

## TECHNOLOGICAL PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM TRADITIONAL PICKLES

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### ABSTRACT

In order to select eligible strains as starter cultures for the production of pickles and other fermented vegetable products, the technological characterization of the 75 lactic acid bacteria (LAB) strains was performed on the basis of salt tolerance, growth at different pH values, acid production ability, enzymatic profile and biogenic amine production. The technological characterization revealed that, among the tested LAB species (*Lactobacillus plantarum*, *L. brevis*, *L. buchneri*, *L. namurensis*, *L. parabrevis*, *Pediococcus ethanolidurans*, *P. parvulus* and *Enterococcus casseliflavus*), *L. plantarum* species showed the most suitable characteristics. The investigated *L. plantarum* strains, except for one strain (MF219), could be considered as potential starter cultures because of their desirable properties of having high rate and extent of acidification, high tolerance to pH 4.0 and 10% NaCl, and non-production of biogenic amines. It was also important to note that the tested *P. ethanolidurans* strains showed high salt tolerance and acid production. Most of the tested strains shared similar enzymatic characteristics including absence of proteolytic and lipolytic activities, and presence of peptidase, glucosidase and galactosidase activities.

**Keywords:** Lactic acid bacteria, autochthonous, pickle, starter culture, technological property, enzymatic profile

## GELENEKSEL TURŞULARDAN İZOLE EDİLEN LAKTİK ASİT BAKTERİLERİNİN TEKNOLOJİK ÖZELLİKLERİ

### ÖZ

Turşu ve diğer fermente sebze ürünleri için uygun starter kültür seçimi amacıyla, 75 adet laktik asit bakterisi (LAB) suşunun tuza dayanıklılık, farklı pH değerlerinde gelişme, asit üretim yeteneği, enzimatik profil ve biyojen amin üretimi gibi teknolojik özellikleri araştırılmıştır. Test edilen LAB türleri (*Lactobacillus plantarum*, *L. brevis*, *L. buchneri*, *L. namurensis*, *L. parabrevis*, *Pediococcus ethanolidurans*, *P. parvulus* and *Enterococcus casseliflavus*) arasında *L. plantarum* suşlarının en uygun teknolojik özelliklere sahip olduğu görülmüştür. Bir suş (MF219) dışındaki tüm *L. plantarum* suşlarının, yüksek asit üretim hızı ve yetenekleri, pH 4.0 ve %10 NaCl'e karşı dayanıklılık ve biyojen amin üretmeme gibi, starter için arzulanan özelliklere sahip oldukları belirlenmiştir. Çalışmada denenen *P. ethanolidurans* suşlarının yüksek tuz toleransına ve asit üretim yeteneğine sahip oldukları da belirlenmiştir. Deneme kapsamındaki LAB suşlarının peptidaz, glikozidaz ve galaktozidaz aktivitelerinin varlığı, proteolitik ve lipolitik aktivitelerinin bulunmaması gibi, enzimatik aktivite yönünden benzer özelliklere sahip oldukları görülmüştür.

**Anahtar kelimeler:** Laktik asit bakterileri, yerel; turşu, starter kültür, teknolojik özellik, enzimatik profili

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## INTRODUCTION

Pickles refer, in the most general sense, to any vegetable or fruit that is stabilised with salt and lactic acid produced by lactic acid bacteria (LAB) (Hutkins, 2006; Çon and Karasu, 2009). Cucumbers, cabbages, green peppers, green tomatoes and carrots are the most common vegetables used for pickling in Turkey (Çetin, 2011; Kabak and Dobson, 2011). These plant-based substrates harbor not only LAB responsible for lactic fermentation, but also undesirable microbial communities that could cause several technological problems and product defects such as bloating, floating and softening (Hutkins, 2006; Wouters et al., 2013b). However, the harsh conditions of pickle environment, typically characterized by high concentrations of salt and organic acids and low pH (less than 4.5), are inhibitory to coliforms, pseudomonads, bacilli, clostridia, and other non-lactic acid bacteria, while favourable for lactic acid bacteria (Hutkins, 2006).

The manufacture of pickles still relies on a system of small and medium enterprises, with uncontrolled fermentation. The fermentation of pickles, in contrast to meat and dairy products, is a natural and spontaneous process that occurs on the raw material by the indigenous lactic flora (Hutkins, 2006; Bağder Elmacı et al., 2015). However, spontaneous fermentation can lead to variations in the sensory quality, the safety, and the stability of the final product, since the indigenous LAB flora varies depending on the quality of the raw material, temperature and harvesting conditions (Gardner et al., 2001; Bevilacqua et al., 2010). The main obstacles with regard to the application of starter cultures in fermented vegetables and fruits are the natural microbial succession that occurs during the course of fermentation and the inability to inactivate endogenous microbiota by pasteurization without causing adverse effects to the product texture (Josephsen and Jespersen, 2004; Wouters et al., 2013a,b). Nevertheless, the use of starter cultures for the production of fermented pickles is becoming increasingly necessary to ensure safety and to standardize product properties (Bonomo et al., 2008; Essid et al., 2009). It will also be of great interest if the

appropriate starter cultures are selected from indigenous LAB of traditional pickles, since these strains are more competitive and well adapted to stressful conditions of the pickle, in comparison with industrial bulk starters, often from various origins. In addition, because of their high metabolic capacities, they can beneficially affect product quality and safety, preserving the typical sensory quality of traditional fermented product (Bonomo et al., 2008; Beganović et al., 2014).

The selection of starter cultures for use in controlled fermentation of vegetables is based on several criteria, including minimum nutritional requirements, ability to grow at low temperatures, ability to ferment diverse carbohydrate substrates, ability to compete against wide array of organisms, ability to produce desirable flavor, rapid growth and acid production, tolerance to acids, low pH, salt and antimicrobial phenolics, resistance to bacteriophage, lack of pectinolytic activity, inability to produce dextrans or other polysaccharides and biogenic amines and minimum loss of viability during storage (Hutkins, 2006).

In Turkey, the use of starter culture in the production of pickles is not a common practice. In addition, only a few studies have attempted to study the selection of appropriate starter cultures for pickle fermentation (e.g., Çon and Karasu, 2009; Karasu et al., 2010). In our recent study (Bağder Elmacı et al., 2015), we isolated and identified 152 LAB strains from fermented pickles produced in Çubuk region of Ankara, Turkey. Seventy five of these, which were selected based on their growth ability in MRS broth, were evaluated for some important technological traits, including growth at different salt concentrations and pH values, the rate and extent of acid production, enzymatic profile and biogenic amine production. In this way, it was aimed to select autochthonous LAB strains that can be potential candidates as starter cultures in the manufacture of pickles and other fermented vegetable products.

## MATERIAL AND METHODS

### Bacterial strains

Seventy five indigenous LAB strains isolated from pickles produced in Ankara-Çubuk region were used in this study. The tested strains included 26 *L. plantarum*, 10 *L. brevis*, 6 *L. buchneri*, 2 *L. namurensis*, 1 *L. parabrevis*, 24 *P. etbanolidurans*, 5 *P. parvulus* and 1 *E. casseliflavus* strains which were previously identified by molecular methods, and maintained in the Culture Collection of the Food Engineering Department of Ankara University. The GenBank accession numbers for the 16S rRNA gene sequences of the strains were reported previously (Bağder Elmacı et al., 2015).

