way α and β diversity indexes could be expressed in the same units. This study was carried out considering the results from the five table olive fermentation *patios* included in matrix Xc (see above). The analysis was done for each fermentation stage.

We define local diversity (γ_1) as the total species richness observed at each surveyed *patio*, while regional diversity (γ_2) is the total species richness taking into account the five *patios* studied here all together. At each of these levels, γ diversity index can be calculated as the sum of α and β diversity indexes. This is,

$$\gamma = \alpha + \beta$$

Where α diversity index, also called "community diversity", is defined as the mean of the species richness (number of different species) in the total surveyed fermenters at each sampled area (region or *patio*) and can be calculated as follows:

$$\alpha = \frac{1}{n}\sum_{i=1}^{n} S_i$$

where n represents the number of fermenters in the considered area and S_i represents the species richness in each fermenter. In turn, β diversity is defined as the variability in species composition among sampling units for a specific area (region or *patio*) and can be calculated as follows:

$$\beta = \gamma - \alpha$$

In addition, β diversity can be decomposed into two components: β_N, which quantifies the degree of nestedness of species richness, i.e. the degree to which species richness differs between fermenters within a specific region or *patio* from the most species-rich fermenter; and β_R, reflecting the difference in species composition among the fermenters. These components can be calculated as follows:

$$\beta_N = \frac{1}{n}\sum_{i=1}^{n} (S_{max} - S_i)$$

$$\beta_R = \beta - \beta_N$$

where $S_{\max}$ represents the species number in the richest fermenter.

Finally, the species diversity for the five *patios,* for each fermentation stage, was averaged to represent the species diversity at the local level.

2.6.3. Analyses of microbial community composition at local and regional levels

Microbial community composition was analyzed using a three-dimensional nonmetric multidimensional scaling (NMDS) with Bray-Curtis similarity matrix calculated from the matrix Xc (see above) to visualize dissimilarity between brine samples. Stress < 0.2 (Kruskal's stress formula #1) was used as an acceptable threshold (Clarke, 1993). Differences in taxa composition among *patios* and at each fermentation stage were evaluated separately using a one-way permutational multivariate analysis of variance (PERMANOVA). The p values were corrected for multiple comparisons using Bonferroni correction. Values of $p < 0.05$ were considered statistically significant. Moreover, similarity percentage (SIMPER) analyses (Clarke, 1993) were used to identify taxa that were responsible for the dissimilarities between groups identified using the NMDS and PERMANOVA analyses at each fermentation stage. Finally, the occurrence stability index of each isolated species was calculated as the number of fermenters containing that species divided by the total number of sampled fermenters at each fermentation stage, and was evaluated following the criteria of classification of Skorupski et al. (2009), i.e. euconstants (≥75% samples), constants (50–74% samples), accessory (25–49% samples), and accidents (≤24% samples). All calculations were performed using the PAST v.3 software (Hammer et al., 2001).

3. Results

Original data of this study, including frequency of isolation and counts of bacterial and yeast taxa, are available at Mendeley Data (see dataset at Lucena-Padrós and Ruiz-Barba, 2019).

3.1. Physico-chemical parameters

NaCl concentration in the brines at equilibrium showed values of 5.69 (± 0.21), 6.94 (± 0.08) and 5.70 (± 0.28) % (w/v) in the fermenters at *patios* A, B and C, respectively. The brines from *patio* A showed a mean pH value of 5.45 (± 0.42) at the initial fermentation stage, and evolved to 4.50 (± 0.00) and 4.11 (± 0.05) at the middle and final fermentation stages, respectively. In *patio* B, mean pH values were 4.50 (± 0.50) and 3.97 (± 0.08) at the middle and final fermentation stages, respectively, while in *patio* C, the corresponding values were 4.70 (± 0.46) and 4.20 (± 0.07). In average, titratable acidity at the final stage was 0.91 (± 0.07), 1.02 (± 0.10) and 0.79 (± 0.07) g/100 mL in the fermenters at *patios* A, B and C, respectively. Finally, averaged combined acidity was 0.12 (± 0.00), 0.10 (± 0.01) and 0.14 (± 0.01) Eq/100 mL for *patios* A, B and C, respectively. The values of all these parameters were considered normal for this table olive preparation.

3.2. Microbiological analyses

Averages of total counts of microorganisms isolated in the culture media used in this study are shown in Table 1. Significant differences could be found among *patios* in most culture media, except for OGYE and RCM media at the middle and final fermentation stages, respectively. On the other hand, statistical significance of time effect in the fermentation within each *patio* was never found for OGYE counts (mostly yeast). However, the dynamics of bacterial populations along the time was observed in MRS-BPB, BHI and RCM media. In these cases, the highest counts were obtained at the middle stage and decreased at the final stage. Finally, growth of halophilic/alkaliphilic bacteria was detected in GYECN in all brine samples of *patio* A at the initial stage. No presence of *Enterobacteriaceae* was detected in VRBG, although microbial growth was observed in 3 of the samples in this medium corresponding, in all cases, to isolates of *Vibrio furnissii*.

