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ORIGINAL PAPER

Lactic acid bacteria population dynamics during spontaneous fermentation of radish (*Raphanus sativus* L.) roots in brine

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Abstract The aim of the present study was to assess the microecosystem development and the dynamics of the lactic acid bacteria population during spontaneous fermentation of radish (*Raphanus sativus* L.) roots in brine at 20 and 30 °C. In both temperatures, lactic acid bacteria prevailed the fermentation; as a result, the pH value was reduced to ca. 3.6 and total titrable acidity increased to ca. 0.4% lactic acid. Enterococci population increased and formed a secondary microbiota while pseudomonads, *Enterobacteriaceae* and yeasts/molds populations were below enumeration limit already before the middle of fermentation. *Pediococcus pentosaceus* dominated during the first days, followed by *Lactobacillus plantarum* that prevailed the fermentation until the end. *Lactobacillus brevis* was also detected during the final days of fermentation. A succession at sub-species level was revealed by the combination of RAPD-PCR and rep-PCR analyses. Glucose and fructose were the main carbohydrates detected in brine and were metabolized into lactic acid, acetic acid and ethanol.

Keywords Spontaneous fermentation · *Raphanus sativus* · *Pediococcus pentosaceus* · *Lactobacillus plantarum* · *Lactobacillus brevis*

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Introduction

Lactic acid fermentation of fruits and vegetables has been extensively studied over the last decades with fermented olives, cucumbers, kimchi and sauerkraut being in the epicenter due to their commercial significance (Paramithiotis et al. 2017b).

An increased interest on indigenous fermented fruits and vegetables such as caper berries (Pulido et al. 2005) cauliflower (Paramithiotis et al. 2010), eggplant (Nguyen et al. 2013), leek (Wouters et al. 2013a), asparagus (Paramithiotis et al. 2014a), green tomatoes (Paramithiotis et al. 2014b) and turnips (Maifreni et al. 2004) has taken place over the last decade in an attempt to characterize the micro-ecosystems, exploit their dynamics and improve our understanding on the factors that play decisive roles in their development.

Radish (*Raphanus sativus* L.) is a member of the Brassicaceae family. Radish root is considered to possess high medicinal and nutritional value; it is rich in antioxidants, vitamin C, B-complex vitamins and minerals like calcium, phosphorus, potassium, magnesium etc. (Pushkala et al. 2013) and its consumption has been associated with positive effects on human health and is suggested as an alternative treatment for various illnessess such as hyperlipidemia, cancer and coronary heart diseases (Talalay and Fahey 2001; Curtis 2003; Beevi et al. 2012). The health promoting properties are mainly attributed to the presence of glucosinolates and their degradation products such as isothiocyanates, but also to natural antioxidants like polyphenolic compounds as well as flavonoid and ascorbic acid (Beevi et al. 2012; Goyeneche et al. 2015).

Currently in Europe, radish roots are consumed raw as a part of fresh mixed salads, contributing their strong and unique flavor. On the other hand, in China, Japan and Korea

they are also consumed as an ingredient of lactic acid fermented products such as Kimchi and Pao Cai (Yan et al. 2008; Patra et al. 2016). Moreover, in India, Nepal, and Bhutan the consumption of Sinki, a product prepared by pit fermentation of radish roots, is quite common (Tamang and Sarkar 1993). In Greece, and more accurately in southern Attica, radish roots are very often subjected to lactic acid fermentation in brine, which is the most widespread type of fermentation in Greece, resulting in a product of unique sensorial properties. In that district, outdoor cultivation is possible throughout the year due to the mild climatic conditions. Thus, the ambient temperatures in which the fermentation takes place may extend from <20 to more than 30 °C. To the best of our knowledge, there is currently no literature available regarding the spontaneous brine fermentation of radish roots. Therefore, the aim of the present study was to monitor the microbial population dynamics during spontaneous fermentation of radish roots in brine, at 20 and 30 °C and to taxonomically characterize the dominating lactic acid microbiota.