### Growth at different salt concentrations

The LAB strains were cultured in MRS broth at 30 °C for 24 h. Aliquots of the culture broth (5 µL) were then inoculated into 5 mL of MRS broth containing 0%, 3%, 6.5%, 10% or 12% NaCl. After 1 week of incubation at 30 °C, bacterial growth was evaluated as absorbance values at 600 nm by using a spectrophotometer (UV-1208 Shimadzu, Japan). The spectrophotometer was set to zero by using uninoculated MRS broth (Chao et al., 2009).

### Growth at different pH values

Five milliliter aliquots of MRS broth adjusted to pH values of 2.0, 3.0, 4.0, 5.0, 6.5 or 9.6 by the addition of 2 N NaOH or 2 N HCl were inoculated with active LAB cultures at an inoculum size of 0.1% (v/v). After incubation at 30 °C for 48 h, bacterial growth was evaluated as absorbance values at 600 nm (Vinderola and Reinheimer, 2003).

### The rate and extent of acid production

Ten milliliter aliquots of MRS broth were inoculated with active LAB cultures at an inoculum size of 0.1% (v/v). The culture tubes were incubated at 30 °C, and the acid production was determined by measuring the titratable acidity on the 24<sup>th</sup> and 48<sup>th</sup> hour of incubation. The titratable acidity, expressed as g lactic acid/100 mL, was calculated by titrating the culture broth with 0.01 N NaOH with 0.1% (w/v) phenolphthalein as the indicator.

### Enzymatic profile

The enzymatic profile of LAB strains were assayed using commercial API-ZYM galleries (BioMérieux, France) following the manufacturer's instructions.

### Production of biogenic amines

Production of biogenic amines from histidine, lysine and tyrosine was assessed by the improved medium described by Bover-Cid and Holzapfel (1999). The result was considered as positive for biogenic amine production if the colour of the medium changed from yellow to purple-violet.

### Statistical analyses

All experiments were conducted in two biological replicates, each with two technical replicates. Experimental data were analysed with one- or two-way ANOVA using the Minitab statistical software, version 14 (Minitab Inc., State College, PA, USA). Statistical differences among means were determined by the Duncan's multiple range tests at the 5% significance level.

## RESULTS AND DISCUSSION

### Growth at different salt concentrations

During vegetable fermentations, one of the important stress conditions to which LAB are exposed is high salt concentrations, and therefore high osmotic pressures (Hutkins, 2006). The salt concentration is a major environmental factor which influences the type and numbers of microorganisms carrying out the fermentation (Daeschel and Fleming, 1984; Reina et al., 2005). In cabbage fermentations where low salt (approximately 2% NaCl) is prevalent, the fermentation is initiated by heterofermentative LAB such as *Leuconostoc* or *Weissella* spp., and followed by homofermentors. High salt concentration, which is typical for cucumber and olive fermentations (6% or greater NaCl), favors the growth of homofermentative LAB such as *L. plantarum* (Reina et al., 2005).

Table 1 shows the results for the growth at different NaCl concentrations (0%, 3%, 6.5%, 10% and 12%). Within each data set referred to each LAB species, there were significant two-way interactions between LAB strains and salt

concentration for OD<sub>600</sub> values ( $P < 0.05$ ). Almost all tested strains were able to grow in the presence of 3% and 6.5% NaCl, with the exception of a few strains of *E. casseliflavus* and *L. buchneri* which were sensitive to 6.5% NaCl. Most *E. casseliflavus*, *L. brevis*, *L. buchneri*, *L. namurensis*, *L. parabrevis* and *P. parvulus* strains tested showed weak or no growth at 10% NaCl whereas *L. plantarum* and *P. ethanolidurans* strains were able to maintain growth to different extent. In accordance with the present results, Seseña et al. (2005) reported that *L. plantarum* and *L. pentosus* strains obtained from brines of spontaneous fermentation of “Almagro” eggplants showed higher resistance to salt compared to *L. brevis* and *L. fermentum* strains. Similarly, Papamanoli et al. (2003) reported that *L. plantarum* strains which were isolated from naturally fermented dry sausages could grow at 6.5-10% NaCl. On the other hand, Karasu et al. (2010) showed that *L. plantarum* strains isolated from traditionally produced fermented vegetables tolerated up to 8% NaCl. The highest salt concentration tested (12%) exerted a strong effect on LAB strains, as most of the tested strains were completely or significantly inhibited. Among *P. ethanolidurans* strains, resistance to 12% NaCl was significantly higher ( $P < 0.05$ ) in strains MF11, MF196, MF167, MF48 and MF194, with OD<sub>600</sub> values greater than 0.100. For all LAB species tested, the salt tolerance appeared as a strain-dependent property, as it varied significantly among strains within the same species. This suggestion is in agreement with the previous studies (Papamanoli et al., 2003; Benito et al., 2007). However, at the species level, the majority of *L. plantarum* and *P. ethanolidurans* strains showed higher resistance to salt in comparison with *E. casseliflavus*, *L. brevis*, *L. buchneri*, *L. namurensis*, *L. parabrevis* and *P. parvulus* strains.

#### Growth at different pH values

Table 2 represents the ability of the LAB strains to grow at pH values ranging between 2.0 and 9.6 after 48 h of incubation. Within each set of 8 LAB species, there was a significant two-way

interaction for OD<sub>600</sub> values of growth due to LAB strains and pH values ( $P < 0.05$ ). Apart from a few *L. plantarum* strains showing scanty growth, most of the strains could not tolerate the lowest pH values tested, 2.0 and 3.0. Concerning the growth at pH 4.0, almost all tested strains showed varying levels of growth. In particular, *L. plantarum* strains appeared to be the most resistant to acidic conditions, with OD<sub>600</sub> values ranging from 1.266 to 1.916 at pH 4.0. The high acid tolerance of the tested *L. plantarum* strains makes them promising candidates as starter cultures for the production of pickles where the pH varies between 3.1 and 3.5 (Di Cagno et al., 2013). The tolerance of this species to acidic environment is attributed to their ability to maintain pH homeostasis at low external pH. Therefore, *L. plantarum* usually predominates at the end of most vegetable fermentations (McDonald et al., 1990; Mäkimattila et al., 2011). The highest growth was observed at pH 5.0 and 6.5 for all tested strains of each species, with the exception of two strains (*E. casseliflavus* MF535, *L. namurensis* MF275). With a few rare exceptions (*E. casseliflavus* MF535, *L. namurensis* MF275), the tested LAB strains were not able to grow at pH 9.6. In addition, a few *L. plantarum* strains showed scanty growth at pH 9.6, with OD<sub>600</sub> values lower than 0.100. The ability to grow at pH 9.6 is one of the tolerance tests used for the identification of LAB species. In accordance with our results, it is known that *Enterococcus* can grow at pH 9.6, whereas *Lactobacillus* and *Pediococcus* cannot (Axelsson, 2004). From the technological point of view, the ability to grow at extreme alkaline pH (~10) could also be an important selection criteria for the LAB strains intended for use in table olives, since this pH can be found in olive brines throughout the lye treatment or in the first fermentation phase (Bevilacqua et al., 2010; Heperkan, 2013).