3.3. Identification of the isolated microorganisms

Bacterial and yeast species isolated in this study, arranged according to their abundance, are shown in Tables 2 and 3, respectively. A total of 951 microbial isolates were obtained which were clustered according to their RAPD profile with primer OPL5. Clustering was carried out separately for bacilli belonging to the *L. plantarum* group, bacilli not belonging to the *L. plantarum* group, cocci and yeast (Figs. S1–S4, respectively). A total of 376 distinct genotypes were identified, belonging to 57 different microbial species, 41 bacterial and 16 yeast species (Tables 2 and 3). Only the species *Lactobacillus pentosus* showed a ubiquitous presence in all 30 fermenters of this study, being isolated from almost every sample (Table 2). Six other species could be found in every *patio*, detected at similar fermentation stages: *Pediococcus parvulus, Lactobacillus collinoides/paracollinoides, Lactobacillus coryniformis, Lactobacillus plantarum, Pichia manshurica* and *Candida thaimueangensis*. Therefore, it is not surprising that 221 strains (58.8% of the total) belonged to these seven species; although only 15 strains were shared by the three *patios* according to their RAPD profile. These "cosmopolitan" strains belonged to the species *L. pentosus* (12 strains), *P. parvulus* (1), *L. collinoides/paracollinoides* (1) and *P. manshurica* (1). As expected, the maximum number of different species detected, that is 31, was found at the initial stage of fermentation (*patio* A), and 20 of them were isolated only at this fermentation stage. Conversely, among the species detected at the initial fermentation stage, only *L. pentosus, L. coryniformis* group*, Sporolactobacillus inulinus* group*, Virgibacillus proomii* and *Rhodotorula mucilaginosa/alborubescens* were able to persist in the brines till the end of the fermentation. Finally, it is remarkable that, to our knowledge, up to sixteen bacterial species had not been described before in table olives (see Table 2), and the yeast species *Lachancea thermotolerans* has been isolated for the first time from Spanish-style green olive fermentations (Table 3). Eleven of these species could only be detected in one or two of the analyzed samples.

3.4. Analysis of the microbial genetic diversity

As deduced from the analysis of the RAPD profiles (Figs. S1–S4), the

Table 1
Averaged microbial counts along Spanish-style green-olive fermentations in three fermentation yards (*patios*) obtained in the culture media used in this study.

Culture medium	Fermentation stage	patio A	patio B	patio C	*p*-value[a]
GYECm	Initial	6.27 (0.73)[b]	-[c]	–	–
VRBG	Initial	0.24 (0.37)	–	–	–
MRS-BPB	Initial	7.04 (0.95)	–	–	–
	Middle	7.43 (0.34)	7.03 (0.75)	7.86 (0.19)	0.005
	Final	6.89 (0.25)	6.04 (0.40)	6.67 (0.47)	0.001
Sig.[d]			*	*	
BHI	Initial	6.94 (0.90)	–	–	–
	Middle	7.29 (0.33)	6.80 (0.54)	7.88 (0.23)	0.000
	Final	6.40 (0.19)	5.90 (0.41)	5.87 (0.55)	0.029
Sig.		*	*	*	
OGYE	Initial	2.77 (0.43)	–	–	–
	Middle	2.82 (0.59)	3.26 (0.49)	3.14 (0.30)	NS[e]
	Final	2.50 (0.46)	3.14 (0.50)	2.97 (1.08)	0.022
Sig.					
RCM	Initial	1.75 (0.89)	–	–	–
	Middle	2.86 (0.68)	0.84 (0.56)	0.55 (0.58)	0.000
	Final	0.72 (0.66)	0.07 (0.21)	0.44 (0.70)	NS
Sig.		*	*		

[a] Statistical significance considering the three *patios* at each fermentation stage (Kruskal Wallis test; for $p \leq 0.05$).

[b] Mean log CFU/ml (standard deviation), n = 10.

[c] -, no data available.

[d] Sig.: statistical significance of time effect in the fermentation within each *patio* (Friedman test in *patio* A and Wilcoxon's signed-ranks test in *patios* B and C; * for $p < 0,05$).

[e] NS, not significant difference.

Table 2

Bacterial species isolated along Spanish-style green olive fermentations from brine samples at different fermentation stages (initial, middle and final) from three different fermentation yards (*patios* A, B and C) located in the province of Seville, southwestern Spain.