Materials and methods

Pickle preparation and sampling

Fermentation of radish (*R. sativus* L.) roots was performed according to a traditional recipe currently employed in southern Attica. 700 g (±5 g) were thoroughly washed with tap water, cut in half (approx. dimensions: height 2.0 cm, radius 1.5 cm) and submerged into 1.3 L of brine solution (5% NaCl w/v). The surface was covered with olive oil and the mixture left to ferment at 20 °C and at 30 °C for 17 and 11 days, respectively. Brine sampling was performed in regular time intervals; the fermentation was considered as completed when pH and TTA values exhibited no statistically significant ($P < 0.05$) change between two consecutive samplings. Thus, brine samples were analyzed at days 0, 1, 3, 5, 7, 11, 15 and 17 in the case of 20 °C and 0, 1, 3, 5, 7 and 11 in the case of 30 °C. Fermentations were performed in duplicate and the average values are presented.

Physico-chemical and microbiological analyses

Brine pH value and total titratable acidity (TTA) were used to monitor fermentation. Brine samples (10 mL) were aseptically derived from each fermentation jar and the pH value was recorded (WTW, Weilheim, Germany). Then, homogenization with 90 mL of distilled water took place using a Stomacher apparatus (Seward, London, UK). The acidity was titrated using 0.1 N NaOH to a final pH of 8.5 and the TTA was expressed in % lactic acid (%LA = mL 0.1 N NaOH used to titrate 10 mL sample multiplied by 0.09). All analyses were performed in triplicate and the average values are presented.

Microbiological analyses were performed in the brine samples (10 mL) throughout fermentation. Total aerobic mesophilic count, lactic acid bacteria, yeasts/molds, enterococci, *Staphylococcus aureus*, sulphur-reducing clostridia, *Escherichia coli, Enterobacteriaceae* and pseudomonads as well as qualitative and quantitative determination of *Listeria monocytogenes* and *Salmonella* spp. were performed according to Paramithiotis et al. (2010). In brief, brine samples (10 mL) were homogenized with sterile saline (90 mL) containing 0.1% peptone (Merck, Darmstadt, Germany) and 0.85% NaCl (Merck) using a Stomacher apparatus. Serial dilutions were performed in sterile Ringer solution (LAB M, Lancashire, UK). Total aerobic mesophilic count, yeasts/molds, enterococci, pseudomonads and *St. aureus* determination was carried out by spreading 0.1 mL of the diluted sample to the surface of Plate Count Agar (LAB M), Rose Bengal Chloramphenicol Agar (LAB M), Kanamycin Aesculin Azide Agar (LAB M), Pseudomonas Agar base supplemented with Cephalothin, Fucidin and Cetrimide (LAB M) and Baird-Parker selective agar (LAB M) and incubating at 30 °C for 48 h, 25 °C for 5 days, 35 °C for 3 days, 25 °C for 48 h and 35 °C for 24–48 h, respectively. The enumeration limit was 2 log CFU/mL. *Enterobacteriaceae* and *E. coli* determination was performed by pouring 1 mL of the diluted sample in Violet Red Bile Glycose Agar (LAB M) and Chromocult® TBX agar (Merck) and incubation at 35 °C for 24 h. The enumeration limit was 1 log CFU/mL. Enumeration of sulphur-reducing clostridia took place by pouring 10 mL aliquots in 20 mL of molten Sulfite Polymyxin Sulfadiazine agar (Merck) and overlay with 5 mL of sterile paraffin after solidification. Incubation was carried out at 35 °C for 24 h. The enumeration limit was 1 CFU/mL. Qualitative and quantitative determination of *L. monocytogenes* and *Salmonella* spp. were performed according to ISO 11290-1:1996 and ISO 11290-2:1998 in the first case and ISO 6579:2002 and ISO/TS 6579-2:2012 in the second case, respectively. The enumeration limit in both cases was 2 log CFU/mL. All analyses were performed in duplicate and the average values are presented.

Isolation and identification of lactic acid bacteria

Lactic acid bacteria were isolated throughout fermentation with the exception of days 0 and 1. Selection of the colonies was performed according to the representative sampling scheme of Harrigan and McCance (1976), purification was performed by successive sub-culturing on MRS agar and incubation at 30 °C for 48 h. Gram stain and catalase reactions were performed for confirmation.

Clustering of the LAB was performed by random amplified polymorphic DNA–polymerase chain

reaction (RAPD-PCR) with M13 as primer and repetitive sequence-based PCR (rep-PCR) with $(GTG)_5$ as primer, as described by Paramithiotis et al. (2014a, b). Electrophoresis was performed in 1.5% agarose gel in 1.0 × Tris–Acetate EDTA (TAE) at 100 V for 1.5 h with concomitant visualization by ethidium bromide staining. Gels were photographed using the GelDoc system (Bio-Rad, Hercules, CA, USA); conversion, normalization and further analysis were performed with Bionumerics software v. 6.1 (Applied Maths NV, Sint-Martens-Latem, Belgium) using the Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis. Strains were subjected to each analysis at least twice.