Table 1. Growth (OD<sub>600</sub> value) of LAB strains isolated from pickles in MRS broth containing different concentrations of NaCl

| Spc          | Strain no.                | 0% NaCl                       | 3% NaCl                      | 6.50% NaCl                   | 10% NaCl                    | 12% NaCl                  |
|--------------|---------------------------|-------------------------------|------------------------------|------------------------------|-----------------------------|---------------------------|
| <i>E. ca</i> | MF535                     | 0.851±0.021 <sup>n.d.</sup>   | 0.642±0.004 <sup>n.d.</sup>  | 0.000 <sup>n.d.</sup>        | 0.000 <sup>n.d.</sup>       | 0.000 <sup>n.d.</sup>     |
|              | MF105                     | 2.098±0.005 <sup>ABa</sup>    | 1.727±0.024 <sup>BCDb</sup>  | 1.586±0.004 <sup>CDB</sup>   | 0.142±0.032 <sup>ABc</sup>  | 0.020±0.003 <sup>Ac</sup> |
|              | MF158                     | 2.067±0.030 <sup>ABa</sup>    | 1.562±0.018 <sup>DEB</sup>   | 1.694±0.008 <sup>BCb</sup>   | 0.095±0.011 <sup>ABc</sup>  | 0.000 <sup>Ac</sup>       |
|              | MF243                     | 2.079±0.013 <sup>ABa</sup>    | 1.749±0.032 <sup>BCb</sup>   | 1.621±0.006 <sup>BCb</sup>   | 0.106±0.002 <sup>ABc</sup>  | 0.000 <sup>Ac</sup>       |
|              | MF250                     | 2.201±0.006 <sup>Aa</sup>     | 1.615±0.028 <sup>CDc</sup>   | 2.014±0.000 <sup>Ab</sup>    | 0.011±0.006 <sup>Bd</sup>   | 0.010±0.013 <sup>Ad</sup> |
|              | MF314                     | 2.165±0.000 <sup>ABa</sup>    | 1.721±0.004 <sup>BCDb</sup>  | 1.425±0.114 <sup>Dc</sup>    | 0.131±0.021 <sup>ABd</sup>  | 0.021±0.000 <sup>Ad</sup> |
|              | MF343                     | 2.139±0.005 <sup>ABa</sup>    | 1.737±0.011 <sup>BCDb</sup>  | 1.628±0.004 <sup>BCb</sup>   | 0.252±0.007 <sup>Ac</sup>   | 0.012±0.003 <sup>Ad</sup> |
|              | MF354                     | 2.161±0.006 <sup>ABa</sup>    | 1.836±0.008 <sup>ABb</sup>   | 1.798±0.002 <sup>Bb</sup>    | 0.168±0.059 <sup>ABc</sup>  | 0.029±0.004 <sup>Ac</sup> |
|              | MF493                     | 2.232±0.000 <sup>Aa</sup>     | 1.962±0.011 <sup>Ab</sup>    | 1.445±0.013 <sup>Dc</sup>    | 0.053±0.013 <sup>Bd</sup>   | 0.032±0.002 <sup>Ad</sup> |
|              | MF494                     | 2.108±0.009 <sup>ABa</sup>    | 1.903±0.009 <sup>ABb</sup>   | 1.664±0.556 <sup>BCc</sup>   | 0.075±0.018 <sup>ABd</sup>  | 0.000 <sup>Ad</sup>       |
| MF531        | 1.979±0.014 <sup>Ba</sup> | 1.432±0.085 <sup>Eb</sup>     | 2.116±0.049 <sup>Aa</sup>    | 0.018±0.013 <sup>Bc</sup>    | 0.014±0.019 <sup>Ac</sup>   |                           |
| <i>L. bu</i> | MF12                      | 2.150±0.000 <sup>Aa</sup>     | 1.995±0.000 <sup>Ab</sup>    | 0.858±0.040 <sup>Cc</sup>    | 0.010±0.012 <sup>Ad</sup>   | 0.000 <sup>Ad</sup>       |
|              | MF102                     | 2.034±0.033 <sup>Ca</sup>     | 1.876±0.011 <sup>Cb</sup>    | 0.139±0.040 <sup>Dc</sup>    | 0.008±0.011 <sup>Ad</sup>   | 0.000 <sup>Ad</sup>       |
|              | MF114                     | 2.063±0.008 <sup>BCa</sup>    | 1.948±0.004 <sup>ABb</sup>   | 0.021±0.001 <sup>Ec</sup>    | 0.000 <sup>Ac</sup>         | 0.000 <sup>Ac</sup>       |
|              | MF117                     | 2.121±0.000 <sup>ABa</sup>    | 1.962±0.017 <sup>ABb</sup>   | 0.064±0.005 <sup>Ec</sup>    | 0.000 <sup>Ac</sup>         | 0.000 <sup>Ac</sup>       |
|              | MF271                     | 2.076±0.026 <sup>BCa</sup>    | 1.907±0.004 <sup>BCb</sup>   | 1.473±0.136 <sup>Bc</sup>    | 0.000 <sup>Ad</sup>         | 0.000 <sup>Ad</sup>       |
|              | MF272                     | 1.967±0.024 <sup>Da</sup>     | 1.860±0.000 <sup>Cb</sup>    | 1.729±0.022 <sup>Ac</sup>    | 0.000 <sup>Ad</sup>         | 0.000 <sup>Ad</sup>       |
| <i>L. nu</i> | MF192                     | 2.003±0.011 <sup>a</sup>      | 1.610±0.075 <sup>b</sup>     | 0.836±0.032 <sup>c</sup>     | 0.055±0.008 <sup>d</sup>    | 0.067±0.030 <sup>d</sup>  |
|              | MF275                     | 2.048±0.004 <sup>a</sup>      | 1.315±0.089 <sup>b</sup>     | 0.769±0.083 <sup>c</sup>     | 0.658±0.081 <sup>c</sup>    | 0.119±0.044 <sup>d</sup>  |
| <i>L. pd</i> | MF231                     | 2.081±0.000 <sup>n.d.</sup>   | 1.812±0.002 <sup>n.d.</sup>  | 1.626±0.009 <sup>n.d.</sup>  | 0.015±0.004 <sup>n.d.</sup> | 0.000 <sup>n.d.</sup>     |
|              | MF4                       | 2.490±0.011 <sup>ABCDEa</sup> | 2.301±0.000 <sup>BCDEb</sup> | 2.185±0.017 <sup>BCDEc</sup> | 1.693±0.013 <sup>Ad</sup>   | 0.036±0.004 <sup>Ac</sup> |
|              | MF33                      | 2.507±0.012 <sup>ABCDa</sup>  | 2.290±0.000 <sup>CDEFb</sup> | 2.146±0.006 <sup>CDEc</sup>  | 1.346±0.051 <sup>Cd</sup>   | 0.050±0.003 <sup>Ac</sup> |
|              | MF99                      | 2.571±0.000 <sup>ABa</sup>    | 2.466±0.000 <sup>Aa</sup>    | 2.223±0.013 <sup>BCDEb</sup> | 1.075±0.049 <sup>Fc</sup>   | 0.034±0.004 <sup>Ad</sup> |
|              | MF118                     | 2.533±0.000 <sup>ABCa</sup>   | 2.270±0.000 <sup>DEFb</sup>  | 2.346±0.000 <sup>Ab</sup>    | 0.854±0.249 <sup>Gc</sup>   | 0.068±0.009 <sup>Ad</sup> |
|              | MF143                     | 2.395±0.018 <sup>DEFa</sup>   | 2.189±0.023 <sup>EFb</sup>   | 2.358±0.000 <sup>Aa</sup>    | 1.272±0.177 <sup>CDc</sup>  | 0.032±0.045 <sup>Ad</sup> |
|              | MF150                     | 2.459±0.011 <sup>BCDEa</sup>  | 2.415±0.010 <sup>ABa</sup>   | 2.146±0.006 <sup>CDEb</sup>  | 1.257±0.047 <sup>CDc</sup>  | 0.100±0.033 <sup>Ad</sup> |
|              | MF169                     | 2.459±0.011 <sup>BCDEa</sup>  | 2.422±0.000 <sup>ABa</sup>   | 2.158±0.022 <sup>BCDEb</sup> | 1.305±0.177 <sup>CDc</sup>  | 0.022±0.002 <sup>Ad</sup> |
|              | MF178                     | 2.499±0.023 <sup>ABCDEa</sup> | 2.265±0.007 <sup>DEFb</sup>  | 2.214±0.013 <sup>BCDEb</sup> | 0.362±0.258 <sup>Kc</sup>   | 0.052±0.005 <sup>Ad</sup> |
|              | MF205                     | 2.552±0.027 <sup>ABa</sup>    | 2.459±0.011 <sup>Aa</sup>    | 2.232±0.000 <sup>BCDb</sup>  | 1.280±0.075 <sup>CDc</sup>  | 0.041±0.008 <sup>Ad</sup> |
| <i>L. pl</i> | MF213                     | 2.323±0.000 <sup>Fa</sup>     | 2.245±0.021 <sup>DEFa</sup>  | 2.210±0.018 <sup>BCDEa</sup> | 1.500±0.014 <sup>Bb</sup>   | 0.033±0.000 <sup>Ac</sup> |
|              | MF219                     | 2.098±0.005 <sup>Ga</sup>     | 1.838±0.011 <sup>Gc</sup>    | 1.948±0.004 <sup>Fb</sup>    | 0.248±0.059 <sup>KLd</sup>  | 0.030±0.018 <sup>Ac</sup> |
|              | MF232                     | 2.451±0.000 <sup>BCDEa</sup>  | 2.459±0.011 <sup>Aa</sup>    | 2.099±0.051 <sup>Eb</sup>    | 0.482±0.045 <sup>Jc</sup>   | 0.016±0.008 <sup>Ad</sup> |
|              | MF239                     | 2.571±0.000 <sup>ABa</sup>    | 2.474±0.011 <sup>Aa</sup>    | 2.140±0.036 <sup>CDEb</sup>  | 0.794±0.190 <sup>Gc</sup>   | 0.015±0.004 <sup>Ad</sup> |
|              | MF265                     | 2.490±0.011 <sup>ABCDEa</sup> | 2.318±0.008 <sup>BCDb</sup>  | 2.260±0.000 <sup>ABCb</sup>  | 0.385±0.073 <sup>Ic</sup>   | 0.046±0.007 <sup>Ad</sup> |
|              | MF303                     | 2.552±0.000 <sup>ABa</sup>    | 2.358±0.000 <sup>ABCb</sup>  | 2.166±0.044 <sup>BCDEc</sup> | 0.308±0.036 <sup>KLd</sup>  | 0.023±0.003 <sup>Ac</sup> |
|              | MF305                     | 2.408±0.000 <sup>CDEFa</sup>  | 2.466±0.000 <sup>Aa</sup>    | 2.166±0.033 <sup>BCDEb</sup> | 0.761±0.174 <sup>Gc</sup>   | 0.063±0.004 <sup>Ad</sup> |
|              | MF322                     | 2.382±0.000 <sup>EFa</sup>    | 2.270±0.000 <sup>DEFb</sup>  | 2.260±0.000 <sup>ABCb</sup>  | 0.369±0.138 <sup>Ic</sup>   | 0.034±0.005 <sup>Ad</sup> |
|              | MF352                     | 2.474±0.011 <sup>BCDEa</sup>  | 2.165±0.000 <sup>Fb</sup>    | 1.853±0.032 <sup>Fc</sup>    | 0.399±0.021 <sup>Ijd</sup>  | 0.045±0.004 <sup>Ac</sup> |
|              | MF357                     | 2.498±0.000 <sup>ABCDEa</sup> | 2.312±0.000 <sup>BCDEb</sup> | 2.214±0.013 <sup>BCDEb</sup> | 1.210±0.034 <sup>DEc</sup>  | 0.023±0.006 <sup>Ad</sup> |
|              | MF376                     | 2.552±0.000 <sup>ABa</sup>    | 2.323±0.000 <sup>BCDb</sup>  | 2.165±0.000 <sup>BCDEc</sup> | 1.123±0.095 <sup>EFd</sup>  | 0.035±0.001 <sup>Ac</sup> |
|              | MF377                     | 2.533±0.000 <sup>ABCa</sup>   | 2.312±0.000 <sup>BCDEb</sup> | 2.162±0.016 <sup>BCDEc</sup> | 1.536±0.001 <sup>Bd</sup>   | 0.065±0.011 <sup>Ac</sup> |
|              | MF380                     | 2.474±0.011 <sup>BCDEa</sup>  | 2.291±0.015 <sup>CDEFb</sup> | 2.280±0.000 <sup>ABb</sup>   | 0.626±0.138 <sup>Hc</sup>   | 0.000 <sup>Ad</sup>       |
|              | MF404                     | 2.482±0.000 <sup>ABCDEa</sup> | 2.335±0.016 <sup>BCDb</sup>  | 1.691±0.083 <sup>Gc</sup>    | 0.872±0.028 <sup>Gd</sup>   | 0.058±0.006 <sup>Ac</sup> |
|              | MF513                     | 2.571±0.000 <sup>ABa</sup>    | 2.482±0.000 <sup>Aa</sup>    | 2.150±0.021 <sup>CDEb</sup>  | 1.097±0.007 <sup>EFc</sup>  | 0.020±0.001 <sup>Ad</sup> |
|              | MF548                     | 2.498±0.000 <sup>ABCDEa</sup> | 2.408±0.000 <sup>ABCa</sup>  | 2.143±0.011 <sup>CDEb</sup>  | 0.204±0.028 <sup>Lc</sup>   | 0.026±0.004 <sup>Ad</sup> |
|              | MF556                     | 2.602±0.016 <sup>Aa</sup>     | 2.251±0.027 <sup>DEFb</sup>  | 2.125±0.005 <sup>DEc</sup>   | 1.353±0.023 <sup>Cd</sup>   | 0.067±0.001 <sup>Ac</sup> |