Bacterial species	Fermentation stage										
	Initial	Middle			Final			Total	Total	No.	Count range
	A	A	B	C	A	B	C	isolates[a]	strains[b]	ferm[c]	(log CFU/ml)[d]
Lactobacillus pentosus	38(6)[e]	49(10)	67(10)	62(10)	49(10)	51(10)	82(10)	398	138	30	1–7
Pediococcus parvulus	0	8(4)	0	0	19(8)	22(10)	11(8)	60	27	26	2–6
Lactobacillus collinoides/paracollinoides	0	12(8)	8(5)	4(2)	21(10)	17(9)	0	62	25	21	2–6
Sporolactobacillus inulinus group [f]	5(3)	35(10)	4(3)	0	7(5)	0	0	51	16	13	1
Lactobacillus coryniformis group [g]	2(1)	6(3)	2(2)	2(2)	0	2(1)	7(4)	22	8	12	3–6
Lactobacillus plantarum	12(8)	0	1(1)	1(1)	0	0	0	14	6	10	2–6
Leuconostoc mesenteroides group [h]	23(10)	0	0	0	0	0	0	23	9	10	1–7
Enterococcus casseliflavus	24(9)	0	0	0	0	0	0	24	16	9	2–4
Lactobacillus parafarraginis	0	2(2)	0	0	7(6)	1(1)	0	10	6	8	4–6
Lactobacillus rhamnosus	0	15(7)	0	0	2(2)	0	0	17	5	8	4–6
Marinilactibacillus psychrotolerans [i]	21(7)	0	0	0	0	0	0	21	6	7	2–5
Vibrio caribbeanicus [j]	15(7)	0	0	0	0	0	0	15	3	7	1–4
Lactobacillus rapi	0	1(1)	0	0	0	6(5)	0	7	5	6	3–5
Virgibacillus proomii [j]	8(5)	0	0	0	1(1)	0	1(1)	10	3	6	1
Staphylococcus epidermidis group [k]	4(3)	0	0	2(2)	0	0	2(1)	8	6	5	1–4
Pediococcus ethanolidurans	0	0	0	0	0	0	4(4)	4	2	4	4–5
Weissella paramesenteroides	9(4)	0	0	0	0	0	0	9	5	4	4–6
Bacillus niacini/drentensis [j]	0	0	3(3)	0	0	0	0	3	3	3	1
Vibrio furnissii [l]	3(3)	0	0	0	0	0	0	3	1	3	1
Staphylococcus saprophyticus group [j] [m]	0	0	2(2)	1(1)	0	0	0	3	3	3	1
Staphylococcus warneri group [j] [n]	4(1)	0	0	2(2)	1(1)	0	3(2)	10	7	6	1–5
Bacillus cereus group [o]	1(1)	0	0	1(1)	0	0	0	2	2	2	1
Lactobacillus acidipiscis	0	0	3(2)	0	0	0	0	3	2	2	5–6
Paenibacillus barengoltzii [j]	1(1)	0	0	0	0	0	0	1	1	1	1
Paenibacillus vini [j]	0	0	0	1(1)	0	0	0	1	1	1	1
Bacillus aerius [p]	0	1(1)	0	0	0	0	0	1	1	1	6
Bacillus circulans	1(1)	0	0	0	0	0	0	1	1	1	1
Bacillus nealsonii [j]	0	0	1(1)	0	0	0	0	1	1	1	1
Bacillus subtilis group [q]	0	1(1)	1(1)	0	0	0	0	2	2	2	1
Clostridium botulinum group [r] [s]	2(1)	0	0	0	0	0	0	2	1	1	1
Clostridium jejuense [r]	2(1)	0	0	0	0	0	0	2	2	1	1
Corynebacterium simulans [j]	1(1)	0	0	0	0	0	0	1	1	1	1
Enterococcus faecalis	0	0	0	0	0	1(1)	0	1	1	1	1–2
Lactococcus formosensis/garviae [j]	1(1)	0	0	0	0	0	0	1	1	1	1
Paenibacillus lautus [j] [r]	0	0	1(1)	0	0	0	0	1	1	1	1
Paenibacillus sp. [j] [t]	0	0	1(1)	0	0	0	0	1	1	1	1
Paenibacillus xylanilyticus [r]	1(1)	0	0	0	0	0	0	1	1	1	1
Pantoea eucrina [j]	0	0	0	0	0	1(1)	0	1	1	1	2
Staphylococcus lugdunensis [j]	1(1)	0	0	0	0	0	0	1	1	1	1
Staphylococcus simulans [j]	1(1)	0	0	0	0	0	0	1	1	1	1
Vibrio variabilis group [j] [u]	1(1)	0	0	0	0	0	0	1	1	1	2

[a] Total isolates of a specific bacterial species.

[b] Total strains of a specific bacterial species as derived from clustering of RAPD profiles.

[c] Number of fermenters, out of a total of 30, from which a specific bacterial species was isolated.

[d] Colony count range at which a specific bacterial species was isolated, expressed as logarithms.

[e] Total isolates at that fermentation stage; in brackets, number of fermenters (out of 10) from which that species was isolated.

[f] Includes the species *Sporolactobacillus inulinus* and *Sporolactobacillus terrae.*

[g] Includes the species *Lactobacillus coryniformis* subsp. *coryniformis* and *L. coryniformis* subsp. *torquens*.

[h] Includes the subspecies *mesenteroides, dextranicum, cremoris* and *suionicum*.

[i] Includes the species *Marinilactibacillus psychrotolerans* and *Marinilactibacillus piezotolerans*.

[j] Bacterial species which have not been previously reported from table olive fermentations.

[k] Includes the species *Staphylococcus epidermidis, S. saccharoliticus, S. capitis* and *S. caprae*.

[l] Bacteria species showing maximum similarity (99,67%). These strains were subjected to (almost) complete sequencing of their 16S rRNA.

[m] Includes the species *Staphylococcus saprophyticus* and *Staphylococcus xylosus*.