One to three representative strains from each cluster were subjected to 16S rRNA gene sequencing according to Cocolin et al. (2004) for taxonomic assignment. Sequences were aligned with those in GenBank using the BLAST program to determine the closest known relatives.

Analysis of metabolites

Carbon sources (glucose and fructose) and metabolites (lactic acid, acetic acid, ethanol, glycerol) were determined in brine samples by high-performance liquid chromatography according to Paramithiotis et al. (2006).

Statistical analysis

One-way analysis of variance (ANOVA) (MS Excel, 2010) was used to statistically assess the differences between pH, TTA and microbial population dynamics during spontaneous fermentation of radish roots at 20 and 30 °C. The Simpson's discrimination (*D*) index was used to determine the discrimination power of the typing methods applied (Hunter and Gaston 1988).

Results

In Table 1, the physico-chemical and microbiological changes during spontaneous fermentation of radish roots at 20 and 30 °C are shown. The initial pH values were 7.04 and 6.95 and decreased to 3.62 and 3.60, respectively. The initial TTA was 0.01%LA in both cases and increased to 0.40 and 0.35%LA, respectively. Brine acidification was faster at 30 °C. Indeed, during the third and fifth days of fermentation at 30 °C the pH value was significantly ($P < 0.05$) lower than the respective at 20 °C (Table 1). In addition, TTA was significantly higher during the third day of fermentation at 30 °C.

Pseudomonads, *Enterobacteriaceae* and yeasts/molds were enumerated already from the beginning of fermentation (day 0) in both temperatures. On the contrary, lactic acid bacteria, enterococci, *St. aureus*, sulphur-reducing clostridia, *E. coli, L. monocytogenes* and *Salmonella*

Table 1 Physico-chemical and microbiological changes during spontaneous fermentation of radish roots at 20 and 30 °C

Day	pH	TTA (%LA)	TAMC	LAB	Pseudomonads	*Enterobacteriaceae*	Yeasts/molds	Enterococci
Fermentation at 20 °C								
0	7.04 (0.24)[a]	0.01 (0.01)[a]	5.78 (0.44)[a]	<1.00	4.78 (0.36)[a]	2.52 (0.32)[a]	4.00 (0.35)[a]	<2.00
1	6.51 (0.32)[a]	0.01 (0.01)[a]	5.77 (0.58)[a]	<1.00	5.12 (0.37)[a]	2.70 (0.22)[a]	3.85 (0.22)[a]	<2.00
3	5.86 (0.12)[a]	0.04 (0.01)[a]	6.37 (0.64)[a]	5.12 (0.38)[a]	4.70 (0.36)[a]	4.20 (0.43)[a]	3.80 (0.34)[a]	3.28 (0.36)[a]
5	4.12 (0.17)[a]	0.17 (0.02)[a]	6.45 (0.41)[a]	7.07 (0.42)[a]	4.48 (0.45)[a]	3.70 (0.33)[a]	3.68 (0.24)[a]	3.90 (0.43)[a]
7	3.78 (0.15)[a]	0.26 (0.11)[a]	7.69 (0.48)[a]	7.71 (0.34)[a]	4.39 (0.57)	3.97 (4.12)	4.35 (0.57)[a]	4.82 (0.40)[a]
11	3.68 (0.19)[a]	0.28 (0.04)[a]	6.81 (0.47)[a]	6.90 (0.28)[a]	3.36 (0.52)	<1.00	3.57 (0.48)[a]	5.72 (0.52)[a]
15	3.60 (0.05)	0.41 (0.01)	6.56 (0.54)	6.54 (0.42)	<2.00	<1.00	<2.00	5.13 (0.25)
17	3.62 (0.07)	0.40 (0.01)	6.40 (0.42)	6.31 (0.31)	<2.00	<1.00	<2.00	5.26 (0.23)
Fermentation at 30 °C								
0	6.95 (0.20)[a]	0.01 (0.01)[a]	5.54 (0.48)[a]	<1.00	4.78 (0.42)[a]	2.58 (0.35)[a]	4.24 (0.57)[a]	<2.00
1	6.44 (0.30)[a]	0.02 (0.01)[a]	5.96 (0.44)[a]	4.90 (0.37)	5.38 (0.46)[a]	3.46 (0.51)[a]	2.85 (0.33)[a]	3.42 (0.54)
3	4.46 (0.17)[b]	0.09 (0.01)[b]	6.87 (0.23)[a]	7.73 (0.42)[b]	4.85 (0.42)[a]	2.95 (0.38)[a]	2.69 (0.43)[a]	3.08 (0.36)[a]
5	3.68 (0.23)[a]	0.28 (0.10)[a]	7.76 (0.62)[a]	7.78 (0.43)[a]	3.48 (0.32)[a]	2.48 (0.24)[b]	3.12 (0.55)[a]	3.70 (0.50)[a]
7	3.53(0.22)[a]	0.34 (0.02)[a]	7.79 (0.51)[a]	7.63 (0.54)[a]	<2.00	<1.00	2.60 (0.40)[a]	3.70 (0.41)[a]
11	3.60 (0.07)[a]	0.35 (0.03)[a]	6.84 (0.42)[a]	6.71 (0.47)[a]	<2.00	<1.00	2.48 (0.36)[a]	3.85 (0.43)[a]

