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Diversity and enumeration of halophilic and alkaliphilic bacteria in Spanish-style green table-olive fermentations

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Abstract

The presence and enumeration of halophilic and alkaliphilic bacteria in Spanish-style table-olive fermentations was studied. Twenty 10-tonne fermenters at two large manufacturing companies in Spain, previously studied through both culture dependent and independent (PCR-DGGE) methodologies, were selected. Virtually all this microbiota was isolated during the initial fermentation stage. A total of 203 isolates were obtained and identified based on 16S rRNA gene sequences. They belonged to 13 bacterial species, included in 11 genera. It was noticeable the abundance of halophilic and alkaliphilic lactic acid bacteria (HALAB). These HALAB belonged to the three genera of this group: *Alkalibacterium, Marinilactibacillus* and *Halolactibacillus.* Ten bacterial species were isolated for the first time from table olive fermentations, including the genera *Amphibacillus, Natronobacillus, Catenococcus* and *Streptohalobacillus*. The isolates were genotyped through RAPD and clustered in a dendrogram where 65 distinct strains were identified. Biodiversity indexes found statistically significant differences between both *patios* regarding genotype richness, diversity and dominance. However, Jaccard similarity index suggested that the halophilic/alkaliphilic microbiota in both *patios* was more similar than the overall microbiota at the initial fermentation stage. Thus, up to 7 genotypes of 6 different species were shared, suggesting adaptation of some strains to this fermentation stage. Morisita-Horn similarity index indicated a high level of codominance of the same species in both *patios*. Halophilic and alkaliphilic bacteria, especially HALAB, appeared to be part of the characteristic microbiota at the initial stage of this table-olive fermentation, and they could contribute to the conditioning of the fermenting brines in readiness for growth of common lactic acid bacteria.

Keywords: olive fermentation, biodiversity, halophilic bacteria, alkaliphilic bacteria, HALAB.

1. Introduction

Table olives represent a typical component of the Mediterranean diet and their production has a great economical and social impact in these countries (IOOC, 2014). This vegetable fermentation can be elaborated by a wide variety of traditional procedures, although the three most common industrial processing methods for the international trade market are Spanish-style green olives, California-style oxidised black olives and Greek-style natural black olives (Rejano *et al.,* 2010). In Spain, the world leading table olive producing country, Spanish-style green olives is the most popular preparation. It is characterised by an initial treatment of the green fruits with a dilute (2.5-3.0 %, w/v) sodium hydroxide solution ("lye") as a fast de-bittering procedure, involving the hydrolysis of oleuropein, followed by one or more water washing step to remove the excess of lye (De Castro *et al.,* 2002; Aponte *et al.,* 2012). Finally, the treated fruits are placed into 10,000 to 15,000-kg glass-fiber containers and covered with a brine of a salt concentration ranging 10–12 % (w/v). These conditions allow a multistep spontaneous fermentation where at least three distinct stages can be defined (Garrido-Fernández et al., 1995). This fermentation is carried out by strains of the species *Lactobacillus pentosus*, although other lactic acid bacteria (LAB) can be also involved (De Castro *et al.,* 2002; Lucena-Padrós et al., 2014b; Rejano *et al.,* 2010; Ruiz-Barba and Jiménez-Díaz, 2012). However, during the first fermentation stage, lasting 3-10 days, a heterogeneous microbiota is usually present which takes advantage of the high salt and pH values of these brines at that moment (De Castro et al., 2002). Actually, several authors have isolated (Abdelkafi et al., 2006; De Castro et al., 2002; Ntougias and Russel, 2000; Quesada et al., 2007) or detected through culture-independent techniques (Abriouel et al., 2011; Cocolin et al., 2013) halophilic and/or alkaliphilic bacteria from table olive fermentations, including the effluents derived from their preparation, as it is the case of *Alkalibacterium olivoapovliticus* (Ntougias and Russel, 2001).

Recently, several comprehensive studies on the microbial ecology associated to Spanish-style green table-olive fermentations at the industrial level have been reported (Lucena-Padrós et al., 2014b, 2014c, 2015b). Both culture-dependent and independent techniques were used on the same samples in an attempt to update our knowledge on this fermentation. When PCR-DGGE was used to examine this fermentations, results revealed that several halophilic and alkaliphilic bacterial species, not isolated before

from table-olive fermentations, could play a relevant role in Spanish-style olive fermentations, as they were widespread in the fermenters and fermentation yards (*patios*) under study (Lucena-Padrós et al., 2015b). It was remarkable the presence of halophilic and alkaliphilic LAB (HALAB), a bacterial group which includes the genera *Alkalibacterium, Halolactibacillus* and *Marinilactibacillus* (Ntougias, 2012). As in the cognate, previous culture-dependent studies no selective culture medium was used to specifically examine the presence of this group of bacteria, only a few halophilic and/or alkaliphilic bacteria were isolated on that occasion, including *Aerococcus viridans/urinaeequi, Enterococcus olivae* (previously identified as *Enterococcus saccharolyticus*), *Enterococcus casseliflavus* and *Vibrio olivae* (previously identified as *Vibrio furnisii/fluvialis*). The aim of this study is to corroborate the presence of halophilic/alkalophilic bacteria, previously detected through PCR-DGGE in Spanish-style green table-olive fermentations, as well to assess their presence through the fermentation time and estimate their possible role. For this, we have used the same fermenting-brine samples which were used before in the mentioned PCR-DGGE study and specific selective culture media designed to rescue such microbiota.

2. Materials and Methods

2.1. Origin of the samples and sampling strategy

Samples of Spanish-style green-olive fermenting brines were obtained from 20 10-tonne fermenters at two large (4,000-8,000 t olives handled per season) manufacturing companies in the province of Sevilla, southern Spain. At each company, fermentation was followed in ten fermenters, each of them of a total capacity of 10 tonnes of olives and 5,500-6,000 litres of brine, made in polyester and glass fibre. These fermenters were located outdoor, buried in the ground of the respective fermentation yards, what it is traditionally called in Spain a "*patio*". The traditional Spanish-style procedure to prepare green olives (Rejano et al., 2010) was followed, and a detailed description was made previously (Lucena-Padrós et al., 2014b). Olives were all of the Manzanilla variety and no starter culture was used.Three consecutive 50-ml samples were taken from approximately the geometric centres of each fermenter at approximately monthly intervals, in coincidence with the initial, middle and final stages of the green table-olive fermentation. More specifically, fermentation had taken place for 1 to 14 (first two weeks), 35 to 48 (5th to 7th week), and 69 to 82 (10th to 12th

week) days after brining, for the initial, middle and final sampling points, respectively. Samples were stored at -80 ºC in 20% (v/v) glycerol until analysed.These same fermenting brines had been analyzed previously through culture-dependent (Lucena-Padrós *et al.,* 2014b, 2014c) and independent (PCR-DGGE; Lucena-Padrós et al 2015b) techniques. Fermentation time, pH and NaCl concentration of these samples are shown in Table S1.

2.2. Isolation and enumeration of microorganisms

Aliquots of brine samples were defrost at room temperature. After vigorous vortexing, serial 10-fold dilutions were performed in 0.1% (w/v) peptone water and plated in duplicates onto agar plates of culture media. Two different alkaline and high salt-content media were used as follows: a) RCMAS, consisting of Reinforced Clostridial Medium (RCM; Biokar Diagnostics) containing 100mM $NaHCO_3/Na_2CO_3$ buffer (pH 10) supplemented with 7 % (w/v) NaCl; b) GYECS, based on GYEC medium (Ntougias and Russel, 2001) and composed of 1% (w/v) glucose (Sigma), 0.5% (w/v) yeast extract (Oxoid), 7% NaCl (w/v), 0.1% (w/v) L-cysteine (AppliChem), and a buffer (100mM Na_2CO_3/1mM K_2HPO_4, pH10.5) containing 0.1% (w/v) NH_4SO_4 plus 0.1mM $MgSO_4*7H_2O$. Agar was added to the broth media at 1.5 % (w/v). Seeded plates were incubated anaerobically at 30 ºC for three days, using a DG250 Anaerobic Workstation (Don Whitley Scientific Ltd., Shipley, West Yorkshire, UK), with a gas mixture consisting of 10 % H_2–10 % CO_2–80 % N_2.

Isolate colonies appearing in the plates were classified attending to their shape, colour, texture, size, etc., as well to their cell morphology, cell arrangement, motility and spore forming ability as observed under a phase-contrast microscope (Olympus Optical Co., Tokyo, Japan). For further studies, a single colony of each different morphotype identified in both culture media at each sampling point was selected from plates with low counts and purified by repeated subculturing. For long-term storage, purified isolates were preserved at -80 ºC in the culture medium they were initially isolated containing glycerol (20% v/v). All isolates were subjected to genotyping through the RAPD technique as described below.

154 *2.3. Molecular identification techniques*

155 Total DNA of the isolates was extracted directly from colonies by the rapid
156 chloroform method described by Ruiz-Barba et al. (2005). Genotyping and molecular
157 identification of the isolates was carried out as described below.

158 *2.3.1. Genotyping through RAPD*

159 Genotyping was carried out by RAPD using the primer OPL5 (5'-
160 ACGCAGGCAC-3') as described by Maldonado-Barragán et al. (2013). Amplification
161 products were electrophoretically resolved through 2% (w/v) agarose gels (SeaKem,
162 Biowhittaker Molecular Applications, USA) in 1x TAE buffer, stained with ethidium
163 bromide (0.5 μg/ml), visualized under UV light and digitally recorded. DNA molecular
164 weight marker 1-kb Plus DNA Ladder (Invitrogen) was used as size standard and as a
165 normalization reference. Reference strains *E. olivae* IGG16.11^T (Lucena-Padrós et al.,
166 2014a and 2014c; previously identified as *E. saccharolyticus* in Lucena-Padrós et al.,
167 2014b) and *V. olivae* IGJ1.11v^T (Lucena-Padrós et al., 2015a; previously identified as *V.*
168 *furnissii/fluvialis* J1.11v in Lucena-Padrós *et al.,* 2014b and 2014c) were included in the
169 cluster analysis of the RAPD profiles in order to produce an improved distinction
170 among species. The resulting RAPD profiles were normalized and analyzed for
171 clustering with the Bionumerics 7.0 software package (Applied Maths, Sint-Martens-
172 Latem, Belgium). Only bands representing amplicons between 150 and 5,000 bp in size
173 were included in the analysis. Similarity dendrograms were constructed by the UPGMA
174 clustering method, using the band-based Dice similarity coefficient. The quality of the
175 cluster analysis was verified by calculating the cophenetic correlation value (in
176 percentage) for each dendrogram, using the BioNumerics 7.0 software. Interpretation of
177 values obtained for the similarity coefficients was as follows: 1.0, genetically
178 indistinguishable isolates; 0.99 to 0.80, closely related isolates that are highly similar
179 but not identical, which could be considered the same strain; 0.79 to 0.50, related
180 isolates; <0.50, unrelated isolates (Tenover et al., 1995; Soll, 2000). As a control,
181 reproducibility of the PCR fingerprinting experiments was verified with a reduced
182 number of strains.