[n] Includes the species *Staphylococcus warneri* and *Staphylococcus pasteuri*.

[o] Includes *B. anthracis, B. cereus, B. thuringiensis, B. mycoides, B. pseudomycoides,* and *B. cytotoxicus*, among others.

[p] Bacteria species showing maximum similarity (100%). This strain was subjected to (almost) complete sequencing of their 16S rRNA.

[q] Includes the species *B. subtilis, B. mojavensis, B. halotolerans, B. tequilensis, B. methylotrophicus, B. amyloliquefaciens, B. aerophilus, B. altitudinis, B. atrophaeus, B. axarquiensis, B. licheniformis, B. malacitensis, B. pumilus, B. safensis, and B. sonorensis, among others.*

[r] The relatively low (< 98.7%) 16S rDNA homology of these isolates with other bacterial species suggests that they might be novel species.

[s] Includes the species *Clostridium botulinum, Eubacterium combesii* and *Clostridium sporogenes*.

[t] Maximum 16S rDNA homology to the species *Paenibacillus peoriae, P. polymyxa, P. brasilensis* and *P. jamilae*.

[u] Includes the species *Vibrio variabilis* and *Vibrio maritimus*.

Table 3
Yeast species isolated along Spanish-style green olive fermentations from brine samples at different fermentation stages (initial, middle and final) from three different fermentation yards (*patios* A, B and C) located in the province of Seville, southwestern Spain.

Yeast species	Fermentation stage										
	Initial	Middle			Final			Total isolates[a]	Total strains[b]	Total ferm.[c]	Count range (log CFU/ml)[d]
Patio	A	A	B	C	A	B	C				
Pichia manshurica	0	6(5)[e]	8(8)	6(6)	2(2)	4(4)	6(4)	32	5	21	[1–2]
Candida thaimueangensis	0	9(7)	4(4)	1(1)	10(10)	0	0	24	12	15	1
Pichia kudriavzevii[f]	0	2(2)	0	16(10)	1(1)	0	4(3)	23	4	13	[1–2]
Candida aaseri/pseudoaaseri	10(10)	0	0	0	0	0	0	10	2	10	2
Saccharomyces cerevisiae	0	0	11(9)	0	0	0	0	11	4	9	[1–2]
Kluyveromyces lactis/marxianus	0	0	0	6(6)	0	0	0	6	3	6	[1–2]
Torulaspora delbrueckii	0	6(6)	0	0	0	0	0	6	1	6	[1–2]
Candida tropicalis	4(4)	0	0	0	0	0	0	4	3	4	[1–2]
Wickerhamomyces sp.[g]	2(2)	3(3)	0	0	0	0	0	5	3	4	1
Rhodotorula mucilaginosa/alborubescens	3(3)	0	0	0	1(1)	0	0	4	2	4	1
Candida diddensiae/dendronema	1(1)	0	0	2(2)	0	0	0	3	2	3	[1–2]
Hanseniaspora sp.[h]	3(3)	0	0	0	0	0	0	3	2	3	2
Lachancea thermotolerans[ij]	0	2(2)	0	0	0	0	0	2	1	2	1
Schwanniomyces etchellsii[k]	0	0	0	0	0	0	2(2)	2	1	2	[1–2]
Meyerozyma guilliermondii[l]	1(1)	0	0	0	0	0	0	1	1	1	1
Pichia membranifaciens	0	0	0	0	0	10(10)	5(4)	15	6	–14	[1-2]

[a] Total isolates of a specific yeast species.

[b] Total strains of a specific yeast species as derived from clustering of RAPD profiles.

[c] Number of fermenters, out of a total of 30, from which a specific yeast species was isolated.

[d] Colony count range at which that yeast species was isolated, expressed as logarithms.

[e] Total isolates of a specific yeast species at that fermentation stage; in brackets, number of fermenters, out of a total of ten, from which that yeast species was isolated in each *patio* at each fermentation stage.

[f] Formerly *Issatchenkia orientalis*.

[g] The most homologous species were *Wickerhamomyces anomalus (formerly Pichia anomala), W. lynferdii* and *W. subpelliculosa*.

[h] The most homologous species were *Hanseniaspora guilliermondii, H. lachancei, H. opuntiae, H. meyeri, H. uvarum, H. clermontiae* and *H. nectarophila*.

[i] Formerly *Kluyveromyces thermotolerans*.

[j] Yeast species which have never been reported from Spanish-style green table olive fermentations.

[k] Formerly *Debaryomyces etchellsii*.

[l] Formerly *Pichia guilliermondii*.

Table 4
Richness (Margalef), dominance (Simpson) and diversity (Shannon-Weaver) indexes of microbial genotypes in three Spanish-style table-olive fermentation yards (*patios*) at each fermentation stage (initial, middle and final).