Microbial populations are presented in log CFU/mL; standard deviation is given in parenthesis

In each column, different superscript letters denote significant differences between the same sampling days of fermentation at 20 and 30 °C

TAMC total aerobic mesophilic count, *LAB* lactic acid bacteria

Table 2 Carbon sources consumption and metabolite production during spontaneous fermentation of radish roots at 20 and 30 °C

Day	Carbon sources		Metabolites		
	Glucose	Fructose	Lactic acid	Acetic acid	Ethanol
Fermentation at 20 °C					
0	1.92 (0.30)[a]	0.84 (0.23)[a]	nd	nd	nd
1	2.57 (0.42)[a]	1.18 (0.31)[a]	nd	nd	nd
3	2.36 (0.36)[a]	0.82 (0.15)[a]	5.23 (1.32)[a]	nd	nd
5	3.92 (0.85)[a]	0.87 (0.28)[a]	11.47 (2.52)[a]	nd	3.25 (0.61)[a]
7	4.72 (0.79)[a]	0.93 (0.36)	23.35 (2.74)[a]	nd	13.01 (1.35)[a]
11	3.57 (0.33)	0.65 (0.17)	34.49 (1.33)[a]	1.28 (0.33)[a]	14.34 (1.47)[a]
15	3.61 (0.72)	0.67 (0.22)	36.54 (1.68)	3.87 (0.41)	15.01 (1.08)
17	0.68 (0.22)	0.54 (0.21)	42.01 (2.31)	5.02 (1.06)	16.18 (1.27)
Fermentation at 30 °C					
0	2.08 (0.35)[a]	0.68 (0.27)[a]	nd	nd	nd
1	3.95 (0.72)[a]	1.29 (0.32)[a]	0.34 (0.12)	nd	nd
3	3.81 (0.65)[a]	0.54 (0.27)[a]	12.14 (1.69)[b]	1.87 (0.20)	4.34 (0.42)
5	3.49 (0.34)[a]	0.65 (0.16)[a]	22.27 (2.44)[b]	1.93 (0.48)	14.22 (1.58)[b]
7	0.17 (0.05)[b]	nd	34.28 (3.04)[a]	2.50 (0.41)	16.32 (1.75)[a]
11	nd	nd	38.78 (2.36)[a]	3.29 (0.55)[a]	18.39 (1.63)[a]

In each column, different superscript letters denote significant differences between the same sampling days of fermentation at 20 and 30 °C

nd not detected

sp. were below enumeration limit. Lactic acid bacteria population was detectable from the third and first day of fermentation and increased until the seventh and third day of fermentation, at 20 and 30 °C, respectively, reaching approximately 7.70 log CFU/mL in both cases. Then a reduction in the population to 6.31 and 6.71 CFU/mL, respectively, took place at the end of fermentation.