183

184

185 *2.3.2. Molecular identification through 16S rRNA gene sequence analysis*

186 Bacterial isolates were identified to the genus and/or species level by PCR
187 sequencing of a *ca*. 500-bp fragment of the 16S rRNA gene, using the primer pair
188 plb16/mlb16 (Kullen et al., 2000). PCR conditions were as described by Delgado et al.
189 (2008). Briefly: initial denaturation at 96°C for 30 s, followed by 30 cycles of
190 denaturation at 96 ºC for 30 s, annealing at 50°C for 30 s, and polymerisation at 72°C
191 for 45 s, plus a final polymerisation step at 72°C for 4 min. MyTaq DNA polymerase
192 (Bioline, London, UK) was used according to the manufacturer instructions. The
193 resulting amplicons were purified using a Nucleospin Extract II kit (Macherey-Nagel,
194 Düren, Germany) and sequenced at Newbiotechnic S.A. (Bollullos de la Mitación,
195 Spain). The resulting sequences were used to search for similarities using the BLASTN
196 program on the basis of 16S rRNA gene sequence data obtained (Altschul et al., 1997)
197 against the database containing type strains with updated validly published prokaryotic
198 names, by using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al.,
199 2012). The identities of the representative isolates were determined on the basis of the
200 highest scores (typically ≥98.5%). When necessary, e.g. when the partial sequence of
201 16S rRNA gene was not sufficient for a clear-cut identification, the complete 16S rRNA
202 gene was PCR amplified (*ca.* 1400 bp) with the primer pair 7for (5'-
203 AGAGTTTGATYMTGGCTCAG-3') and 1510r (5'-
204 TACGGYTACCTTGTTACGACTT-3') (Lane, 1991), and the resulting amplicon
205 sequenced and analyzed as described above. In these cases, the almost full-length 16S
206 rRNA gene sequences were assembled using the Seqman software version 5.01
207 (DNASTAR, USA). Finally, sequences (*ca.* 500 or 1400-bp-long 16S rRNA gene
208 sequences) were aligned with CLUSTAL W (Thompson et al., 1994), checked manually
209 and grouped into operational taxonomic units (OTUs) or phylotypes using a ≥98.5%
210 similarity threshold. A representative 16S rRNA gene sequence from each OTU was
211 then archived in the GenBank database.

212 *2.3.3. Phylogenetic analysis of partial 16S rRNA gene sequences*

213 Phylogenetic trees based on the partial 16S rRNA gene sequences were
214 constructed using MEGA version 5.0 (Tamura *et al.,* 2011) with the neighbor-joining
215 method (Saito and Nei, 1987) and 1000 replicates of bootstrap analysis. Phylogenetic
216 analyses were restricted to nucleotide positions that could be unambiguously aligned in

all representative sequences of each OTU selected together with that of their closest relatives, as downloaded from databases.

2.4 Biodiversity analyses

Biodiversity of the overall microbial load was evaluated with Margalef's index of genotypes richness (R), Shannon–Weaver's index of diversity (H′) and Simpson's index of dominance (D), calculated as proposed by Ventorino et al. (2007) for each fermenter. Comparisons of mean values of biodiversity indexes between *patios* were done by t-Student's tests. Levene tests were used to check for homogeneity of the variance, while Shapiro-Wilk test was used to check for normality. A probability value of $P < 0.05$ was regarded to be statistically significant. These analyses were performed using the SPSS 21.0 statistical software (SPSS Inc., Chicago, USA). Venn diagram was drawn using the Venn Diagram Plotter (Pacific Northwest National Laboratory, Richland, WA, U.S.A.). The number of halophilic/alkaliphilic species shared between *patios* along the fermentation was estimated using Jaccard qualitative similarity index (Magurran, 1988). Morisita-Horn similarity index (Magurran, 1988) was also calculated as a quantitative index weighing shared species by their relative genotype diversity using the following formula:

$$C_{MH} = 2\Sigma (an_i * bn_i) / (Da + Db)(aN)(bN)$$

Where an_i and bn_i is the total number of different genotypes in the ith species in *patio* 1 and *patio* 2, respectively; Da and Db is the Simpson's index of dominance calculated as proposed by Ventorino et al. (2007) in *patio* 1 and *patio* 2, respectively; aN and bN is the total number of genotypes in *patio* 1 and *patio* 2, respectively.

2.5. Statistical analyses

Total counts of microorganisms were expressed as the mean values of colony forming units (CFU) per millilitre of brine based on duplicate analyses made to each sample. The resulting values were transformed to logarithmic values before statistical analyses were performed. To compare paired population densities quantified on RCMAS and GYECS media, Wilcoxon's signed-ranks test for two groups was applied. The Spearman rank coefficient of correlation was also calculated. Finally, to determine statistically significant differences between the microbial counts in both *patios* at each

sampling point and for each culture media (RCMAS and GYECS) U Mann-Whitney test was used. These analyses were performed using the SPSS 21.0 statistical software (SPSS Inc., Chicago, USA).

3. Results

3.1. Total counts and evolution of halophilic and alkaliphilic bacteria in the fermenting olive brines

Total counts of the microbial population isolated on RCMAS and GYECS culture media during Spanish-style green olive fermentations are shown in Table 1 and Figure S1. At each sampling point, counts were very similar in both culture media, being Pearson's coefficient 0.96, while no significant differences were found by the Wilcoxon test when this statistic was applicable. As expected, the highest counts were obtained at the initial fermentation stage, were pH values and salt concentrations (Table S1) were still high in the fermenting brines. As fermentation progressed, and pH became more acidic, this microbiota decreased dramatically, especially in *patio* 1 (Table 1 and Figure S1). No statistical differences could be found between the results obtained in both culture media, i.e. RCMAS and GYECS, at any sampling point. However, statistically significant differences could be found between both *patios* at the initial fermentation stage, being the halophilic/alkaliphilic microbiota more abundant in *patio* 2 (Table 1). At subsequent fermentation stages, their growth became undetectable or it was so scarce that no statistical tests could be properly carried out.

3.2. Diversity and enumeration of halophilic/alkaliphilic bacteria in green table-olive fermentations.

A total of 203 halophilic/alkaliphilic isolates were selected attending to the morphotyping criteria described above. These isolates could be clustered after UPGMA analysis in a phylogenetic dendrogram according to their RAPD-PCR profiles obtained with primer OPL5 (Figure S2). As a result, up to 65 distinct genotypes (strains) could be distinguished exhibiting similarity indexes ≥80 % (Figure S2). For further molecular identification, up to 92 isolates, belonging to 61 different strains, were selected for partial 16S rRNA gene sequencing (Figure S2). Additionally, in order to improve molecular identification, some strains preliminary identified as *Halolactibacillus* sp. and *Marinilactobacillus* sp. were subjected to (almost) complete sequencing of their 16S

284 rRNA (Figure S2). Subsequently, the 16S rRNA sequences obtained could be grouped
285 into the 13 phylotypes shown in Table 2, where the bacterial species showing maximum
286 similarity is indicated along with additional species exhibiting ≥98.5 % similarity. The
287 partial or complete 16S rRNA gene sequence of one representative strain of each
288 phylotype was submitted to the GenBank database (accession numbers in Table 2).
289 Finally, the phylogenetic relationships between 16S rRNA gene partial sequences of
290 these representative strains and those of closest relative species are illustrated in Figure
291 1. All of the representative strains could be affiliated to at least 13 distinct species,
292 belonging to 11 different genera.
293 A summary of the halophilic/alkaliphilic bacterial species isolated in this study
294 as well as the number of isolates and strains along the three stages of the olive
295 fermentations in the two *patios* studied here is shown in Table 3. Also, the number of
296 fermenters from which a given species could be isolated as well as the count range at
297 which it was present is reported in Table 3. On the other hand, the genotype frequency
298 of these species at the genus level in the 20 fermenters of the two *patios* under study is
299 shown in Figure 2.
300 Very similar species composition was recovered using RCMAS or GYECS
301 culture media. However, some species such as *E. olivae*, which had been isolated in a
302 previous study only in *patio* 2 (Lucena-Padrós et al., 2014c), and two species,
303 *Catenococcus thiocycli* and *Halomonas mongoliensis*, plus an unidentified isolate were
304 obtained only in GYECS (Table 3). Furthermore, it was remarkable the prevalence of
305 isolates belonging to the HALAB group, for 35 (64 %) and 98 (66 %) isolates could be
306 collected from *patio* 1 and 2, respectively. Their presence was ubiquitous in the
307 fermenters under study (Table 3 and Figure 2), although limited to the initial
308 fermentation stage (Table 3). On the other hand, only two species, shared by both
309 *patios*, i.e. *Amphibacillus tropicus* and *Natronobacillus azotifigens*, could be isolated at
310 the middle and/or final fermentation stages (Table 3).
311 Figure 3 shows, through a proportional Venn diagram, the number of microbial
312 genotypes isolated at both *patios* as well as the number of species and genera they
313 belong to. Up to 7 distinct genotypes were shared by both *patios*, belonging to the 6
314 microbial species and 5 different genera also shown in Figure 3. For these shared
315 species, the total number of genotypes found for each of them ranged from 4 to 18
316 (Figure 3).

Finally, it is important to mention that, to our knowledge and with the exception of just three species, i.e. *E. olivae* (Lucena-Padrós et al., 2014c), *V. olivae* (previously described as *Vibrio furnissii/fluvialis* J1.11v in Lucena-Padrós *et al.,* 2014c) and *A. viridans/urinaeequi* (González-Cancho and Durán-Quintana, 1981; Lucena-Padrós et al., 2014b), the rest of bacterial species, i.e. 10 species, had not been isolated before from any table olive fermentation.

3.3. Biodiversity analyses

Comparisons of richness (R), diversity (H') and dominance (D) indexes of the overall genotypes between both *patios* are shown in Figure 4, where H' and D are calculated both at the species and genus level. Statistical differences were found in all indexes between both *patios*. R and H' indexes were lower in *patio* 1 than in *patio* 2. In contrast, the highest concentration of dominance was associated to *patio* 1.

On the other hand, when the bacterial species composition of both *patios* was evaluated using different similarity indexes, the estimated values were 0.43 and 0.86 for Jaccard and Morisita-Horn indexes, respectively. When Jaccard index was re-calculated taking into account all of the bacterial species isolated during the first fermentation stage, previously described for these same samples in Lucena-Padrós et al. (2014b) and excluding repeated species, its value was 0.20. However, Morisita-Horn index could not be re-calculated in this manner because of the existence of highly dominant species such as *L. pentosus* and *A. viridans/urinaeequi* (Lucena-Padrós et al., 2014b) which could bias the result.

4. Discussion

This study has corroborated and expanded previous results obtained through a culture-independent technique such as PCR-DGGE applied to samples of fermenting brines obtained from Spanish-style green table-olive fermentations. Thus, the presence of halophilic and alkaliphilic bacteria in these samples, predicted by PCR-DGGE (Lucena-Padrós et al., 2015b), has been corroborated after the isolation of up to 203 isolates belonging to at least 13 different species. In the previous, cognate culture-dependent study (Lucena-Padrós et al., 2014b) just three of these species could be isolated, indicating the need of special selective media to assess this many times

overlooked part of the characteristic table-olive fermentation microbiota. Although results were very similar with both selective media used here, i.e. RCMAS and GYECS, the fact that some species were only isolated in GYECS suggested that this culture medium could be more appropriated to rescue the halophilic/alkaliphilic microbiota associated to this fermentation.