Index	Ferm. stage	patio A	patio B	patio C	*p*-value
Margalef	Initial	4.96(0.66)[a]	–[b]	–	–
	Middle	4.48(0.32)	3.94(0.63)	3.50(0.46)	0.002[c]
	Final	4.29(0.40)	3.85(0.49)	3.99(0.77)	NS
Sig.[d]					
Simpson	Initial	0.14(0.05)	–	–	–
	Middle	0.21(0.04)	0.37(0.09)	0.40(0.15)	0
	Final	0.27(0.07)	0.30(0.10)	0.48(0.16)	0.007
Sig.		*			
Shannon-Weaver	Initial	2.15(0.26)	–	–	–
	Middle	1.77(0.16)	1.30(0.26)	1.20(0.36)	0
	Final	1.52(0.26)	1.43(0.29)	1.05(0.37)	0.014
Sig.		*(*[e])			

[a] Mean value (standard deviation), n = 10.

[b] not data available.

[c] Significance among patios at each fermentation stage (Kruskal Wallis test; for $p \leq 0.05$).

[d] Significance of time effect along the fermentation; * for $p < 0.05$; for *patio* A, the Friedman test was used; for *patios* B and C, the Wilcoxon's signed-ranks test was used.

[e] In brackets, statistical significance between the middle and final stages in each *patio* (Wilcoxon's signed-ranks test) in each *patio*; * for $p < 0.05$. NS: not significant difference.

number of observed different genotypes per sample ranged from 7 to 21, whereas the number of species was from 2 to 14. The genetic diversity of the microbial communities detected in the brine samples was estimated using the various indexes showed in Table 4. The values of these indexes were significantly different among the selected *patios*, except for genotype richness (Margalef index) at the final fermentation stage. In this sense, the highest values of dominance (Simpson index) and the lowest values of diversity (Shannon-Weaver) were observed in *patio* C. On the other hand, no statistical differences in any of studied indexes were detected between the middle and final fermentation stages, except for Shannon-Weaver's index of diversity in *patio* A, where we observed a progressive reduction of diversity along the time. Finally, we didn't observe the time effect in genotype richness in *patio* A, most probably due to the fact that the number of isolates obtained at the initial and middle fermentation stages was similar and higher than the number of isolates obtained at the final stage. This difference in sampling effort was due to a lower number of different colony morphotypes observed at the final stage in this *patio*.

3.5. Microbial species diversity at the regional level

An additive partitioning study of the microbial species diversity found in the five *patios* included in matrix Xc is shown in Table 5. In general, at the local (*patio*) level, the average number of species per fermenter, i.e. the α component of diversity, decreased from the initial to the middle fermentation stages in all *patios*, with the exception of *patio* 2 (Table 5). This component was similar at the middle and final fermentation stages, with the exception of *patio* A, which showed a decrease (Table 5). In contrast, the averaged local diversity (γ_1) as well as the regional diversity (γ_2) decreased steeply from the initial to the final fermentation stages (Table 5).

Table 5
Additive partitioning of species diversity, both at local and regional levels, considering five different fermentation yards (*patios*) producing Spanish-style green table olives in the province of Seville, Spain.

		Fermentation stage														
		Initial					Middle					Final				
Patio	Index[a]	α	B[b]	β_N	β_R	γ	α	β	β_N	β_R	γ	α	β	β_N	β_R	γ
A		10.3	20.7	3.7	17.0	31.0	7.2	8.8	0.8	8.0	16.0	5.7	6.3	2.3	4.0	12.0
B		–	–	–	–	–	5.3	9.7	1.7	8.0	15.0	5.2	4.8	1.8	3.0	10.0
C		–	–	–	–	–	4.7	9.3	2.3	7.0	14.0	4.4	6.6	2.6	4.0	11.0
1[c]		9.0	19.0	3.0	16.0	28.0	4.3	7.7	2.7	5.0	12.0	4.4	8.6	2.6	6.0	13.0
2[c]		8.2	12.8	1.8	11.0	21.0	5.3	8.7	2.7	6.0	14.0	5.4	7.6	0.6	7.0	13.0
Local level[d]		9.2	17.5	2.8	14.7	26.7[e]	5.4	8.8	2.0	6.8	14.2[e]	5.0	6.8	2.0	4.8	11.8[e]
Regional level[e]		9.2	51.8	4.8	47.0	61.0[f]	5.4	32.6	2.6	30.0	37.0[f]	5.0	21.0	3.0	18.0	26.0[f]

[a] Diversity indexes: the diversity values are the mean of species number recorded in ten fermenters at each *patio*.

[b] β diversity has been decomposed into two components, i.e. β nestedness diversity (β_N) and β replacement diversity (β_R).

[c] The microbiota associated to the fermenters at these *patios* was described previously in Lucena-Padrós et al. (2014a and 2014b) and Lucena-Padrós and Ruiz-Barba (2016).

[d] Averaged diversity indexes of the five *patios* surveyed.

[e] Corresponds to γ_1.

[f] Corresponds to γ_2.

Finally, the β component of diversity showed a slightly greater contribution to γ diversity than the α component, both at local and regional levels (Table 5). Actually, at the regional level, we found a higher contribution to heterogeneity (represented by the corresponding β_R value) due to differences among local sites (different *patios*) than the average local heterogeneity observed among communities in each site (fermenters at a specific *patio*). The contribution of β diversity was mainly due to changes in β replacement diversity (β_R), while β nestedness diversity (β_N) showed little changes (Table 5).