Table 1 continued

|              |       |                                |                              |                            |                              |                             |
|--------------|-------|--------------------------------|------------------------------|----------------------------|------------------------------|-----------------------------|
|              | MF11  | 2.033±0.000 <sup>GHIa</sup>    | 1.836±0.008 <sup>CDEb</sup>  | 0.992±0.010 <sup>FGc</sup> | 1.016±0.081 <sup>FGc</sup>   | 0.391±0.042 <sup>Ad</sup>   |
|              | MF14  | 2.072±0.013 <sup>FGHIa</sup>   | 1.915±0.003 <sup>ABCDb</sup> | 1.456±0.040 <sup>CDc</sup> | 1.294±0.005 <sup>BCd</sup>   | 0.000 <sup>De</sup>         |
|              | MF48  | 2.045±0.008 <sup>FGHIa</sup>   | 1.983±0.047 <sup>ABCa</sup>  | 1.293±0.004 <sup>Eb</sup>  | 1.158±0.001 <sup>CDEc</sup>  | 0.140±0.025 <sup>BCDd</sup> |
|              | MF50  | 2.128±0.010 <sup>DEFGHa</sup>  | 1.853±0.011 <sup>CDEb</sup>  | 1.110±0.017 <sup>Fd</sup>  | 1.311±0.013 <sup>ABc</sup>   | 0.000 <sup>De</sup>         |
|              | MF78  | 1.890±0.008 <sup>JKa</sup>     | 1.396±0.021 <sup>Gb</sup>    | 0.493±0.042 <sup>Id</sup>  | 0.651±0.023 <sup>Hc</sup>    | 0.000 <sup>De</sup>         |
|              | MF82  | 1.954±0.000 <sup>IJKa</sup>    | 1.522±0.006 <sup>FGb</sup>   | 0.508±0.037 <sup>Ic</sup>  | 0.541±0.077 <sup>Hc</sup>    | 0.026±0.000 <sup>Dd</sup>   |
|              | MF83  | 1.874±0.002 <sup>Ka</sup>      | 1.488±0.001 <sup>FGb</sup>   | 0.538±0.159 <sup>Ic</sup>  | 0.310±0.000 <sup>Id</sup>    | 0.011±0.005 <sup>De</sup>   |
|              | MF86  | 1.864±0.006 <sup>Ka</sup>      | 1.472±0.022 <sup>FGb</sup>   | 0.430±0.099 <sup>Ic</sup>  | 0.298±0.038 <sup>Id</sup>    | 0.000 <sup>De</sup>         |
|              | MF107 | 2.223±0.000 <sup>ABCDEa</sup>  | 1.927±0.000 <sup>ABCDb</sup> | 1.775±0.035 <sup>Bc</sup>  | 1.329±0.040 <sup>ABd</sup>   | 0.071±0.000 <sup>CDc</sup>  |
|              | MF115 | 2.312±0.000 <sup>Aa</sup>      | 1.858±0.003 <sup>BCDEb</sup> | 0.943±0.016 <sup>Gd</sup>  | 1.289±0.025 <sup>BCc</sup>   | 0.049±0.022 <sup>De</sup>   |
|              | MF136 | 2.232±0.000 <sup>ABCDEa</sup>  | 1.927±0.000 <sup>ABCDb</sup> | 1.372±0.189 <sup>DEc</sup> | 0.653±0.015 <sup>Hd</sup>    | 0.087±0.001 <sup>CDc</sup>  |
| <i>P. et</i> | MF167 | 2.214±0.000 <sup>ABCDEa</sup>  | 1.886±0.008 <sup>ABCDb</sup> | 1.104±0.139 <sup>Fc</sup>  | 1.162±0.028 <sup>CDEc</sup>  | 0.195±0.033 <sup>BCd</sup>  |
|              | MF179 | 2.237±0.020 <sup>ABCDa</sup>   | 1.734±0.033 <sup>Eb</sup>    | 0.924±0.011 <sup>GHd</sup> | 1.428±0.011 <sup>Ac</sup>    | 0.035±0.001 <sup>De</sup>   |
|              | MF180 | 2.173±0.011 <sup>BCDEFa</sup>  | 1.955±0.006 <sup>ABCDb</sup> | 1.372±0.030 <sup>DEc</sup> | 1.352±0.049 <sup>ABc</sup>   | 0.000 <sup>Dd</sup>         |
|              | MF183 | 2.005±0.000 <sup>HIIa</sup>    | 1.813±0.020 <sup>DEb</sup>   | 1.264±0.048 <sup>Ec</sup>  | 0.916±0.014 <sup>Gd</sup>    | 0.000 <sup>De</sup>         |
|              | MF185 | 2.027±0.000 <sup>GHIa</sup>    | 1.591±0.006 <sup>Fb</sup>    | 0.813±0.018 <sup>Hd</sup>  | 1.114±0.023 <sup>EFc</sup>   | 0.015±0.004 <sup>De</sup>   |
|              | MF187 | 2.181±0.000 <sup>ABCDEFa</sup> | 1.950±0.000 <sup>ABCDb</sup> | 1.534±0.260 <sup>Cc</sup>  | 1.142±0.035 <sup>DEFd</sup>  | 0.056±0.037 <sup>De</sup>   |
|              | MF194 | 2.101±0.000 <sup>EFGHa</sup>   | 1.847±0.003 <sup>CDEb</sup>  | 1.248±0.033 <sup>Ec</sup>  | 1.135±0.006 <sup>DEFc</sup>  | 0.119±0.043 <sup>BCDd</sup> |
|              | MF196 | 2.161±0.006 <sup>BCDEFGa</sup> | 1.899±0.021 <sup>ABCDb</sup> | 1.032±0.095 <sup>FGd</sup> | 1.223±0.001 <sup>BCDEc</sup> | 0.237±0.072 <sup>Be</sup>   |
|              | MF214 | 2.135±0.000 <sup>CDEFGHa</sup> | 2.025±0.012 <sup>Ab</sup>    | 1.937±0.039 <sup>Ab</sup>  | 0.316±0.016 <sup>Ic</sup>    | 0.045±0.007 <sup>Dd</sup>   |
|              | MF229 | 2.143±0.011 <sup>CDEFGHa</sup> | 1.962±0.004 <sup>ABCb</sup>  | 1.837±0.038 <sup>ABc</sup> | 0.104±0.002 <sup>Jd</sup>    | 0.007±0.008 <sup>Dd</sup>   |
|              | MF230 | 1.851±0.030 <sup>Ka</sup>      | 1.573±0.035 <sup>Fb</sup>    | 1.109±0.436 <sup>Fc</sup>  | 0.331±0.007 <sup>Id</sup>    | 0.043±0.023 <sup>De</sup>   |
|              | MF251 | 2.286±0.022 <sup>ABa</sup>     | 1.939±0.016 <sup>ABCDb</sup> | 1.092±0.013 <sup>Fd</sup>  | 1.248±0.020 <sup>BCDc</sup>  | 0.054±0.005 <sup>De</sup>   |
|              | MF269 | 2.270±0.000 <sup>ABCa</sup>    | 2.000±0.007 <sup>ABb</sup>   | 1.010±0.051 <sup>FGd</sup> | 1.302±0.025 <sup>ABc</sup>   | 0.030±0.001 <sup>De</sup>   |
| <i>P. pa</i> | MF152 | 2.260±0.000 <sup>Aa</sup>      | 1.964±0.107 <sup>ABb</sup>   | 0.230±0.010 <sup>Cc</sup>  | 0.000 <sup>Dd</sup>          | 0.000 <sup>Ad</sup>         |
|              | MF233 | 2.057±0.017 <sup>Ba</sup>      | 1.900±0.000 <sup>Bb</sup>    | 1.585±0.003 <sup>Ac</sup>  | 0.329±0.063 <sup>Bd</sup>    | 0.036±0.012 <sup>Ae</sup>   |
|              | MF244 | 2.189±0.000 <sup>Aa</sup>      | 2.016±0.000 <sup>Ab</sup>    | 1.538±0.000 <sup>Ac</sup>  | 0.703±0.010 <sup>Ad</sup>    | 0.073±0.103 <sup>Ae</sup>   |
|              | MF245 | 2.177±0.006 <sup>Aa</sup>      | 1.925±0.107 <sup>ABb</sup>   | 1.274±0.061 <sup>Bc</sup>  | 0.147±0.066 <sup>Cd</sup>    | 0.014±0.020 <sup>Ae</sup>   |
|              | MF249 | 2.206±0.012 <sup>Aa</sup>      | 1.984±0.007 <sup>ABb</sup>   | 1.609±0.008 <sup>Ac</sup>  | 0.699±0.092 <sup>Ad</sup>    | 0.000 <sup>Ae</sup>         |