Pseudomonads population remained without statistically significant change for 7 and 3 days of fermentation at 20 and 30 °C, respectively. Then, it diminished and was below enumeration limit during the 15th and seventh days of fermentation at 20 and 30 °C, respectively. *Enterobacteriaceae* population increased at the beginning of fermentation and reached 4.20 and 3.46 log CFU/mL during the third and first days of fermentation at 20 and 30 °C, respectively. Then, the population was reduced and was below enumeration limit during the 11th and the seventh days of fermentation at 20 and 30 °C, respectively. The population of yeasts/molds remained stable at approximately 4.00 log CFU/mL during the first 11 days of spontaneous fermentation at 20 °C but then reduced below enumeration limit until the end. On the contrary, during fermentation at 30 °C, yeasts/molds population remained between 2.48 and 3.12 log CFU/mL, slightly reduced from the initial 4.24 log CFU/mL. *St. aureus*, sulphur-reducing clostridia, *E. coli, L. monocytogenes* and *Salmonella* sp. remained below enumeration limit throughout fermentation. Absence of the last two foodborne pathogens was also verified.

The carbon sources detected during spontaneous fermentation of radish roots were glucose and fructose (Table 2); their initial concentration ranged from 1.92 to 2.08 mM and from 0.68 to 0.84 mM, respectively. During fermentation at 20 °C their concentration ranged from 2.36 to 4.72 mM and from 0.65 to 1.18 mM, respectively, with the exception of day 17 in which they were reduced to 0.68 and 0.54 mM, respectively. During fermentation at 30 °C, glucose ranged from 3.49 to 3.95 mM during the first 5 days, reduced to 0.17 mM in day 7 and was not detected in day 11. Fructose ranged from 0.65 to 1.29 during the first 5 days and was not detected in days 7 and 11. Lactic acid, acetic acid and ethanol were the metabolites detected (Table 2); at the end of fermentation at 20 °C they reached 42.01, 5.02 and 16.18 mM, respectively, while at the end of fermentation at 30 °C they reached 38.78, 3.29 and 18.39 mM, respectively. On the contrary, glycerol was not detected throughout fermentation.

A total of 230 lactic acid bacteria isolates were obtained throughout the study, subjected to RAPD and rep-PCR analyses and effectively separated into many clusters (Fig. 1). Representative strains from each cluster were subjected to sequencing of their 16S-rRNA gene and the resulting phylogenetic affiliation is exhibited in Table 3. The majority of the isolates, i.e. 135 were assigned to *Lactobacillus plantarum*, 77 to *Pediococcus pentosaceus* and 18 to *Lactobacillus brevis*.