3.6. Microbial community diversity composition at the regional level

Considering the five *patios* included in matrix Xc, the total number of microbial species isolated was 89, which fell into 30 bacterial and 12 yeast genera. Approximately a third of these species was detected in one unique brine sample, while two-thirds of them showed low relative abundance. Conversely, in addition to *L. pentosus*, only *L. collinides/paracollinoides, P. parvulus* and *C. thaimueangensis* were present at the five *patios* considered here; being all of them detected virtually at the same fermentation stages.

Statistical analyses (PERMANOVA) of the microbial community composition found in the five *patios* revealed significant differences in their structure at the species level at every fermentation stage (Table S1). These differences were also shown in all pairwise comparisons among *patios* at every fermentation stage (data not shown). However, SIMPER analyses confirmed that, as the fermentation progressed, the overall dissimilarities among the *patios* were reduced (Table 6). This is easily observed in the NMDS plot shown in Fig. 1, where microbial communities exhibited a clear spatial concentration towards the final fermentation stage. Actually, the good quality of this ordination is indicated by a low stress value (0.16). Moreover, the number of discriminant microbial species (those contributing more than 5% to the overall dissimilarities among *patios*) decreased along the time (Table 6). It was also detected a change in the actual species responsible for such discrimination, which was more marked in the transition from the initial to the middle fermentation stages (Table 6). It is worth to highlight that among these discriminant species, *L. pentosus* and *P. parvulus* could be classified as “eu-constant”, while *L. collinoides/paracollinoides* and *Marinilactibacillus psychrotolerans* group were classified as “constant” species, based on the occurrence stability index in at least one fermentation stage (Table 7). Therefore, the relative abundance of these species (see Table S1 in Lucena-Padrós and Ruiz-Barba, 2019) could help us to effectively discriminate among the different *patios* at each fermentation stage. However, *Candida aaseri/pseudoaaseri* and *C. thaimueangensis*, which could be classified as “constant” species at the initial and final stages, respectively, contributed less than 5% to the dissimilarities among *patios* because their relative abundance is quite similar in all of them. Also, their low relative abundance makes them worse fermentation-stage-discriminant species than the above cited species. Finally, some of the species that could be classified as “accidental”, such as *L. coryniformis*, *Lactobacillus rhamnosus, Lactobacillus paracasei*, *Lactobacillus rapi* and *Pediococcus ethanolidurans* among others, might help us to distinguish among *patios* and characterize them (see Tables 6 and 7).

Table 6
Percentage contribution (> 5%) of microbial species to the average dissimilarity among five table olive fermentation yards (*patios*) in the province of Seville, Spain, assessed by similarity percentage analysis (SIMPER).

Taxon	Fermentation stage		
	Initial	Middle	Final
	(84.5%)[a]	(59.8%)	(49.5%)
Leuconostoc mesenteroides group	**8.03**[b]	0	0
Aerococcus viridans/urinaeequi	**7.37**	0	0
Enterococcus olivae	**7.13**	0	0
Lactobacillus pentosus	**6.15**	**6.87**	4.51
Alkalibacterium indicireducens group	**5.88**	0	0
Marinilactibacillus sp.	**5.60**	0	0
Marinilactibacillus psychrotolerans group	**5.42**	0	0
Lactobacillus plantarum	**5.23**	1.15	0
Enterococcus casseliflavus	**5.00**	0	0
Lactobacillus collinoides/paracollinoides	0	**14.71**	**16.8**
Pediococcus parvulus	0	**8.87**	**14.31**
Lactobacillus coryniformis group	0.93	**8.70**	**7.81**
Lactobacillus rhamnosus	0	**7.05**	3.81
Lactobacillus paracasei	0	**6.57**	0.98
Saccharomyces cerevisiae	2.22	**5.38**	1.72
Lactobacillus parafarraginis	0	**5.04**	**8.87**
Pediococcus ethanolidurans	0	0.92	**7.85**
Lactobacillus rapi	0	2.80	**5.89**
Staphylococcus epidermidis group	0	1.81	**5.04**

[a] Overall average dissimilarity among *patios* analyzed at each fermentation stage.

[b] Percentage contribution of each species to the total dissimilarity among *patios* at each fermentation stage. Bold figures, discriminant species, i.e. those contributing ≥5% to dissimilarity at each fermentation stage.