Values are expressed in mean ± standard deviation.

Values with different capital letters within a column indicate significant differences between LAB strains ( $P < 0.05$ ). Values with different lower case letters within a row indicate significant differences between NaCl concentrations ( $P < 0.05$ ).

Spc: Species, *E. ca*: *E. casseliflavus*, *L. br*: *L. brevis*, *L. bu*: *L. buchneri*, *L. na*: *L. namurensis*, *L. pa*: *L. parabrevis*, *L. pl*: *L. plantarum*, *P. et*: *P. ethanolidurans*, *P. pa*: *P. parvulus*

### The rate and extent of acid production

Rapid acid production by lactic acid bacteria is one of the primary criterion in the selection of starter cultures used for vegetable fermentation technology (Çon and Karasu, 2009), as that is essential for lowering of pH and, thus, inhibiting the growth undesirable bacteria during the initial stage of fermentation (Daeschel and Fleming, 1984). As shown in Table 3, there were significant differences ( $P < 0.05$ ) between the strains of each LAB species with respect to both the rate of acidification, taken as the total acid production after 24 h, and the extent of acidification, taken as the total acid production after 48 h. The only exception was that no significant differences ( $P$

$> 0.05$ ) were found between *L. buchneri* strains with respect to the extent of acidification. On the basis of acid production, the LAB strains were classified into three groups: fast ( $> 0.6\%$  acidity), moderate (0.5-0.6% acidity) and slow acidifiers ( $< 0.5\%$  acidity). Only the strains of *L. plantarum* (except for strain MF219) showed fast acidifying activity after 24 h of incubation in MRS broth, whereas the other strains ascribed to the species *P. ethanolidurans*, *P. parvulus*, *L. brevis*, *L. buchneri*, *L. parabrevis*, *L. namurensis* and *E. casseliflavus* were characterized as slow acid producers. The acidification developed by strains of *L. plantarum*, *P. ethanolidurans*, *P. parvulus* and *L. brevis* was higher than that produced by the strains of *E. casseliflavus*,

*L. parabrevis*, *L. buchneri* and *L. namurensis* after 48 h of incubation. Although *P. ethanolidurans* and *P. parvulus* strains produced low amount of lactic acid after 24 h of incubation, the acid production was enhanced after 48 h, and the final acidity was similar to that of *L. plantarum*. This kind of behaviour was also observed in LAB strains

isolated from cheese (Aquilanti et al., 2007). Consequently, twenty-five *L. plantarum* strains with the highest rate and extent of acid production revealed to be suitable as starter culture for fermented vegetable products.

Table 2. Growth (OD<sub>600</sub> value) of LAB strains isolated from pickles in MRS broth adjusted to different pH values