A statistically significant difference was found in the total counts of halophilic/alkaliphilic bacteria between *patios* 1 and 2 (Table 2). In addition, species richness was higher in *patio* 2 (12 species) than in *patio* 1 (7 species) (Table 3). This result could be due to the fact that in *patio* 1 brines were routinely acidified with HCl as soon as alkali-treated olives were covered in brine (Lucena-Padrós et al., 2014b). This practise, however, is not carried out at that moment of the fermentation in *patio* 2. At this initial stage, averaged pH values were 5.7 and 7.43 in the fermentation brines of *patios* 1 and 2, respectively (Table S1). Therefore, as otherwise it would be anticipated, early acidification appeared to reduce both growth and diversity of halophilic/alkaliphilic bacteria in Spanish-style olive fermentations. On the other hand, NaCl concentration in the brines at equilibrium (first week of fermentation) was 7.76 and 5.88 in the fermenters of *patio* 1 and 2, respectively (Table S1). The less stringent conditions regarding NaCl concentration in *patio* 2 could also contribute to explain the higher counts and halophilic/alkaliphilic species richness observed in this *patio*. As expected, virtually all this microbiota could be isolated only at the initial fermentation stage, i.e. when salt concentration and alkalinic pH are still adequate. In fact, the two only exceptions were the species *A. tropicus* and *N. azotifigens*, which have been described as obligate alkaliphilic and highly salt tolerant (Zhilina et al., 2001; Sorokin et al., 2008). The fact of isolating these two species at fermentation stages when pH values were about 4.3 in both *patios* (Lucena-Padrós et al., 2014b) could be actually due to their ability to form resistant endospores, for they have been described to grow at pH ranges 8.5-11.5 and 7.5-10.6 for *A. tropicus* and *N. azotifigens*, respectively (Zhilina et al., 2001; Sorokin et al., 2008).

It was remarkable the ubiquitous presence of HALAB in both *patios*, whose metabolism, especially the production of lactic acid under alkaline conditions (Ntougias, 2012), undoubtedly contributed to the reduction of the initial highly alkaline pH values of the brines. This in turn should have facilitated the creation of more adequate conditions for the growth of common LAB, such as *L. pentosus*, which can then take over and complete the fermentation. As far as we know, up to 10 bacterial

384 species had not been isolated before from any table olive fermentation, thus
385 demonstrating the value of microbial ecology studies where combined culture-
386 dependent and independent techniques synergistically enhance our knowledge of the
387 real situation in a complex ecosystem such as olive fermentation. In addition, one of the
388 species isolated in both *patios* has been tentatively classified as *Marinilactibacillus* sp.
389 However, the very low homology (96.1 %) of the complete 16S rRNA gene of these
390 isolates to other bacterial species suggested that this could constitute at least a novel
391 species. We are currently working out this subject.
392 Biodiversity at the strain level was assessed through RAPD. In general, strains
393 clustered well into a dendrogram (Figure S2), showing discrete groups which could well
394 correspond to single species. However, the fact that in some cases it was not possible to
395 distinguish among two or three different species of the same genus using just 16S rRNA
396 gene sequence made it impossible to determine whether this clustering corresponded to
397 actual different species. As expected, the value obtained for the diversity index (H') was
398 significantly higher in *patio* 2, while dominance was more characteristic of *patio* 1,
399 where a few species such as *Marinilactibacillus psychrotolerans* and *V. olivae*
400 dominated in most of the fermenters. In contrast, up to 4 species appeared to be
401 ubiquitous in the fermenters of *patio* 2 (Table 3). The value obtained for Jaccard index
402 when considering just the halophilic/alkaliphilic microbiota (0.43) was ca. double that
403 obtained when considering the overall bacterial microbiota during the initial
404 fermentation stage in these same fermenters (0.20; Lucena-Padrós et al., 2014b). This
405 could indicate that the halophilic/alkaliphilic microbiota was more similar between both
406 *patios* than the overall microbiota at this stage. Such observation is probably a
407 consequence of the dominance of these species at the first fermentation stage, reflecting
408 a good adaptation to the high salt/high pH conditions which are characteristic of this
409 table olive preparation at this stage. In addition, that indication was reinforced by the
410 detection of up to 7 genotypes which were shared between both *patios*, perhaps
411 indicating that specialised strains are necessary due to the extreme environmental
412 conditions at this stage of the Spanish-style table-olive fermentations. Also, these results
413 could indicate a common origin of these strains and this point is currently under
414 investigation in our laboratory. Finally, the relatively high value (0.86) obtained for
415 Morisita-Horn index, used to quantitatively compare the similarity of species
416 composition, suggested that codominance in both *patios* was carried out by the same
417 species.

418 This study revealed that the presence of halophilic and alkaliphilic bacteria was
419 widespread among the fermenters of Spanish-style green table olives at the initial
420 fermentation stage. The source of these bacteria is most probably the actual
421 fermentation environment where, selected by the very stringent conditions of pH and
422 salt content at the initial fermentations stages, these halophilic and alkaliphilic bacteria
423 remain season after season in the same *patio*. A suggested origin of these microbiota is
424 the salt supply which, in Spain, is usually of marine origin. The marine origin of many
425 of these species has been indicated by several authors (Ishikawa et al. 2003, 2005, 2009,
426 among others). However, a number of alkaliphilic and/or highly alkali and halo-tolerant
427 bacterial species have been detected or isolated in effluents such as the lyes and washing
428 water employed in the processing steps previous to the actual Spanish-style table olive
429 fermentation (De Castro et al., 2002; Quesada et al., 2007; Ntougias and Russel, 2000,
430 2001). This fact could suggest that the raw olive fruits could also be a source of some
431 typical halophilic and alkaliphilic species found at the initial fermentation stage of
432 Spanish-style green olives. Some authors have actually associated this microbiota to
433 plant material as it is the case of *Alkalibacterium* species in indigo fermentation liquor
434 (Yumoto et al., 2004, 2008; Nakajima et al., 2005; Aino et al., 2010) or dark fire-cured
435 tobacco leaves (Di Giacomo et al., 2007). Known the relatively high similarity at the
436 species as well the strain levels shown by the fermentation brines at both *patios* studied,
437 it appeared that this table-olive elaboration process and its special conditions have
438 selected specific species and genotype patterns due to their specific, well adapted
439 metabolism. In this sense, the profuse isolation of HALAB, which are the only known
440 microorganisms able to achieve lactate fermentation under highly alkaline conditions
441 while being quite halotolerant (Ntougias, 2012), was noteworthy. These bacteria can
442 certainly contribute to the conditioning of the fermenting brines so that the microbiota
443 characteristic of the middle fermentation stage, i.e. LAB such as *L. pentosus*, can thrive
444 and accomplish characteristic Spanish-style table-olive fermentations. Finally,
445 considering the results obtained in this study, we suggest the need of routinely introduce
446 specific, selective media to study the evolution of the halophilic/alkaliphilic microbiota
447 during, at least, the initial fermentation stage of Spanish-style green table-olive
448 fermentations. The presence of this bacterial group appears to be a characteristic of this
449 food fermentation at that stage, and its decline can indicate that the middle, or second,
450 fermentation stage has started.
451

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References

Abdelkafi, S., Labat, M., Casalot, L., Chamkha, M., Sayadi, S., 2006. Isolation and characterization of *Halomonas* sp. strain IMPC, a p-coumaric acid-metabolising bacterium that decarboxylates other cinnamic acids under hypersaline conditions. FEMS Microbiology Letters 255, 108–114.

Abriouel, H., Benomar, N., Lucas, R., Gálvez, A., 2011. Culture-independent study of the diversity of microbial populations in brines during fermentation of naturally-fermented Aloreña green table olives. International Journal of Food Microbiology 144, 487-496

Aino, K., Narihiro, T., Minamida, K., Kamagata, Y., Yoshimune, K., Yumoto, I., 2010. Bacterial community characterization and dynamics of indigo fermentation. FEMS Microbiology Ecology 74, 174–183.

Altschul, S. F., Madden, T. L., Schaeffer, A. A., Zhang, J., Zhang, Z., Miller, W., Lipman, D. J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25, 3389-3402.

Aponte, M., Blaiotta, G., La Croce, F., Mazzaglia, A., Farina, V., Settanni, L., Moschetti, G., 2012. Use of selected autochthonous lactic acid bacteria for Spanish-style table olive fermentation. Food Microbiology 30, 8–16.

Cocolin, L., Alessandria, V., Botta, C., Gorra, R., De Filippis, F., Ercolini, D., Rantsiou, K., 2013. NaOH-debittering induces changes in bacterial ecology during table olives fermentation. PLoS One 8 (7), e69074. http://dx.doi.org/10.1371/journal.pone.0069074.

De Castro, A., Montaño, A., Casado, F.J., Sánchez, A.H., Rejano, L., 2002. Utilization of *Enterococcus casseliflavus* and *Lactobacillus pentosus* as starter culture for Spanish-style green olive fermentation. Food Microbiology 19, 637–644.

Delgado, S., Arroyo, R., Martín, R., Rodríguez, J.M., 2008. PCR-DGGE assessment of the bacterial diversity of breast milk in women with lactational infectious mastitis. BMC Infectious Diseases 8, 51.

Di Giacomo, M., Paolino, M., Silvestro, D., Vigliotta, G., Imperi, F., Visca, P., Alifano, P., Parente, D., 2007. Microbial community structure and dynamics of dark fire-cured tobacco fermentation. Applied and Environmental Microbiology 73, 825-837.

Garrido Fernández, A., García García, P., Brenes Balbuena, M., 1995. Olive fermentations. In: Rehm, H.-J., Reed, G. (Eds.), Biotechnology: Enzymes, Biomass, Food and Feed, VCH, New York, pp. 593–627.

González-Cancho, F., Durán-Quintana, M.C., 1981. Bacterias cocáceas del ácido láctico en el aderezo de aceitunas verdes. Grasas y Aceites 32, 373-379.

IOOC (International Olive Oil Council), 2014. World and European Table Olive Figures. Available Online at: http://www.internationaloliveoil.org/ (Accessed May 2014).

Ishikawa, M., Nakajima, K., Yanagi, M., Yamamoto, Y., Yamasato, K., 2003. *Marinilactibacillus psychrotolerans* gen. nov., sp. nov., a halophilic and alkaliphilic

515 marine lactic acid bacterium isolated from marine organisms in temperate and
516 subtropical areas of Japan. International Journal of Systematic and Evolutionary
517 Microbiology 53, 711–720.

518

519 Ishikawa, M., Nakajima, K., Itamiya, Y., Furukawa, S., Yamamoto, Y., Yamasato, K.,
520 2005. *Halolactibacillus halophilus* gen. nov., sp. nov. and *Halolactibacillus miurensis*
521 sp. nov., halophilic and alkaliphilic marine lactic acid bacteria constituting a
522 phylogenetic lineage in *Bacillus* rRNA group 1. International Journal of Systematic and
523 Evolutionary Microbiology 55, 2427–2439.

524

525 Ishikawa, M., Tanasupawat, S., Nakajima, K., Kanamori, H., Ishizaki, S., Kodama, K.,
526 Okamoto-Kainuma, A., Koizumi, Y., Yamamoto, Y., Yamasato, K., 2009.
527 *Alkalibacterium thalassium* sp. nov., *Alkalibacterium pelagium* sp. nov.,
528 *Alkalibacterium putridalgicola* sp. nov. and *Alkalibacterium kapii* sp. nov., slightly
529 halophilic and alkaliphilic marine lactic acid bacteria isolated from marine organisms
530 and salted foods collected in Japan and Thailand. International Journal of Systematic
531 and Evolutionary Microbiology 59, 1215–1226 .

532

533 Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S.C., Jeon, Y. S.,
534 Lee, J. H., Yi, H., Won, S., Chun, J., 2012. Introducing EzTaxon-e: a prokaryotic 16S
535 rRNA Gene sequence database with phylotypes that represent uncultured species.
536 International Journal of Systematic and Evolutionary Microbiology 62, 716–721.

537

538 Kullen, M.J., Sanozky-Dawes, R.B., Crowell, D.C., Klaenhammer, T.R., 2000. Use of
539 the DNA sequence of variable regions of the 16S rRNA gene for rapid and accurate
540 identification of bacteria in the *Lactobacillus acidophilus* complex. Journal of Applied
541 Microbiology 89, 511-516.