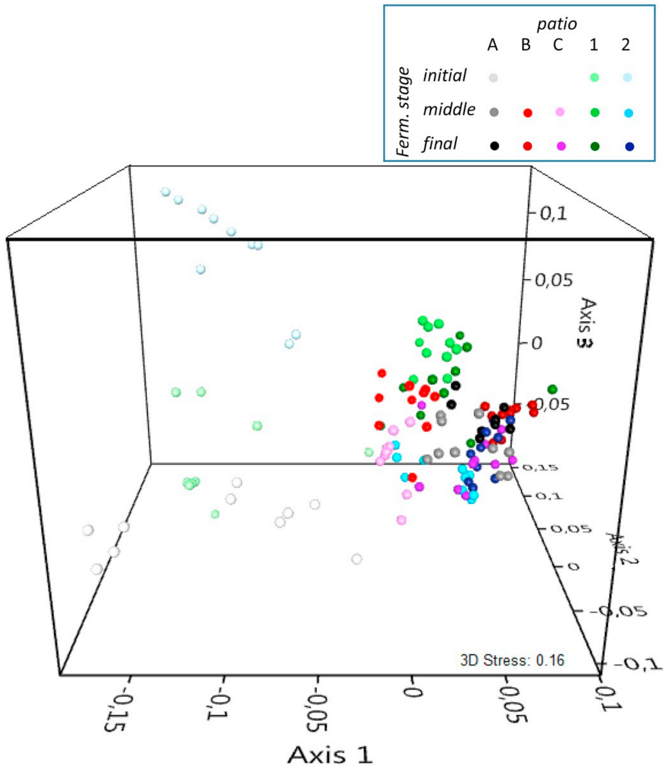

Fig. 1. 3D-Nonmetric multidimensional scaling (NMDS) plot based on Bray-Curtis similarity matrixes of Spanish-style table olive microbial communities located at five different fermentation yards (*patios*) in the province of Seville, Spain. The 3D stress value is indicated. Points represent the composition of the microbial community in a specific fermentation brine sample, i.e. a fermenter in a specific *patio*. The distance between any two points represents the difference in the microbial composition between those two communities. Color key is shown at the top of the Figure.

Table 7

Occurrence Stability Index of microbial species isolated from Spanish-style green olive fermentations at five different fermentation yards (*patios*) in the province of Seville, Spain.

Taxon	Fermentation stage						No. Fermenters[a]
	Initial		Middle		Final		
Lactobacillus pentosus	83.33[b]	A[c]	100	A	100	A	50
Pediococcus parvulus	0	-	20	D	76	A	38
Lactobacillus collinoides/ paracollinoides	0	-	46	C	56	B	33
Candida thaimueangensis	3.33	D	36	C	54	B	32
Saccharomyces cerevisiae	33.33	C	40	C	6	D	26
Pichia manshurica	0	-	40	C	26	C	25
Pichia kudriavzevii	26.67	C	24	D	8	D	21
Marinilactibacillus psychrotolerans group	60	B	0	-	0	-	18
Lactobacillus coryniformis group	3.33	D	20	D	16	D	17
Candida aaseri/pseudoaaseri	53.33	B	0	-	0	-	16
Lactobacillus parafarraginis	0	-	12	D	22	D	16
Staphylococcus epidermidis group	20	D	10	D	14	D	16
Enterococcus casseliflavus	46.67	C	0	-	0	-	14
Pichia membranifaciens	0	-	0	-	28	C	14
Sporolactobacillus inulinus group	10	D	28	C	10	D	14
Lactobacillus plantarum	33.33	C	4	D	0	-	12
Alkalibacterium indicireducens group	36.67	C	0	-	0	-	11
Amphibacillus tropicus	6.67	D	20	D	8	D	11
Lactobacillus rapi	0	-	6	D	16	D	11
Lactobacillus rhamnosus	0	-	18	D	8	D	11
Aerococcus viridans/ urinaeequi	33.33	C	0	-	0	-	10
Candida tropicalis	33.33	C	0	-	0	-	10
Enterococcus olivae	33.33	C	0	-	0	-	10
Kluyveromyces lactis/ marxianus	0	-	20	D	0	-	10
Leuconostoc mesenteroides group	33.33	C	0	-	0	-	10
Weissella paramesenteroides	33.33	C	0	-	0	-	10
Halolactibacillus halophilus/ miurensis	30	C	0	-	0	-	9
Marinilactibacillus sp.	30	C	0	-	0	-	9
Pediococcus ethanolidurans	0	-	2	D	18	D	9

[a] Number of fermenters, out of a total of 50, from which each species was isolated during the three fermentation stages studied.

[b] Occurrence Stability Index of the microbial species per fermentation stage (%).

[c] Category of the species: A, euconstant; B, constant; C, accessory; and D, accident. Omitted species in this table were classified as accidental (D), being detected in ≤8 of the fermenters analyzed.

4. Discussion

Microbial biogeography is the study of microbial diversity distribution across space and time (Martiny et al., 2006). With this work, we wanted to elucidate if there might be a microbial biogeography non-randomly associated with Spanish-style green olive fermentations in the province of Seville, Spain, considering this geographical area as a very specific agroecosystem. In such a case, we also wanted to identify which are the responsible microbial key taxa. For this, culture dependent techniques have been used as our further goal is to find out what role plays this microbiota in the final quality and safety of the product, as well as contributing to the preservation of such characteristic microbiota. This characteristic, and presumably well adapted, microbiota can be truly considered a valuable natural resource that should be preserved conveniently. Also, this specific microbiota might be considered a sort of “virtuous microbiota”, shaping the “terroir” aspects of table olives prepared through the renowned Sevillian style.