| Spc          | Strain no | pH 2.0                       | pH 3.0                     | pH 4.0                      | pH 5.0                        | pH 6.5                       | pH 9.6                      |
|--------------|-----------|------------------------------|----------------------------|-----------------------------|-------------------------------|------------------------------|-----------------------------|
| <i>E. ca</i> | MF535     | 0                            | 0.0195±0.002               | 0.005±0.007                 | 0.04±0.033                    | 1.266±0.010                  | 1.782±0.007                 |
|              | MF105     | 0.027±0.000 <sup>ABd</sup>   | 0.033±0.003 <sup>Bd</sup>  | 0.800±0.001 <sup>Bc</sup>   | 1.022±0.035 <sup>Fa</sup>     | 0.972±0.016 <sup>Fb</sup>    | 0.000 <sup>Ad</sup>         |
| <i>L. br</i> | MF158     | 0.000 <sup>Bd</sup>          | 0.005±0.007 <sup>Bd</sup>  | 0.846±0.021 <sup>Ac</sup>   | 1.056±0.013 <sup>Ea</sup>     | 1.025±0.084 <sup>Db</sup>    | 0.000 <sup>Ad</sup>         |
|              | MF243     | 0.052±0.016 <sup>Ad</sup>    | 0.009±0.012 <sup>Bc</sup>  | 0.705±0.035 <sup>Cc</sup>   | 0.952±0.024 <sup>Gb</sup>     | 1.121±0.011 <sup>Ca</sup>    | 0.000 <sup>Ac</sup>         |
|              | MF250     | 0.030±0.023 <sup>ABdc</sup>  | 0.043±0.030 <sup>Bd</sup>  | 0.584±0.008 <sup>Ec</sup>   | 1.009±0.006 <sup>Fa</sup>     | 0.788±0.018 <sup>Fb</sup>    | 0.000 <sup>Ac</sup>         |
|              | MF314     | 0.000 <sup>Bd</sup>          | 0.000 <sup>Bd</sup>        | 0.633±0.010 <sup>Dc</sup>   | 1.185±0.017 <sup>Ca</sup>     | 1.115±0.001 <sup>Cb</sup>    | 0.000 <sup>Ad</sup>         |
|              | MF343     | 0.011±0.016 <sup>Bc</sup>    | 0.079±0.020 <sup>Ad</sup>  | 0.640±0.014 <sup>Dc</sup>   | 1.136±0.009 <sup>Db</sup>     | 1.247±0.005 <sup>Aa</sup>    | 0.000 <sup>Ac</sup>         |
|              | MF354     | 0.000 <sup>Bc</sup>          | 0.000 <sup>Bc</sup>        | 0.797±0.052 <sup>Bb</sup>   | 1.223±0.018 <sup>Ca</sup>     | 1.199±0.020 <sup>Ba</sup>    | 0.000 <sup>Ac</sup>         |
|              | MF493     | 0.000 <sup>Bd</sup>          | 0.000 <sup>Bd</sup>        | 0.657±0.016 <sup>Dc</sup>   | 1.385±0.024 <sup>Aa</sup>     | 1.208±0.008 <sup>Bb</sup>    | 0.000 <sup>Ad</sup>         |
|              | MF494     | 0.000 <sup>Bd</sup>          | 0.000 <sup>Bd</sup>        | 0.655±0.007 <sup>Dc</sup>   | 1.314±0.011 <sup>Ba</sup>     | 1.185±0.028 <sup>Bb</sup>    | 0.000 <sup>Ad</sup>         |
|              | MF531     | 0.000 <sup>Bd</sup>          | 0.033±0.003 <sup>Bd</sup>  | 0.436±0.006 <sup>Fc</sup>   | 0.887±0.001 <sup>Hb</sup>     | 1.201±0.005 <sup>Ba</sup>    | 0.000 <sup>Ad</sup>         |
|              | MF12      | 0.024±0.034 <sup>Ad</sup>    | 0.040±0.007 <sup>Ad</sup>  | 0.852±0.059 <sup>Ac</sup>   | 1.121±0.013 <sup>Aa</sup>     | 1.016±0.003 <sup>Ab</sup>    | 0.000 <sup>Ad</sup>         |
| <i>L. bu</i> | MF102     | 0.000 <sup>Ac</sup>          | 0.000 <sup>Ac</sup>        | 0.063±0.037 <sup>Cb</sup>   | 0.639±0.030 <sup>Da</sup>     | 0.670±0.037 <sup>Ca</sup>    | 0.000 <sup>Ac</sup>         |
|              | MF114     | 0.008±0.011 <sup>Ad</sup>    | 0.055±0.015 <sup>Ad</sup>  | 0.385±0.001 <sup>Bc</sup>   | 0.953±0.004 <sup>Ba</sup>     | 0.710±0.013 <sup>Cb</sup>    | 0.000 <sup>Ad</sup>         |
|              | MF117     | 0.000 <sup>Ac</sup>          | 0.000 <sup>Ac</sup>        | 0.000 <sup>Dc</sup>         | 0.682±0.025 <sup>Da</sup>     | 0.439±0.035 <sup>Db</sup>    | 0.000 <sup>Ac</sup>         |
|              | MF271     | 0.040±0.008 <sup>Ac</sup>    | 0.000 <sup>Ac</sup>        | 0.048±0.005 <sup>CDc</sup>  | 0.851±0.064 <sup>Ca</sup>     | 0.329±0.013 <sup>Eb</sup>    | 0.000 <sup>Ac</sup>         |
|              | MF272     | 0.023±0.033 <sup>Ad</sup>    | 0.000 <sup>Ad</sup>        | 0.379±0.008 <sup>Bc</sup>   | 0.805±0.081 <sup>Cb</sup>     | 0.957±0.052 <sup>Ba</sup>    | 0.000 <sup>Ad</sup>         |
| <i>L. na</i> | MF192     | 0.013±0.018 <sup>e</sup>     | 0.076±0.001 <sup>d</sup>   | 0.688±0.006 <sup>b</sup>    | 0.858±0.034 <sup>a</sup>      | 0.570±0.004 <sup>c</sup>     | 0.000 <sup>e</sup>          |
|              | MF275     | 0.000 <sup>f</sup>           | 0.092±0.018 <sup>e</sup>   | 0.842±0.017 <sup>c</sup>    | 1.076±0.031 <sup>b</sup>      | 0.598±0.005 <sup>d</sup>     | 1.595±0.057 <sup>a</sup>    |
| <i>L. pa</i> | MF231     | 0.000                        | 0.032±0.015                | 0.331±0.031                 | 0.683±0.007                   | 0.578±0.001                  | 0.000                       |