542

543 Lane, D. J., 1991. 16/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M.
544 (Eds.), Nucleic Acid Techniques in Bacterial Systematics, John Wiley and Sons,
545 Chichester, UK, pp. 115-175.
546
547 Lucena-Padrós, H., González, J.M., Caballero-Guerrero, B., Ruiz-Barba, J.L.,
548 Maldonado-Barragán, A. 2014a. *Enterococcus olivae* sp. nov., isolated from Spanish-
549 style green-olive fermentations. International Journal of Systematic and Evolutionary
550 Microbiology, 64, 2534–2539.
551
552 Lucena-Padrós, H., Caballero-Guerrero, B., Maldonado-Barragán, A., Ruiz-Barba,
553 J.L., 2014b. Microbial diversity and dynamics of Spanish-style green table-olive
554 fermentations in large manufacturing companies through culture-dependent techniques.
555 Food Microbiology 42, 154–165.
556
557 Lucena-Padrós, H., Caballero-Guerrero, B., Maldonado-Barragán, A., Ruiz-Barba,
558 J.L., 2014c. Genetic diversity and dynamics of bacterial and yeast strains associated to
559 Spanish-style green table-olive fermentations in large manufacturing companies.
560 International Journal of Food Microbiology 190, 72-78.
561
562 Lucena-Padrós, H., González, J.M., Caballero-Guerrero, B., Ruiz-Barba, J.L.,
563 Maldonado-Barragán, A., 2015a. *Vibrio olivae* sp. nov., isolated from Spanish-style
564 green-olive fermentations. International Journal of Systematic and Evolutionary
565 Microbiology 65: 1895-1901.
566
567 Lucena-Padrós, H., Jiménez, E., Maldonado-Barragán, A., Rodríguez, J.M., Ruiz-
568 Barba, J.L., 2015b. PCR-DGGE assessment of the bacterial diversity in Spanish-style
569 green table-olive fermentations. International Journal of Food Microbiology 205, 47-53.
570
571 Magurran, A. E., 1988. A variety of diversities. In: Magurran, A.E. (Ed.), Ecological
572 Diversity and Its Measurement, Princeton University Press, Princeton, N.J., pp. 81-99.
573
574 Maldonado-Barragán, A., Caballero-Guerrero, B., Lucena-Padrós, H., Ruiz-Barba, J.L.,
575 2013. Induction of bacteriocin production by coculture is widespread among

plantaricin-producing *Lactobacillus plantarum* strains with different regulatory operons. Food Microbiology 33, 40–47.

Nakajima, K., Hirota, K., Nodasaka, Y., Yumoto, I., 2005. *Alkalibacterium iburiense* sp. nov., an obligate alkaliphile that reduces an indigo dye. International Journal of Systematic and Evolutionary Microbiology 55, 1525-1530.

Ntougias, S., Russell, N.J., 2000. *Bacillus* sp. WW3-SN6 a novel facultatively alkaliphilic bacterium isolated from the washwaters of edible olive. Extremophiles 4, 201–8.

Ntougias, S., Russell, N. J., 2001. *Alkalibacterium olivoapovliticus* gen. nov., sp. nov., a new obligately alkaliphilic bacterium isolated from edible-olive wash waters. International Journal of Systematic and Evolutionary Microbiology 51, 1161–1170.

Ntougias, S., 2012. Alkaliphilic Lactic Acid Bacteria: Novel Sources for Genetic Engineering and Biotechnology. Gene Technology 1:e102. doi: 10.4172/2329-6682.1000e102.

Quesada, T., Aguilera, M., Morillo, J. A., Ramos-Cormenzana, A., Monteoliva-Sánchez, M., 2007. *Virgibacillus olivae* sp. nov., isolated from waste wash-water from processing of Spanish-style green olives. International Journal of Systematic and Evolutionary Microbiology 57, 906–910.

Rejano, L., Montaño, A., Casado, F.J., Sánchez, A.H., de Castro, A., 2010. Table Olives: Varieties and Variations. In: Preedy, V.R., Watson, R.R. (Eds.), Olives and Olive Oil in Health and Disease Prevention, Academic Press, Oxford, pp. 5-15.

Ruiz-Barba, J.L., Maldonado, A., Jiménez-Díaz, R., 2005. Small-scale total DNA extraction from bacteria and yeast for PCR applications. Analytical Biochemistry 347, 333-335.

Ruiz-Barba, J.L., Jiménez-Díaz, R., 2012. A novel *Lactobacillus pentosus*-paired starter culture for Spanish-style green olive fermentation. Food Microbiology 30, 253-259.

Saito, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4, 406–425.

Soll, D. R., 2000. The ins and outs of DNA fingerprinting the infectious fungi. Clinical Microbiology Reviews 13, 332–370.

Sorokin, I. D., Zadorina, E. V., Kravchenko, I. K., Boulygina, E. S., Tourova, T. P., Sorokin, D. Y., 2008. *Natronobacillus azotifigens* gen. nov., sp. nov., an anaerobic diazotrophic haloalkaliphile from sodarich habitats. Extremophiles 12, 819–827.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28, 2731-2739.

Tenover, F. C., Arbeit, R. D., Goering, R.V., Mickelsen, P. A., Murray, B. E., Persing, D. H., Swaminathan, B., 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial typing. Journal of Clinical Microbiology 33, 2233–2239.

Thompson, J. D., Higgins, D. G., Gibson, T. J., 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22, 4673–4680.

Ventorino, V., Chiurazzi, M., Aponte, M., Pepe, O., Moschetti, G., 2007. Genetic diversity of a natural population of *Rhizobium leguminosarum* bv. *viciae* nodulating plants of *Vicia faba* in the vesuvian area. Current Microbiology 55, 512-517.

Yumoto, I., Hirota, K., Nodasaka, Y., Yolota, Y., Hocino, T., Nakajima, K., 2004. *Alkalibacterium psychrotolerans* sp. nov., a psychrotolerant obligate alkaliphile that

642 reduces an indigo dye. International Journal of Systematic and Evolutionary
643 Microbiology 54, 2379-2383.

644

645 Yumoto, I., Hirota, K., Nodasaka, Y., Tokiwa, Y., Nakajima, K., 2008. *Alkalibacterium*
646 *indicireducens* sp. nov., an obligate alkaliphile that reduces an indigo dye. International
647 Journal of Systematic and Evolutionary Microbiology 58, 901-905.

648

649 Zhilina, T. N., Garnova, E. S., Tourova, T. P., Kostrikina, N. A., Zavarzin, G. A., 2001.
650 *Amphibacillus fermentum* sp. nov. and *Amphibacillus tropicus* sp. nov., new
651 alkaliphilic, facultatively anaerobic, saccharolytic bacilli from Lake Magadi.
652 Microbiology 70, 711–722.

Legends of the Figures.

Figure 1. Phylogenetic relationships based on comparison of partial 16S rRNA gene sequences (427 nucleotide positions) of halophilic/alkaliphilic bacterial strains isolated in this study and the type strains of the most closely related species. Strain names are shown in boldface. GenBank accession numbers are given in parentheses. Bootstrap values (%), calculated from 1,000 resamplings using the neighbour-joining method, are shown at the nodes for values ≥50%. The number of strains sharing a similar (≥ 98.5%) partial 16S rRNA gene sequence is shown in square brackets. Bar, 0.05 changes per nucleotide position.

Figure 2. Genotype frequency of halophilic/alkaliphilic bacterial genera in the overall Spanish-style green table-olive fermentations detected in a total of 20 fermenters located at two different fermentation yards (*patios*).

Figure 3. Number of microbial genotypes, and the species and genus they belong to, shared between the fermenting brines at two fermentation yards (*patios*) during Spanish-style green olive fermentation. The proportional Venn diagram indicates the number of genotypes which have only been isolated at each *patio*, along with the number of species (in brackets) and genera (in square brackets) they belong to. The intersection of this Venn diagram represents the number of genotypes which are shared by both *patios*, as well as the number of species and genera they belong to. The text box indicates the species and the number of genotypes of these species shared by both *patios*. In brackets, the total number of genotypes found for each species. [1]Included *Alkalibacterium indicireducens/pelagium/thalassium*; [2] Included *Halolactibacillus halophilus/miurensis.;*[3]Included *Marinilactibacillus psychrotolerans/piezotolerans*.; [4]Possible novel species, whose closest relative are *Marinilactibacillus psychrotolerans/piezotolerans*.

Figure 4. Richness, diversity and dominance indexes of halophilic/alkaliphilic microbial genotypes found in the fermentation brines at two Spanish-style table-olive fermentation yards (*patios*) (n=10 at each *patio*). Panel A: Margalef's index of genotype richness (R); Panel B: Shannon–Weaver's index of diversity (H′); Panel C: Simpson's index of dominance (D). H' and D indexes are calculated at the species as well as the

684 genus levels, as indicated. Data are shown as mean values with SEM. *Statistically
685 significant difference ($p < 0.05$).

Table

Table 1. Averaged halophilic/alkaliphilic bacterial loads in twenty fermenters along the three (initial, middle and final) fermentation stages of Spanish-style green olive fermentations in two fermentation yards (*patios*) obtained in the culture media used in this study (GYECS and RCMAS).

Fermentation yard	Fermenter	Fermentation Stage					
		Initial		Middle		Final	
		GYECS	RCMAS	GYECS	RCMAS	GYECS	RCMAS
Patio #1	1	5.73 (0.00)[a]	5.72 (0.01)	ND[b]	ND	ND	ND
	2	5.11 (0.03)	5.11 (0.00)	ND	ND	ND	ND
	3	5.80 (0.01)	5.77 (0.00)	ND	ND	ND	ND
	4	1.88 (0.15)	2.00 (0.00)	ND	ND	ND	ND
	5	6.41 (0.01)	6.52 (0.01)	ND	ND	ND	ND
	6	5.16 (0.03)	5.18 (0.04)	ND	ND	ND	ND
	7	2.18 (0.15)	2.40 (0.00)	ND	ND	ND	ND
	8	5.30 (0.01)	5.28 (0.02)	ND	ND	ND	ND
	9	5.19 (0.03)	5.10 (0.00)	ND	ND	ND	ND
	10	4.44 (0.00)	4.48 (0.00)	2.54 (0.00)	2.70 (0.00)	ND	ND
	average[c]	4.72 (1.43) [n=10]	4.76 (1.38) [n=10]	2.54 (0.00) [n=1]	2.70 (0.00) [n=1]	[d]-	-
Patio #2	1	7.31 (0.02)	6.08 (0.01)	2.40 (0.00)	2.18 (0.00)	2.48 (0.00)	2.65 (0.00)
	2	7.31 (0.02)	7.26 (0.01)	2.40 (0.00)	2.54 (0.00)	ND	ND
	3	6.12 (0.01)	5.99 (0.01)	ND	ND	ND	ND
	4	6.41 (0.01)	6.34 (0.01)	ND	2.18 (0.00)	ND	ND
	5	6.14 (0.04)	6.16 (0.01)	1.70 (0.00)	1.70 (0.00)	ND	ND
	6	7.60 (0.01)	7.48 (0.01)	ND	2.18 (0.00)	ND	ND
	7	7.43 (0.00)	7.32 (0.03)	2.40 (0.00)	2.30 (0.00)	2.88 (0.03)	3.06 (0.02)
	8	7.55 (0.01)	7.32 (0.02)	3.15 (0.00)	3.20 (0.01)	ND	1.70 (0.02)
	9	7.26 (0.02)	5.84 (0.00)	2.48 (0.00)	2.65 (0.00)	1.70 (0.00)	2.18 (0.00)
	10	5.65 (0.03)	6.28 (0.03)	2.90 (0.00)	2.98 (0.02)	ND	ND
	average[c]	6.88 (0.68) [n=10]	6.61 (0.62) [n=10]	2.49 (0.42) [n=7]	2.43 (0.43) [n=9]	2.35 (0.49) [n=3]	2.40 (0.51) [n=4]
	Sig.	*	*	-	-	-	-

[a]Total counts are expressed as the mean values of log CFU/ml based on duplicate analyses made for each sample; standard deviation of the mean (SEM) is shown in parentheses; [b]ND, not detected; [c]Averaged halophilic/alkaliphilic bacterial loads, considering only those fermenters (number in square brackets) showing growth of these bacteria; [d]- not enough data to carry out the statistical test. Sig.: statistical significance considering both *patios* (U Mann-Whitney's test; *for $P < 0.05$).