At a first glance, when we consider the results shown here (*patios* A, B and C) along with those obtained previously (*patios* 1 and 2) (Lucena-Padrós et al., 2014a; Lucena-Padrós and Ruiz-Barba, 2016), it is remarkable the great microbial heterogeneity exhibited among *patios* within the same region (the province of Seville) at each fermentation stage. This result was somehow expected since olive fermentation remains a spontaneous process nowadays, for raw materials cannot be previously sterilized. However, it has been shown that heterogeneity across *patios* reached a maximum at the initial fermentation stage and decreased as fermentation progressed. Differences in the actual elaboration practices among the different *patios*, such as NaCl addition during the alkali treatment, the number and duration of water washes to remove the excess of alkali or the acidification of the initial brines, could explain in part a great microbial heterogeneity and dissimilarities at the initial fermentation stage. Also, seasonal variations affecting the fruits (Sánchez-Gómez et al., 2006), the persistence of a specific microbiota in the factory facilities (Grounta et al., 2015) or a certain degree of “provincialism” (Martiny et al., 2006) add, most probably, extra heterogeneity to this ecosystem. For example, whereas at *patio* A the only halophilic and alkaliphilic lactic acid bacteria (HALAB) detected at the initial fermentation stage was *Marinilactibacillus psychrotolerans/ piezotolerans,* in our previous studies we found a wide diversity of species and genera of HALABs with a ubiquitous presence and relative high abundance (Lucena-Padrós et al., 2015; Lucena-Padrós and Ruiz-Barba, 2016). Also, the species of the *Vibrio* genus detected now are different from those in the cited studies. Incidentally, *Vibrio* species were isolated from VRBG plates. In this regard, Rosen and Levin (1970) already pointed out that violet red bile agar, recommended for the selectively detection of coliforms from food product of nonmarine origin, was ineffective as a primary enumeration medium of coliforms from fishing industry samples because of the nonselective growth of large numbers of other Gram-negative microorganisms. Therefore, it is advisable to check the actual identity of the isolates on this culture medium through molecular techniques, as we did, and not trusting just on growth in this supposedly "specific" medium. It was also remarkable the ubiquitous presence of *Leuconostoc mesenteroides* at the initial fermentation stage in *patio* A, although this species was not detected in our previous analogous study (Lucena-Padrós et al., 2014a). It has been reported that this heterofermentative lactic coccus is typical from the first fermentation stage but is also able to persist in the brines for a longer period of time (González Cancho and ;Durán Quintana, 1981).

A total of 47.4% (27 out of 57) of the microbial species isolated in this study had been also recovered previously by our own research group in fermentation yards of the same region during earlier seasons (Lucena-Padrós et al., 2014a; Lucena-Padrós and Ruiz-Barba, 2016). Therefore, these species appeared to be less sensitive to seasonal and environmental changes. So, when focused on the results of species diversity partitioning shown in Table 5, and more specifically in α and β nestedness (β_N) diversity indexes, we should be able to find out an expected "minimum common species" along the fermentation. This observation points to eu-constant and constant species (Table 7) as the key responsible taxa of this vegetable fermentation. In this context, it is worth to highlight the wide ecological plasticity of the species *L. pentosus,* which is the eu-constant and dominant species at all fermentation stages. This should be extended to other species such as those of the *M. psychrotolerans* group, *L. collinoides/paracollinoides* and *P. parvulus*. However, we should consider also those *patio*-discriminant, "accessory" or "accidental", species competing with the (eu)constant species for dominating the fermentation brines. In other words, the presence of such accessory/accidental strains might affect the particular relative abundance of these (eu)constant species in each *patio*, thus making these species discriminative in this way. The higher contribution of β replacement diversity (β_R) than that of β nestedness diversity (β_N) to the total β diversity (Table 5) reflected somehow the need to preserve a larger geographical area to protect the indigenous and characteristic microbiota of this food fermentation.

Additionally, this study highlighted the importance of the genetic diversity in this fermentation, i.e. considering the strain level to discriminate and characterize different *patios*. Thus, considering the five *patios* analyzed here at the "regional" or agroecosystem level, we observed differences in the values of the genetic indexes of diversity and dominance at the middle and final fermentation stages (Table 4) that had not been detected in previously reported studies (Lucena-Padrós et al., 2014b).

Finally, once identified the discriminative (Table 6) and common taxa (Table 7), and preferably using the "cosmopolitan" genotypes identified of these species (Figs. S1–S4), we could study their ability to drive this table olive fermentation and their impact in safety and quality of the final product. However, further studies on the occurrence of these species (and strains) in other *patios* and other seasons in the agroecosystem "province of Seville" are needed to include microbial biogeography aspects on food specifications aiming to a possible Protected Geographical Indication (Vigentini et al., 2015).

To our knowledge, this is the first study on the microbial biogeography associated to table olive fermentations. Among other novel results, up to 16 bacterial species have been isolated that had not been described before in table olive fermentations. Microbial key taxa, i.e. those most probably responsible for the specific characteristics of this table olive fermentation in this precise geographic area, have been identified as the species *L. pentosus, P. parvulus, L. collinides/paracollinoides* and those of the *Marinilactibacillus psychrotolerans* group, although a number of accessory species could further discriminate among different *patios*.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.02.004.

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