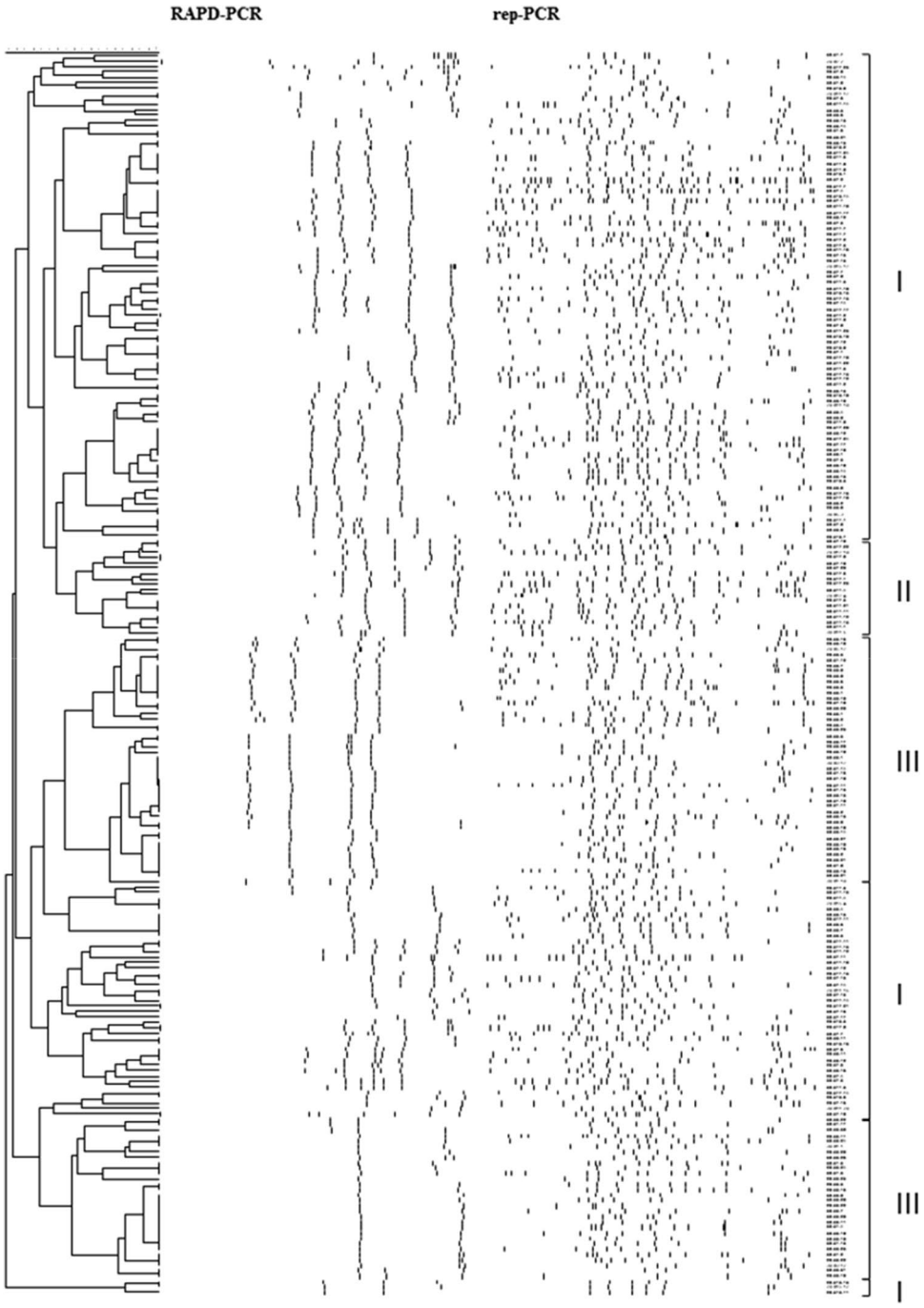

Fig. 1 Cluster analysis of rep-PCR and RAPD-PCR patterns of LAB isolates. Distance is indicated by the mean correlation coefficient [*r* (%)] and clustering was performed by UPGMA analysis. Strain origin is indicated by the Latin numerals; the first indicates the fermentation temperature (20: fermentation at 20 °C; 30: fermentation at 30 °C), the second the day of isolation (d1–d17) and the third the isolate number. Representative strains selected for 16S-rRNA sequencing are marked in *bold*. Latin numerals designate lactic acid bacteria species (I: *Lb. plantarum*, II: *Lb. brevis*, III: *Pd. pentosaceus*)

In Table 4, the Simpson's index of diversity of the typing techniques applied is shown. rep-PCR resulted in optimal differentiation; isolates assigned to the same species produced several similar but not identical genotypic profiles, therefore the Simpson's index was 1. On the contrary, several identical genotypic profiles were generated by RAPD-PCR analysis and thus the Simpson's index was <1.

In Fig. 2, the population dynamics during the spontaneous radish fermentation at 20 and 30 °C is presented. In both temperatures *Pd. pentosaceus* prevailed the microecosystem during the first days of fermentation. Then domination of *Lb. plantarum* was observed during the seventh day of fermentation at 20 °C and the fifth day of fermentation at 30 °C. Finally, *Lb. brevis* was detected during the final day (17th) of fermentation at 20 °C and during the seventh and 11th days of fermentation at 30 °C.

Table 3 Phylogenetic affiliation of selected strains based on sequencing of the 16S-rRNA gene

Strain number	Closest relative	Query cover (%)	Identity (%)	Accession number
20.d11.10	*Lactobacillus plantarum*	98	99	KX388384
20.d11.17	*Lactobacillus plantarum*	98	99	KX388384
20.d11.18	*Lactobacillus plantarum*	99	99	AB973176
20.d15.12	*Lactobacillus plantarum*	98	99	KP317711
20.d15.17	*Lactobacillus plantarum*	98	99	KX388384
20.d15.4	*Lactobacillus plantarum*	98	99	KX388384
20.d7.2	*Lactobacillus plantarum*	98	99	KP317711
30.d11.20	*Lactobacillus plantarum*	98	99	KF806536
30.d5.3	*Lactobacillus plantarum*	98	99	KP388384
20.d7.17	*Pediococcus pentosaceus*	99	100	KU933533
30.d1.1	*Pediococcus pentosaceus*	98	99	KX377684
30.d1.10	*Pediococcus pentosaceus*	99	100	KU933533
30.d3.12	*Pediococcus pentosaceus*	99	99	KU933533
30.d3.17	*Pediococcus pentosaceus*	98	99	KX377684
20.d17.10	*Lactobacillus brevis*	97	99	KU746859
20.d17.4	*Lactobacillus brevis*	97	99	KU746859
30.d11.6	*Lactobacillus brevis*	99	99	KX000271