Table 2. Molecular identification of halophilic/alkaliphilic bacterial strains isolated from Spanish-style green table-olive fermentations through 16S rRNA gene sequence homology.

Strain	Length (bp)	Accession number	Closest relative sequence (accession number)	Similarity (%)
Aerococcus sp. G18.53 (2) [1]	423	KT336460	*Aerococcus urinaeequi* IFO 12173 (D87677)[2]	99.7
Alkalibacterium sp. G17.65 (26)	460	KT336461	*Alkalibacterium pelagium* T143-1-1^T (AB294166)[3]	100
Alkalibacterium psychrotolerans G18.55 (1)	427	KT336462	*Alkalibacterium psychrotolerans* IDR2-2^T (AB125938)	99.7
Amphibacillus tropicus J33.61 (15)	477	KT336463	*Amphibacillus tropicus* Z-7792^T (AF418602)	98.5
Catenococcus thiocycli G20.61.2 (3)	463	KT336464	*Catenococcus thiocycli* DSM 9165^T (HE582778)[4]	99.1
Enterococcus olivae G12.61 (4)	464	KT336465	*Enterococcus olivae* IGG16.11^T (JQ283454)	100
Halolactibacillus sp. G13.57 (9)	1453	KT372895	*Halolactibacillus halophilus* M2-2^T (AB196783)[5]	99.0
Halomonas mongoliensis G20.66 (1)	669	KT336467	*Halomonas mongoliensis* Z-7009^T (AY962236)	99.3
Marinilactibacillus sp. G11.53 (9)	460	KT336468	*Marinilactibacillus psychrotolerans* M13-2^T (AB083406)[6]	100
Marinilactibacillus sp. G13.51 (10)	1407	KT336469	*Marinilactibacillus piezotolerans* LT20^T (AY485792)[7]	96.1
Natronobacillus azotifigens G31.52 (6)	477	KT336471	*Natronobacillus azotifigens* 24KS-1^T (EU143681)	100
Streptohalobacillus salinus G14.54 (2)	424	KT336472	*Streptohalobacillus salinus* H96B60^T (FJ746578)	100
Vibrio sp. J2.62 (4)	464	KT336474	*Vibrio olive* IGJ1.11^T (JQ283456.1)	98.0

[1]In brackets, the number of isolates whose 16S rRNA gene sequence showed a similarity ≥98.5% with the 16S rRNA gene sequence submitted to the GenBank database. Further species that are not distinguishable by 16S rRNA gene sequence and/or have a similarity value ≥98.5%: [2]*Aerococcus viridans*; [3]*Alkalibacterium indicireducens*/*thalassium*; [4]*Vibrio maritimus*/*sagamiensis*; [5]*Halolactibacillus miurensis*; [6]*Marinilactibacillus piezotolerans*; [7]*Marinilactibacillus psychrotolerans*.

Table

Table 3. Halophilic and alkaliphilic bacterial species isolated along Spanish-style green table-olive fermentations in two different fermentation yards ("*patios*").

Patio #1	Fermentation stage			Total [a]	Total [b]	No.[c]	Count range [d]
Bacterial species	Initial	Middle	Final	isolates	strains	ferm.	(log CFU/ml)
Marinilactibacillus psychrotolerans/piezotolerans	28 [e]	0	0	28	1	8	1-4
Vibrio olivae [f,g]	13	0	0	13	3	7	1-3
Amphibacillus tropicus	4	1	0	5	3	2	1-4
Alkalibacterium indicireducens/pelagium/thalassium	4	0	0	4	4	3	2-4
Halolactibacillus halophilus/miurensis	2	0	0	2	2	2	1-2
Natronobacillus azotifigens	2	0	0	2	1	1	1
Marinilactibacillus sp. [h]	1	0	0	1	1	1	3
Total isolates [i]	54	1	0	55 [j]			
Total strains [k]	15	1	0		15 [l]		
Species richness	7	1	0	7 [m]			

Patio #2	Fermentation stage			Total [a]	Total [b]	No. [c]	Count range [d]
Bacterial species	Initial	Middle	Final	isolates	strains	ferm.	(log CFU/ml)
Alkalibacterium indicireducens/pelagium/thalassium	32 [e]	0	0	32	16	8	3-5
Halolactibacillus halophilus/miurensis	31	0	0	31	8	7	3-5
Marinilactibacillus sp. [h]	22	0	0	22	5	8	1-5
Amphibacillus tropicus	0	13	7	20	10	9	1
Streptohalobacillus salinus	10	0	0	10	1	4	1-5
Marinilactibacillus psychrotolerans/piezotolerans	11	0	0	11	6	3	3-5
Enterococcus olivae [f,n,o]	6	0	0	6	1	4	2-5
Natronobacillus azotifigens	0	6	0	6	4	4	1
Aerococcus viridans/urinaeequi [f]	3	0	0	3	1	3	3-5
Alkalibacterium psychrotolerans	2	0	0	2	1	2	3-4
Catenococcus thiocycli [o]	3	0	0	3	2	3	1
Not identified [o]	1	0	0	1	1	1	4
Halomonas mongoliensis [o]	1	0	0	1	1	1	3
Total isolates [i]	122	19	7	148 [j]			
Total strains [k]	43	10	7		57 [l]		
Species richness	10	2	1	12 [m]			

[a]Total isolates of a specific bacterial species; [b]Total strains of a specific bacterial species; [c]Number of fermentors, out of a total of 10, from which a specific bacterial species was isolated in each *patio*; [d]Colony count range at which that bacterial species was isolated; [e]Number of isolates of that bacterial species at that fermentation stage; [f]Bacterial species which have been previously detected and reported in Lucena-Padrós *et al.*, 2014b; [g]*Vibrio olivae* was previously identified as V*ibrio furnissii/fluvialis* in Lucena-Padrós et al., 2014b and 2014c; [h]The relatively low (≤97%) 16S rDNA homology of these isolates with other bacterial species in the data banks could indicate that they might be at least a novel species; [i]Total isolates at each fermentation stage; [j]Total isolates in each *patio*; [k]Total strains at each fermentation stage; [l]Total strains at each *patio*; [m]Total species richness; [n]*Enterococcus olivae* was previously identified as *Enterococcus saccharolyticus* in Lucena-Padrós et al., 2014b; [o]Species which have been isolated only in GYEC media.