|              | MF4       | 0.065±0.013 <sup>CDEFd</sup> | 0.093±0.000 <sup>FGd</sup> | 1.632±0.003 <sup>Kc</sup>   | 2.352±0.008 <sup>EFGHb</sup>  | 2.474±0.011 <sup>CDa</sup>   | 0.082±0.001 <sup>ABd</sup>  |
|              | MF33      | 0.000 <sup>Hc</sup>          | 0.112±0.023 <sup>EFd</sup> | 1.733±0.021 <sup>FGc</sup>  | 2.341±0.025 <sup>FGHb</sup>   | 2.444±0.031 <sup>CDEFa</sup> | 0.000 <sup>De</sup>         |
|              | MF99      | 0.106±0.018 <sup>ABe</sup>   | 0.319±0.040 <sup>Ad</sup>  | 1.895±0.021 <sup>ABc</sup>  | 2.451±0.000 <sup>Aa</sup>     | 2.395±0.000 <sup>FGHb</sup>  | 0.000 <sup>Df</sup>         |
|              | MF118     | 0.081±0.043 <sup>BCDd</sup>  | 0.108±0.001 <sup>EFd</sup> | 1.266±0.022 <sup>Oc</sup>   | 2.201±0.006 <sup>Mb</sup>     | 2.371±0.035 <sup>GHa</sup>   | 0.000 <sup>De</sup>         |
|              | MF143     | 0.097±0.016 <sup>ABCd</sup>  | 0.219±0.026 <sup>CDc</sup> | 1.821±0.040 <sup>DEb</sup>  | 2.409±0.019 <sup>ABCa</sup>   | 2.444±0.011 <sup>CDEFa</sup> | 0.000 <sup>De</sup>         |
|              | MF150     | 0.097±0.016 <sup>ABCe</sup>  | 0.251±0.025 <sup>BCd</sup> | 1.696±0.000 <sup>GHc</sup>  | 2.260±0.000 <sup>KLb</sup>    | 2.307±0.023 <sup>Ia</sup>    | 0.000 <sup>Df</sup>         |
|              | MF169     | 0.109±0.021 <sup>ABd</sup>   | 0.195±0.018 <sup>Dc</sup>  | 1.533±0.071 <sup>Lb</sup>   | 2.346±0.000 <sup>EFGHa</sup>  | 2.365±0.043 <sup>HLa</sup>   | 0.000 <sup>De</sup>         |
|              | MF178     | 0.078±0.017 <sup>BCDd</sup>  | 0.094±0.001 <sup>FGd</sup> | 1.406±0.016 <sup>Nc</sup>   | 2.323±0.000 <sup>GHb</sup>    | 2.482±0.000 <sup>BCa</sup>   | 0.000 <sup>De</sup>         |
|              | MF205     | 0.112±0.004 <sup>ABd</sup>   | 0.309±0.006 <sup>Ac</sup>  | 1.825±0.007 <sup>CDEb</sup> | 2.395±0.000 <sup>BCDa</sup>   | 2.395±0.000 <sup>FGHa</sup>  | 0.009±0.013 <sup>De</sup>   |
|              | MF213     | 0.072±0.012 <sup>BCDEc</sup> | 0.291±0.001 <sup>ABd</sup> | 1.795±0.002 <sup>Ec</sup>   | 2.275±0.007 <sup>IJKb</sup>   | 2.346±0.000 <sup>Ia</sup>    | 0.045±0.005 <sup>BCDe</sup> |
|              | MF219     | 0.059±0.001 <sup>CDEFd</sup> | 0.045±0.003 <sup>Gd</sup>  | 1.421±0.023 <sup>Nc</sup>   | 1.974±0.014 <sup>Oa</sup>     | 1.909±0.000 <sup>Kb</sup>    | 0.000 <sup>De</sup>         |
|              | MF232     | 0.138±0.049 <sup>Ac</sup>    | 0.213±0.003 <sup>CDd</sup> | 1.494±0.025 <sup>LMc</sup>  | 2.162±0.016 <sup>Nb</sup>     | 2.451±0.000 <sup>CDEa</sup>  | 0.000 <sup>Df</sup>         |
|              | MF239     | 0.049±0.008 <sup>DEFGc</sup> | 0.280±0.066 <sup>ABd</sup> | 1.832±0.003 <sup>CDEc</sup> | 2.402±0.009 <sup>BCDb</sup>   | 2.459±0.011 <sup>CDa</sup>   | 0.000 <sup>Df</sup>         |
|              | MF265     | 0.081±0.008 <sup>BCDd</sup>  | 0.190±0.003 <sup>De</sup>  | 1.872±0.000 <sup>BCb</sup>  | 2.370±0.017 <sup>CDEFa</sup>  | 2.358±0.000 <sup>HLa</sup>   | 0.084±0.016 <sup>ABd</sup>  |
|              | MF303     | 0.025±0.035 <sup>EFGHc</sup> | 0.138±0.010 <sup>Ed</sup>  | 1.471±0.034 <sup>Mc</sup>   | 2.256±0.006 <sup>KLb</sup>    | 2.436±0.000 <sup>CDEFa</sup> | 0.033±0.004 <sup>CDc</sup>  |
|              | MF305     | 0.063±0.038 <sup>CDEFc</sup> | 0.123±0.023 <sup>EFd</sup> | 1.752±0.011 <sup>Fc</sup>   | 2.389±0.027 <sup>BCDEb</sup>  | 2.515±0.000 <sup>ABa</sup>   | 0.000 <sup>Df</sup>         |
|              | MF322     | 0.063±0.006 <sup>CDEFc</sup> | 0.309±0.002 <sup>Ad</sup>  | 1.916±0.009 <sup>Ac</sup>   | 2.312±0.000 <sup>HIJb</sup>   | 2.409±0.019 <sup>EFGa</sup>  | 0.058±0.001 <sup>ABCc</sup> |
|              | MF352     | 0.000 <sup>Hc</sup>          | 0.055±0.013 <sup>Gd</sup>  | 1.386±0.013 <sup>Nc</sup>   | 2.223±0.000 <sup>LMb</sup>    | 2.444±0.011 <sup>CDEFa</sup> | 0.038±0.007 <sup>BCDd</sup> |
|              | MF357     | 0.019±0.027 <sup>FGHe</sup>  | 0.105±0.010 <sup>EFd</sup> | 1.610±0.016 <sup>Kc</sup>   | 2.346±0.000 <sup>EFGHb</sup>  | 2.515±0.000 <sup>ABa</sup>   | 0.086±0.013 <sup>Ad</sup>   |
|              | MF376     | 0.024±0.034 <sup>FGHf</sup>  | 0.199±0.003 <sup>Dd</sup>  | 1.511±0.008 <sup>LMc</sup>  | 1.988±0.040 <sup>Ob</sup>     | 2.534±0.026 <sup>Aa</sup>    | 0.063±0.002 <sup>ABCc</sup> |
|              | MF377     | 0.032±0.045 <sup>EFGHc</sup> | 0.178±0.004 <sup>Dd</sup>  | 1.721±0.024 <sup>FGc</sup>  | 2.382±0.000 <sup>BCDEFb</sup> | 2.533±0.000 <sup>Aa</sup>    | 0.036±0.050 <sup>BCDc</sup> |
|              | MF380     | 0.075±0.000 <sup>BCDd</sup>  | 0.095±0.028 <sup>FGd</sup> | 1.667±0.023 <sup>HIJc</sup> | 2.261±0.028 <sup>KLb</sup>    | 2.408±0.000 <sup>EFGa</sup>  | 0.000 <sup>De</sup>         |