Table 4 Simpson's index of diversity of the genotyping techniques applied

Species	Number of isolates	Method	
		RAPD-PCR	rep-PCR
Lactobacillus plantarum	135	0.992	1
Pediococcus pentosaceus	77	0.941	1
Lactobacillus brevis	18	0.967	1

Discussion

The factors that determine the outcome of spontaneous fermentation of fruits and vegetables include abiotic, such as pH value, salt concentration and temperature, and biotic ones, such as the indigenous micro-communities of the raw materials. In the case of fresh fruits and vegetables, yeasts/molds and Gram-negative aerobic bacteria have been reported to dominate the micro-ecosystem (Harris 1998; Paramithiotis et al. 2017a). This was also the case in the present study and was reflected in the composition of the micro-ecosystem during day 0, i.e. upon placing of the radish roots in the brine solution.

As fermentation proceeds, lactic acid bacteria population increases from as low as 2 log CFU/mL or even below enumeration limit, as in the case of the present study, to 7–9 log CFU/mL, due to their metabolic capacity, dominating thus the microecosystem (Sesena and Palop 2007; Wouters et al. 2013a). As a result, pH value is reduced and acidity is developed. At the same time the remaining microbial populations diminish due to the effect of pH,

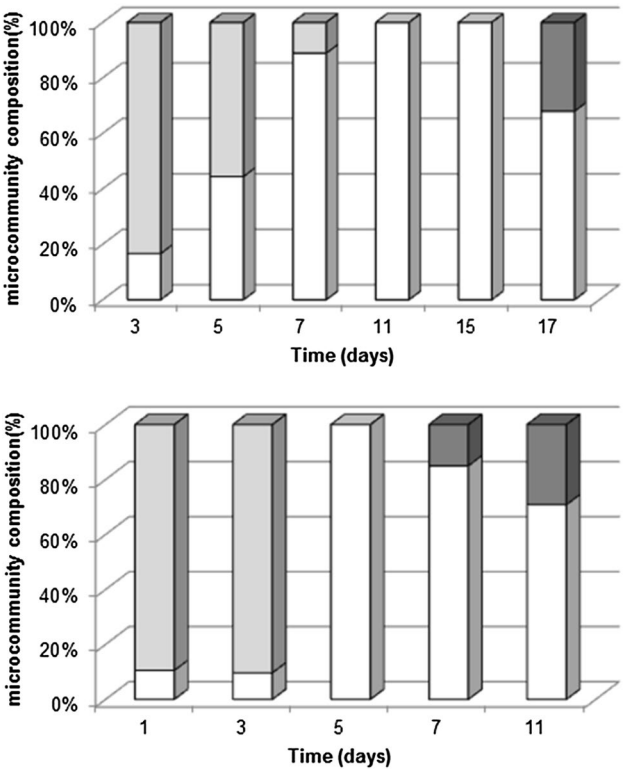

Fig. 2 Population dynamics of *Lb. plantarum* (*white bars*), *Pd. pentosaceus* (*light grey bars*) and *Lb. brevis* (*dark grey bars*) during spontaneous fermentation of radish roots at 20 °C (upper *graph*) and 30 °C (*lower graph*)

acidity and antagonism for nutrients (Pulido et al. 2005; Paramithiotis et al. 2010, 2014a, b; Wouters et al. 2013a, b; Maifreni et al. 2004). This was also the case in the present

study. The final pH and TTA values ranged within the ones usually observed in such fermentations and were justified by the population of the lactic acid bacteria (Paramithiotis et al. 2017b) At higher fermentation temperature (30 °C), faster reduction of the pH value and development of acidity were observed, most likely due to the faster development of the lactic acid microbiota, which concomitantly resulted in faster reduction of pseudomonads, *Enterobacteriaceae* and yeasts/molds population. On the contrary, enterococci population remained at the level of 3–5 log CFU/mL due to their tolerance to acidic conditions that has been adequately exhibited (Fisher and Phillips 2009).