Figure

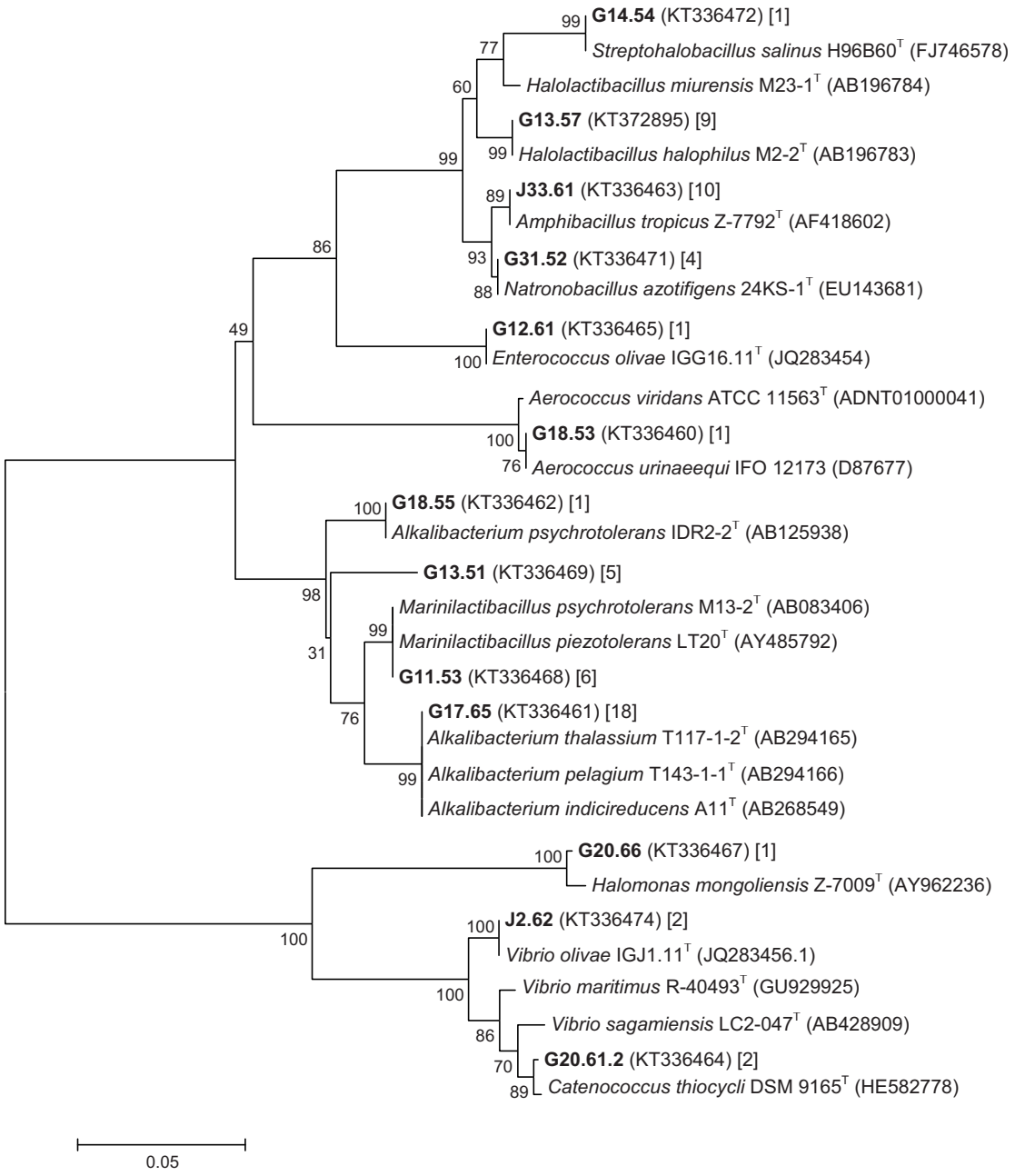


Figure 1. Helena Lucena-Padrós and José Luis Ruiz Barba

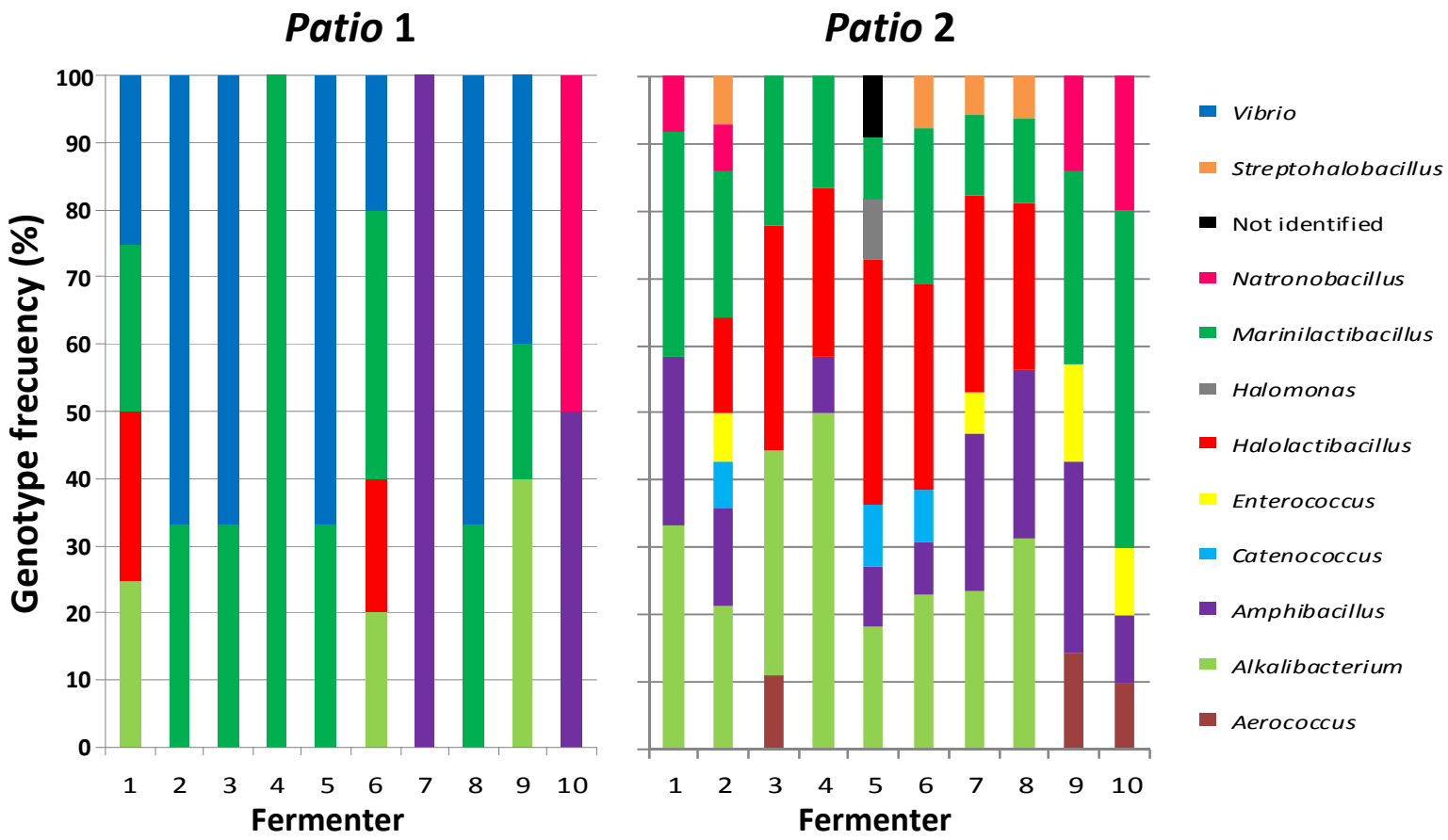


Figure 2. Helena Lucena-Padrós and José Luis Ruiz Barba

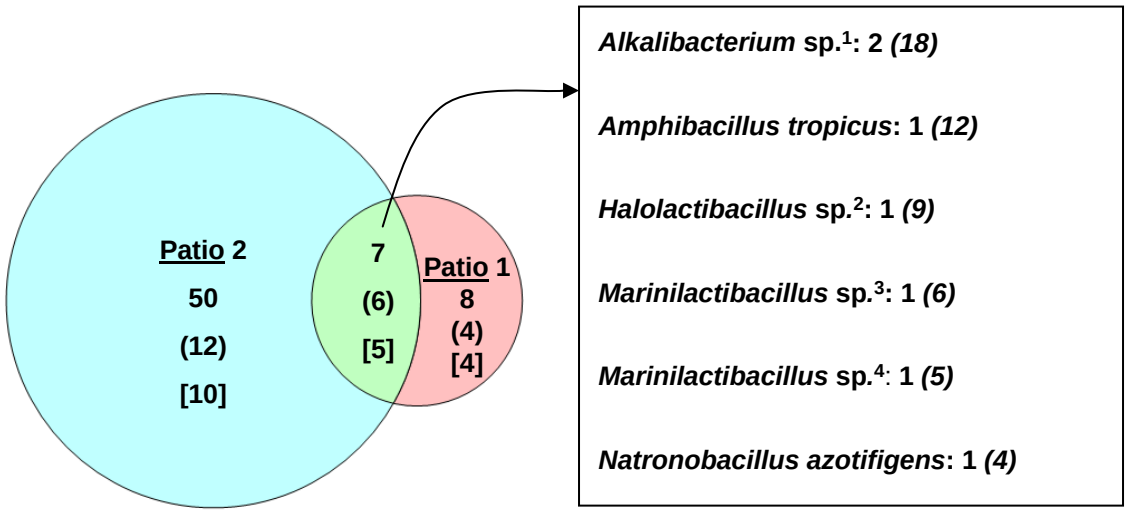


Figure 3. Helena Lucena-Padrós and José Luis Ruiz-Barba

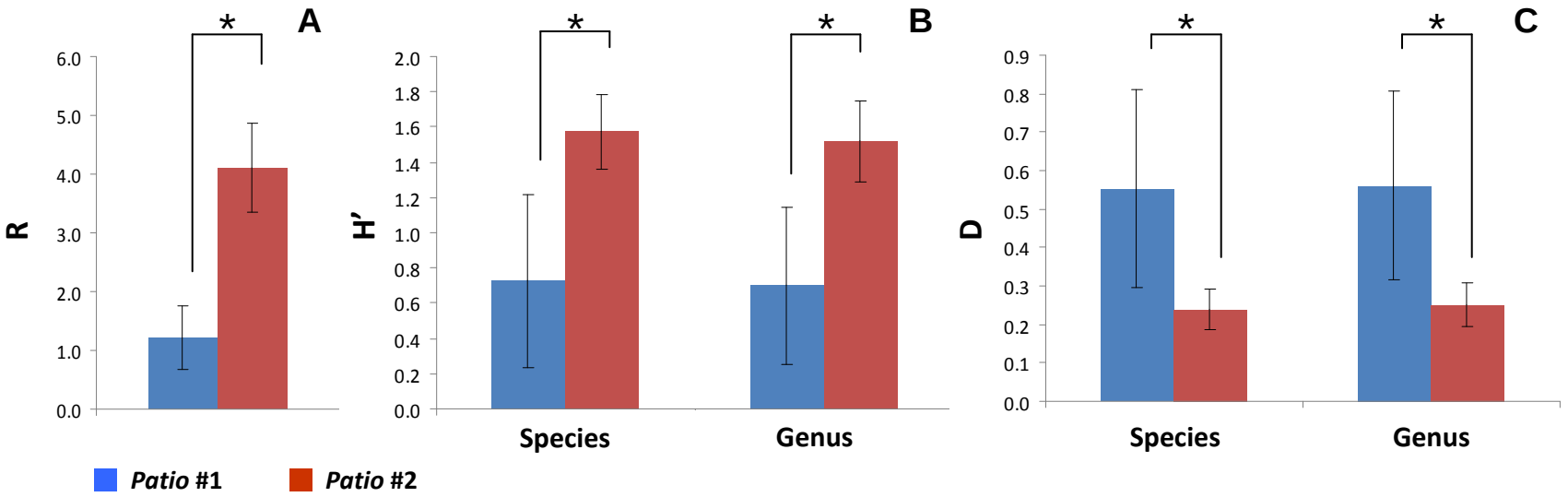


Figure 4. Helena Lucena-Padrós and José Luis Ruiz Barba

Table S1. Evolution of pH values and NaCl concentrations in brine samples from twenty fermenters of two fermentation yards (*patios*) along the three (initial, middle and final) stages of Spanish-style green olive fermentations.

Ferm. yard	Fermenter	Fermentation stage								
		Initial			Middle			Final		
		time (days)	pH	NaCl (%)	time (days)	pH	NaCl (%)	time (days)	pH	NaCl (%)
Patio #1	1	1	5.90	7.51	35	3.96	6.58	69	3.89	6.35
	2	4	6.20	7.88	38	4.15	6.83	72	4.06	6.37
	3	4	6.10	7.64	38	3.98	7.60	72	3.93	7.60
	4	6	5.00	7.79	40	4.02	7.80	74	3.99	7.80
	5	7	5.90	7.97	41	4.00	7.90	75	3.91	7.80
	6	7	5.85	7.42	41	4.04	7.40	75	3.99	7.40
	7	7	6.11	7.93	41	3.90	7.90	75	3.73	7.65
	8	8	5.92	7.91	42	4.02	7.65	76	3.96	7.90
	9	9	6.03	8.05	43	4.16	8.10	77	4.01	7.95
	10	14	4.00	7.54	48	3.75	7.50	82	3.67	7.55
Patio #2	1	2	7.85	6.31	36	4.40	6.30	73	4.38	6.25
	2	2	8.11	5.88	36	4.45	5.90	73	4.45	5.75
	3	2	7.90	6.17	36	4.44	6.20	73	4.45	6.12
	4	2	8.20	5.59	36	4.53	5.50	73	4.54	5.40
	5	2	7.90	5.59	36	4.52	5.60	73	4.51	5.56
	6	4	6.92	7.00	38	4.00	7.02	75	4.00	7.00
	7	4	7.53	5.40	38	4.50	5.36	75	4.45	5.28
	8	4	7.10	5.30	38	4.02	5.26	75	4.01	5.24
	9	8	6.50	6.20	42	4.03	6.15	79	4.01	6.12
	10	9	6.50	5.40	43	4.12	5.30	80	4.10	5.27

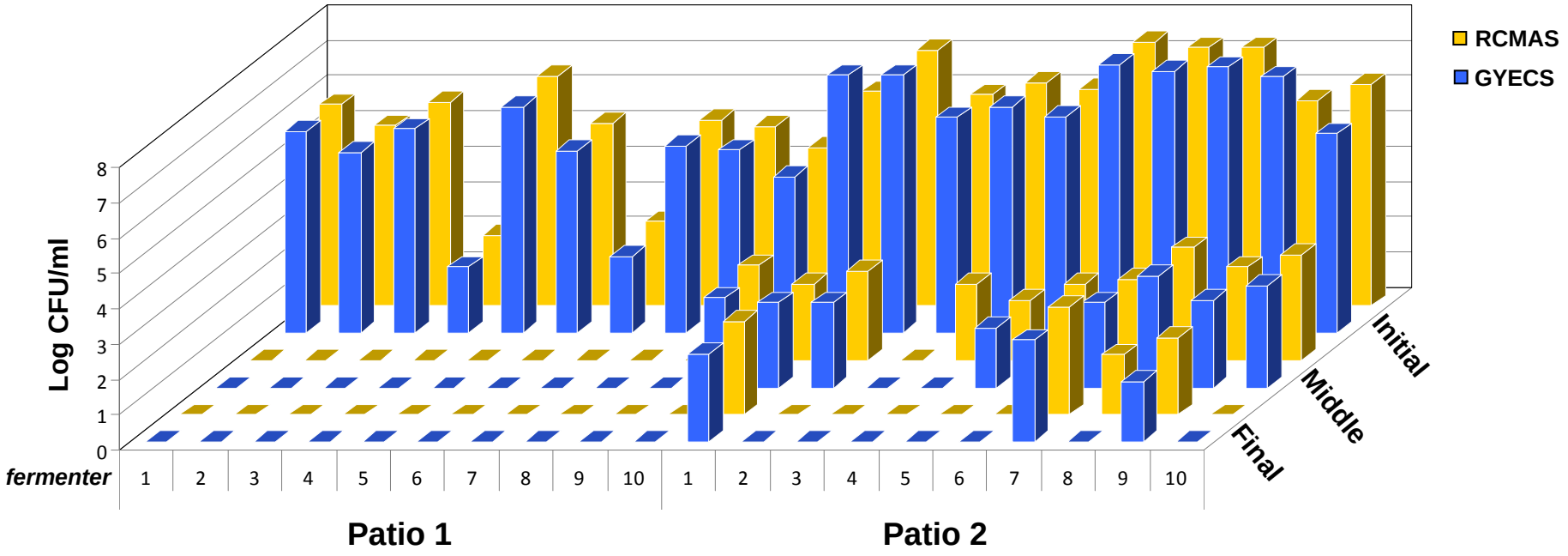
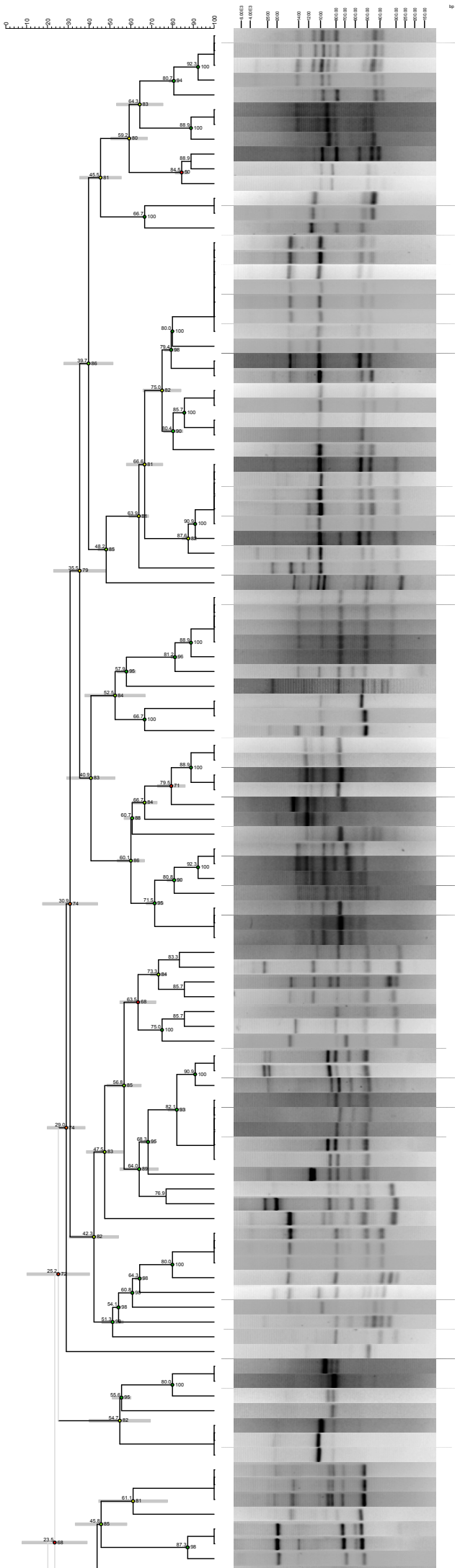


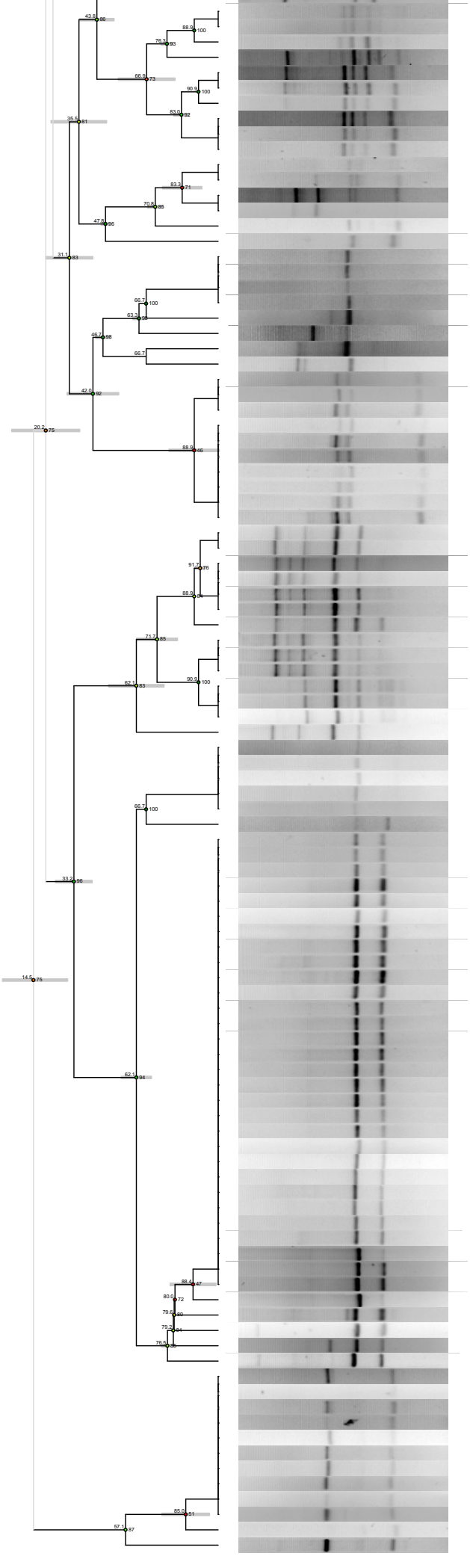
Figure S1. Helena Lucena-Padrós and José Luis Ruiz Barba

Supplementary material - Figure legend

Figure S1. Total counts of halophilic/alkaliphilic bacteria obtained in RCMAS and GYECS culture media along Spanish-style green table-olive fermentations in two different fermentation yards (*patios*). The analysed fermenter, numbered 1-10 at each *patio*, is indicated in the X axys. Values are means of log CFU/ml of duplicate samples at each of the three fermentation stages considered in this study, i.e. initial, middle and final. Standard deviations have been omitted for clarity but are shown in Table 2.



Identification	Isolate	Genotype	Patio	Fermenter	Stage
Alkalibacterium indicireducens/pelagium/thalassium	G16.61*	1	2	1	I
Alkalibacterium indicireducens/pelagium/thalassium	G18.62	1	2	3	I
Alkalibacterium indicireducens/pelagium/thalassium	G17.52	1	2	2	I
Alkalibacterium indicireducens/pelagium/thalassium	G19.53	1	2	4	I
Alkalibacterium indicireducens/pelagium/thalassium	G15.62*	1	2	8	I
Amphibacillus tropicus	J13.51*	1	1	10	I
Amphibacillus tropicus	J13.61*	1	1	10	I
Amphibacillus tropicus	**J33.61***	1	1	10	M
Marinilactibacillus sp.	G16.53*	1	2	1	I
Alkalibacterium indicireducens/pelagium/thalassium	G19.61*	2	2	4	I
Alkalibacterium indicireducens/pelagium/thalassium	G19.62	2	2	4	I
Alkalibacterium psychrotolerans	G16.64	1	2	1	I
Alkalibacterium psychrotolerans	**G18.55***	1	2	3	I
Alkalibacterium indicireducens/pelagium/thalassium	G15.64*	3	2	8	I
Marinilactibacillus sp.	G16.63*	2	2	1	I
Marinilactibacillus sp.	G15.53	2	2	8	I
Marinilactibacillus sp.	G17.53	2	2	2	I
Marinilactibacillus sp.	G17.55	2	2	2	I
Marinilactibacillus sp.	G19.54	2	2	4	I
Marinilactibacillus sp.	G20.55	2	2	5	I
Marinilactibacillus sp.	G20.57	2	2	5	I
Marinilactibacillus sp.	G20.52*	2	2	5	I
Marinilactibacillus sp.	G17.54*	3	2	2	I
Marinilactibacillus sp.	G13.63*	3	2	6	I
Marinilactibacillus sp.	G14.51	4	2	7	I
Marinilactibacillus sp.	G18.51*[a]	4	2	3	I
Marinilactibacillus sp.	**G13.51***[a]	4	2	6	I
Marinilactibacillus sp.	G13.53	4	2	6	I
Marinilactibacillus sp.	G16.62	4	2	1	I
Marinilactibacillus sp.	G14.55*[a]	5	2	7	I
Marinilactibacillus sp.	G17.64*	5	2	2	I
Marinilactibacillus sp.	G15.51	5	2	8	I
Marinilactibacillus sp.	G16.51	5	2	1	I
Marinilactibacillus sp.	G19.52	5	2	4	I
Marinilactibacillus sp.	J10.52*[a]	5	1	6	I
Marinilactibacillus sp.	G18.63	5	2	3	I
Halomonas mongoliensis	**G20.66***	1	2	5	I
Not identified	G20.62	1	2	5	I
Enterococcus olivae	G11.63	1	2	10	I
Enterococcus olivae	G11.64	1	2	10	I
Enterococcus olivae	**G12.61***	1	2	9	I
Enterococcus olivae	G14.62*	1	2	7	I
Enterococcus olivae	G17.67*	1	2	2	I
Enterococcus olivae	G12.62*	1	2	9	I
Enterococcus olivae	IGG16.11[Tb]	2	2	1	I
Catenococcus thiocycli	G13.61.2*	1	2	6	I
Catenococcus thiocycli	**G20.61.2***	1	2	5	I
Catenococcus thiocycli	G17.61.2*	2	2	2	I
Amphibacillus tropicus	G56.61	2	2	1	F
Amphibacillus tropicus	G32.51*	2	2	9	M
Amphibacillus tropicus	G37.51*	2	2	2	M
Amphibacillus tropicus	G34.61	2	2	7	M
Amphibacillus tropicus	G54.51*	3	2	7	F
Amphibacillus tropicus	G36.61*	4	2	1	M
Amphibacillus tropicus	G56.51*	5	2	1	F
Amphibacillus tropicus	G35.61	6	2	8	M
Amphibacillus tropicus	G37.61*	6	2	2	M
Amphibacillus tropicus	G52.51*	6	2	9	F
Amphibacillus tropicus	J14.61*	6	1	7	I
Amphibacillus tropicus	G35.62	7	2	8	M
Amphibacillus tropicus	G40.61	7	2	5	M
Amphibacillus tropicus	G54.61	7	2	7	F
Alkalibacterium indicireducens/pelagium/thalassium	G14.64	4	2	7	I
Alkalibacterium indicireducens/pelagium/thalassium	J6.64*	4	1	9	I
Alkalibacterium indicireducens/pelagium/thalassium	G13.52*	5	2	6	I
Alkalibacterium indicireducens/pelagium/thalassium	G13.58*	5	2	6	I
Alkalibacterium indicireducens/pelagium/thalassium	G15.63*	6	2	8	I
Alkalibacterium indicireducens/pelagium/thalassium	G17.62*	6	2	2	I
Alkalibacterium indicireducens/pelagium/thalassium	G19.55*	7	2	4	I
Alkalibacterium indicireducens/pelagium/thalassium	G13.62*	8	2	6	I
Alkalibacterium indicireducens/pelagium/thalassium	G14.53*	8	2	7	I
Alkalibacterium indicireducens/pelagium/thalassium	J15.61	8	1	1	I
Alkalibacterium indicireducens/pelagium/thalassium	G14.52*	8	2	7	I
Alkalibacterium indicireducens/pelagium/thalassium	G15.55	8	2	8	I
Alkalibacterium indicireducens/pelagium/thalassium	G19.63*	8	2	4	I
Alkalibacterium indicireducens/pelagium/thalassium	G13.61	8	2	6	I
Alkalibacterium indicireducens/pelagium/thalassium	G18.61*	8	2	3	I
Alkalibacterium indicireducens/pelagium/thalassium	G13.55*	9	2	6	I
Alkalibacterium indicireducens/pelagium/thalassium	J6.62*	10	1	9	I
Alkalibacterium indicireducens/pelagium/thalassium	G16.52*	11	2	1	I
Alkalibacterium indicireducens/pelagium/thalassium	G16.54*	12	2	1	I
Alkalibacterium indicireducens/pelagium/thalassium	G15.52*	13	2	8	I
Alkalibacterium indicireducens/pelagium/thalassium	G19.51	13	2	4	I
Alkalibacterium indicireducens/pelagium/thalassium	G20.51	13	2	5	I
Alkalibacterium indicireducens/pelagium/thalassium	G14.61*	13	2	7	I
Alkalibacterium indicireducens/pelagium/thalassium	G14.63*	14	2	7	I
Alkalibacterium indicireducens/pelagium/thalassium	G19.56*	15	2	4	I
Alkalibacterium indicireducens/pelagium/thalassium	J10.65*	16	1	6	I
Alkalibacterium indicireducens/pelagium/thalassium	**G17.65***	17	2	2	I
Alkalibacterium indicireducens/pelagium/thalassium	G20.61*	18	2	5	I
Natronobacillus azotifigens	G31.51	1	2	10	M
Natronobacillus azotifigens	G37.62*	1	2	2	M
Natronobacillus azotifigens	**G31.52***	1	2	10	M
Natronobacillus azotifigens	G32.52*	2	2	9	M
Natronobacillus azotifigens	G36.51*	3	2	1	M
Natronobacillus azotifigens	J13.52*	3	1	10	I
Natronobacillus azotifigens	J13.53	3	1	10	I
Marinilactibacillus physichrotolerans/piezotolerans	**G11.53***	1	2	10	I
Marinilactibacillus physichrotolerans/piezotolerans	G12.51*	1	2	9	I
Marinilactibacillus physichrotolerans/piezotolerans	G12.53	1	2	9	I
Marinilactibacillus physichrotolerans/piezotolerans	G11.62*	2	2	10	I
Aerococcus urinaeequi/viridans	G11.65*	1	2	10	I
Aerococcus urinaeequi/viridans	G12.63	1	2	9	I
Aerococcus urinaeequi/viridans	**G18.53***	1	2	3	I