Glucose and fructose were the main carbohydrates detected during fermentation, in accordance to the literature (Masalkar and Keskar 1998). The end-products of their catabolism were lactic acid, acetic acid and ethanol. Production of lactic acid may be assigned to homofermentative metabolism by lactic acid bacteria and accumulation of acetic acid and ethanol to heterofermentative catabolism by lactic acid bacteria as well as yeasts.

The structure and dynamics of the lactic acid bacteria microcommunity was monitored with RAPD and rep-PCR, an approach commonly applied in similar studies. The former has been extensively used for clustering and differentiation of LAB from a variety of sources (Fontana et al. 2005; Rossetti and Giraffa 2005; Banwo et al. 2012) whereas rep-PCR with $(GTG)_5$ as primer is currently well-known for the discriminatory efficiency at sub-species level (Gevers et al. 2001). 16S rRNA gene sequencing has been extensively used in phylogenetic studies. However, differentiation of closely related species cannot be reliably achieved though sequencing of such a highly conserved genomic region. This is the case of the *Lb. plantarum* group. This group includes six species, namely *Lb. plantarum, Lactobacillus pentosus, Lactobacillus paraplantarum, Lactobacillus fabifermentans, Lactobacillus xiangfangensis* and *Lactobacillus mudanjiangensis* (Gu et al. 2013). In order to accurately assign the phylogenetic affiliation of an isolate within this group, several protocols based on specific PCR have been proposed; with the one developed by Huang et al. (2016) being the latest. In the present study, no such protocol was applied, thus it would be more accurate to refer to these strains as belonging to *Lb. plantarum*-group instead of belonging to *Lb. plantarum* species that was the closest relative in all cases.

At species level, a rather limited LAB biodiversity was revealed during this study. *Lb. plantarum, Lb. brevis* and *Pd. pentosaceus* are among the species that immensely contribute in the fermentation of several fruits and vegetables such as cucumber (Singh and Ramesh 2008), eggplant (Nguyen et al. 2013), caper berries (Pulido et al. 2005) cauliflower (Wouters et al. 2013b), suan-tsai (Chao et al. 2009), sauerkraut (Barrangou et al. 2002; Plengvidhya et al. 2007; Wiander 2017) and kimchi (Cho et al. 2006; Kim and Chun 2005; Lee et al. 2005; Park et al. 2003). Moreover, *Lb. plantarum* and *Lb. brevis* have been reported to dominate several fermentations including cucumber (Tamang et al. 2005), Almagro eggplant (Sesena and Palop 2007) and inziangsang (Tamang et al. 2005) whereas *Pd. pentosaceus* has been reported to prevail in Suan-tsai fermentation (Chen et al. 2006). This was also the case of sinki, a product prepared by pit fermentation of radish roots. Tamang and Sarkar (1993) reported that *Lb. fermentum* initiated the fermentation and substituted sequentially by *Lb. brevis* and *Lb. plantarum*. On the other hand, Tamang et al. (2005) analyzed 12 sinki samples and isolated *Lb. brevis* and *Leuconostoc fallax*. Generally, *Lb. plantarum* is mostly associated with the final stages of fermentation, mostly due to the large metabolic capacity that distinguishes it (Daeschel et al. 1987). On the other hand, occurrence of *Pd. pentosaceus* and *Lb. brevis* in such fermentations is mostly associated with their ability to grow under stressful conditions. The fermentation temperature had no effect on the composition of the lactic acid microecosystem; nonetheless it accelerated the succession at species level.

The commonly reported succession at species level (Paramithiotis et al. 2010, 2014a, b; Wouters et al. 2013a; Chao et al. 2009; Plengvidhya et al. 2007; Cho et al. 2006; Lee et al. 2005; Sesena and Palop 2007; Yeun et al. 2013; Chang et al. 2008) that was observed in the present study was accompanied by a respective at subspecies level. The latter is also frequently reported when a combination of typing techniques is applied (Paramithiotis et al. 2014a, b) providing with an insight to the development of the respective spontaneous micro-ecosystem.

Over-viewing the results obtained in the present study, LAB dominate spontaneous fermentation of radish roots. Fermentation was driven by *Pd. pentosaceus* during the first days and *Lb. plantarum* during the rest of fermentation. *Lb. brevis* was also detected during the final days of fermentation. A succession at sub-species level took place in parallel to the respective at species level.

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