Halolactibacillus halophilus/miurensis	G15.66	1	2	8	I
Halolactibacillus halophilus/miurensis	**G13.57*[a]**	1	2	6	I
Halolactibacillus halophilus/miurensis	G14.58	1	2	7	I
Halolactibacillus halophilus/miurensis	G14.56*[a]	2	2	7	I
Halolactibacillus halophilus/miurensis	G15.54*[a]	3	2	8	I
Halolactibacillus halophilus/miurensis	G14.65	3	2	7	I
Halolactibacillus halophilus/miurensis	G20.63	3	2	5	I
Halolactibacillus halophilus/miurensis	G18.66	3	2	3	I
Halolactibacillus halophilus/miurensis	G15.61	3	2	8	I
Halolactibacillus halophilus/miurensis	G14.57	3	2	7	I
Halolactibacillus halophilus/miurensis	G13.66	4	2	6	I
Halolactibacillus halophilus/miurensis	G14.66	4	2	7	I
Halolactibacillus halophilus/miurensis	G15.68*[a]	4	2	8	I
Halolactibacillus halophilus/miurensis	G20.54	4	2	5	I
Halolactibacillus halophilus/miurensis	G19.64*[a]	5	2	4	I
Halolactibacillus halophilus/miurensis	J10.63*[a]	6	1	6	I
Amphibacillus tropicus	G33.51	8	2	6	M
Amphibacillus tropicus	G35.51*	8	2	8	M
Amphibacillus tropicus	G35.52	8	2	8	M
Amphibacillus tropicus	G39.51	8	2	4	M
Amphibacillus tropicus	G55.51	9	2	8	F
Amphibacillus tropicus	G54.62*	10	2	7	F
Natronobacillus azotifigens	G31.61*	4	2	10	M
Amphibacillus tropicus	J14.51*	11	1	7	I
Streptohalobacillus salinus	G13.56	1	2	6	I
Streptohalobacillus salinus	G17.56	1	2	2	I
Streptohalobacillus salinus	G17.51	1	2	2	I
Streptohalobacillus salinus	G13.64	1	2	6	I
Streptohalobacillus salinus	G13.67	1	2	6	I
Streptohalobacillus salinus	G14.68	1	2	7	I
Streptohalobacillus salinus	G15.65*	1	2	8	I
Streptohalobacillus salinus	G15.61.2	1	2	8	I
Streptohalobacillus salinus	G17.63	1	2	2	I
Streptohalobacillus salinus	**G14.54***	1	2	7	I
Vibrio olivae	J6.55	1	1	9	I
Vibrio olivae	J15.53*	1	1	1	I
Vibrio olivae	IGJ1.11v[Tc]	1	1	2	I
Vibrio olivae	J15.63	1	1	1	I
Vibrio olivae	J1.54	1	1	2	I
Vibrio olivae	J2.52	1	1	8	I
Vibrio olivae	J5.52	1	1	5	I
Vibrio olivae	J6.63*	2	1	9	I
Vibrio olivae	J8.62*	2	1	3	I
Vibrio olivae	J10.64	2	1	6	I
Vibrio olivae	J1.63	2	1	2	I
Vibrio olivae	**J2.62***	2	1	8	I
Vibrio olivae	J5.63	2	1	5	I
Vibrio olivae	J8.53	3	1	3	I
Halolactibacillus halophilus/miurensis	G18.52	7	2	3	I
Halolactibacillus halophilus/miurensis	G17.61*[a]	7	2	2	I
Halolactibacillus halophilus/miurensis	G17.68	7	2	2	I
Halolactibacillus halophilus/miurensis	G19.66	7	2	4	I
Halolactibacillus halophilus/miurensis	G20.64	7	2	5	I
Amphibacillus tropicus	G31.62*	12	2	10	M
Marinilactibacillus physichrotolerans/piezotolerans	J1.61	3	1	2	I
Marinilactibacillus physichrotolerans/piezotolerans	J1.62	3	1	2	I
Marinilactibacillus physichrotolerans/piezotolerans	J2.61	3	1	8	I
Marinilactibacillus physichrotolerans/piezotolerans	J2.63	3	1	8	I
Marinilactibacillus physichrotolerans/piezotolerans	J5.61	3	1	5	I
Marinilactibacillus physichrotolerans/piezotolerans	J5.62	3	1	5	I
Marinilactibacillus physichrotolerans/piezotolerans	J6.61	3	1	9	I
Marinilactibacillus physichrotolerans/piezotolerans	J8.61	3	1	3	I
Marinilactibacillus physichrotolerans/piezotolerans	J10.61*	3	1	6	I
Marinilactibacillus physichrotolerans/piezotolerans	J11.61	3	1	4	I
Marinilactibacillus physichrotolerans/piezotolerans	J15.62*	3	1	1	I
Marinilactibacillus physichrotolerans/piezotolerans	J1.51	3	1	2	I
Marinilactibacillus physichrotolerans/piezotolerans	J1.52	3	1	2	I
Marinilactibacillus physichrotolerans/piezotolerans	J1.53	3	1	2	I
Marinilactibacillus physichrotolerans/piezotolerans	J2.51	3	1	8	I
Marinilactibacillus physichrotolerans/piezotolerans	J5.51	3	1	5	I
Marinilactibacillus physichrotolerans/piezotolerans	J6.51	3	1	9	I
Marinilactibacillus physichrotolerans/piezotolerans	J6.52	3	1	9	I
Marinilactibacillus physichrotolerans/piezotolerans	J6.53	3	1	9	I
Marinilactibacillus physichrotolerans/piezotolerans	J6.54	3	1	9	I
Marinilactibacillus physichrotolerans/piezotolerans	J8.51	3	1	3	I
Marinilactibacillus physichrotolerans/piezotolerans	J8.52	3	1	3	I
Marinilactibacillus physichrotolerans/piezotolerans	J10.51	3	1	6	I
Marinilactibacillus physichrotolerans/piezotolerans	J10.53	3	1	6	I
Marinilactibacillus physichrotolerans/piezotolerans	J11.51	3	1	4	I
Marinilactibacillus physichrotolerans/piezotolerans	J15.51*	3	1	1	I
Marinilactibacillus physichrotolerans/piezotolerans	J15.52	3	1	1	I
Marinilactibacillus physichrotolerans/piezotolerans	G11.54	3	2	10	I
Marinilactibacillus physichrotolerans/piezotolerans	G12.52	3	2	9	I
Marinilactibacillus physichrotolerans/piezotolerans	G12.54	3	2	9	I
Marinilactibacillus physichrotolerans/piezotolerans	G11.52	3	2	10	I
Marinilactibacillus physichrotolerans/piezotolerans	J10.62	3	1	6	I
Marinilactibacillus physichrotolerans/piezotolerans	G11.51*	4	2	10	I
Marinilactibacillus physichrotolerans/piezotolerans	G13.54*	5	2	6	I
Marinilactibacillus physichrotolerans/piezotolerans	G11.61*	6	2	10	I
Halolactibacillus halophilus/miurensis	G18.54*[a]	8	2	3	I
Halolactibacillus halophilus/miurensis	J15.64	8	1	1	I
Halolactibacillus halophilus/miurensis	G14.67	8	2	7	I
Halolactibacillus halophilus/miurensis	G15.67	8	2	8	I
Halolactibacillus halophilus/miurensis	G17.66	8	2	2	I
Halolactibacillus halophilus/miurensis	G18.64	8	2	3	I
Halolactibacillus halophilus/miurensis	G20.65	8	2	5	I
Halolactibacillus halophilus/miurensis	G13.59	8	2	6	I
Halolactibacillus halophilus/miurensis	G13.60	8	2	6	I
Halolactibacillus halophilus/miurensis	G20.53	8	2	5	I
Halolactibacillus halophilus/miurensis	G19.65	8	2	4	I
Halolactibacillus halophilus/miurensis	G13.65*	9	2	6	I

Figure S2. Helena Lucena-Padrós and José Luis Ruiz Barba

Supplementary material - Figure legend

Figure S2. Phylogenetic dendrogram obtained from RAPD-PCR profiles with primer OPL5 of 203 halophilic/alkaliphilic bacterial isolates collected during Spanish-style green table-olive fermentations at two different fermentation yards (*patios*). The different genotypes (similarity coefficients ≥0.8) found for a given species are indicated, as well as the *patio* they were isolated from. The actual fermenter, numbered 1-10 at each *patio*, from which a particular isolate was collected, is indicated in the column labeled "Fermenter". The fermentation stage at which it was isolated is indicated in the column labelled "Stage": I, initial; M, middle; F, final. Scale line at the top indicates the percentage of similarity. The 1 kb Plus DNA ladder (Invitrogen), used to normalize banding patterns, is represented at the top of the figure. In bold, strains whose 16S rRNA sequence has been added to the GenBank database (see accession numbers in Table 1). * Isolates chosen for partial sequencing of the 16S rRNA gene; [a]Isolates whose 16S rRNA gene was (virtually) completely sequenced; [b]Reference strain *Enterococcus olivae* IGG16.11^T (Lucena-Padrós *et al.,* 2014a and 2014c); [c]Reference strain *Vibrio olivae* IGJ1.11v^T (Lucena-Padrós *et al.,* 2015a; previously described as *Vibrio furnissii/fluvialis* J1.11v in Lucena-Padrós *et al.,* 2014c).