Table 2 continued

|              |                            |                              |                              |                             |                              |                             |                             |
|--------------|----------------------------|------------------------------|------------------------------|-----------------------------|------------------------------|-----------------------------|-----------------------------|
| <i>L. pl</i> | MF404                      | 0.014±0.019 <sup>GHe</sup>   | 0.078±0.006 <sup>FGd</sup>   | 1.713±0.060 <sup>FGHe</sup> | 2.364±0.008 <sup>DEFGb</sup> | 2.482±0.000 <sup>BCa</sup>  | 0.063±0.003 <sup>ABCd</sup> |
|              | MF513                      | 0.114±0.006 <sup>ABd</sup>   | 0.212±0.020 <sup>CDc</sup>   | 1.845±0.016 <sup>BCDb</sup> | 2.422±0.000 <sup>ABa</sup>   | 2.430±0.030 <sup>DEFa</sup> | 0.000 <sup>De</sup>         |
|              | MF548                      | 0.070±0.029 <sup>BCDEc</sup> | 0.122±0.004 <sup>EFd</sup>   | 1.663±0.018 <sup>Uc</sup>   | 2.370±0.000 <sup>CDEFb</sup> | 2.552±0.000 <sup>Aa</sup>   | 0.013±0.004 <sup>Df</sup>   |
|              | MF556                      | 0.064±0.014 <sup>CDEFc</sup> | 0.200±0.014 <sup>Dd</sup>    | 1.486±0.025 <sup>LMc</sup>  | 2.265±0.007 <sup>JKb</sup>   | 2.482±0.023 <sup>BCa</sup>  | 0.062±0.001 <sup>ABCe</sup> |
|              | MF11                       | 0.000 <sup>Dd</sup>          | 0.000 <sup>Dd</sup>          | 0.229±0.011 <sup>HJc</sup>  | 1.120±0.025 <sup>Ka</sup>    | 0.942±0.018 <sup>Fb</sup>   | 0.000 <sup>Ad</sup>         |
| <i>P. et</i> | MF14                       | 0.030±0.014 <sup>ABCDd</sup> | 0.019±0.002 <sup>BCDd</sup>  | 0.218±0.011 <sup>HJc</sup>  | 1.169±0.012 <sup>Ja</sup>    | 0.780±0.028 <sup>Fb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF48                       | 0.019±0.027 <sup>BCDe</sup>  | 0.062±0.037 <sup>ABd</sup>   | 0.511±0.021 <sup>Dc</sup>   | 1.368±0.049 <sup>Ea</sup>    | 0.566±0.052 <sup>Kb</sup>   | 0.000 <sup>Ac</sup>         |
|              | MF50                       | 0.040±0.041 <sup>ABCDd</sup> | 0.000 <sup>Dd</sup>          | 0.191±0.008 <sup>Ic</sup>   | 1.258±0.000 <sup>Ha</sup>    | 0.973±0.005 <sup>EFb</sup>  | 0.000 <sup>Ad</sup>         |
|              | MF78                       | 0.000 <sup>Dd</sup>          | 0.000 <sup>Dd</sup>          | 0.433±0.001 <sup>Ec</sup>   | 1.128±0.011 <sup>Ka</sup>    | 0.467±0.028 <sup>Ib</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF82                       | 0.006±0.008 <sup>CDd</sup>   | 0.040±0.019 <sup>ABCDd</sup> | 0.524±0.001 <sup>Db</sup>   | 1.229±0.003 <sup>Ha</sup>    | 0.353±0.004 <sup>Mc</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF83                       | 0.000 <sup>Dd</sup>          | 0.046±0.000 <sup>ABCc</sup>  | 0.439±0.009 <sup>Eb</sup>   | 1.049±0.006 <sup>Ma</sup>    | 0.444±0.037 <sup>Lb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF86                       | 0.000 <sup>Dc</sup>          | 0.048±0.005 <sup>ABCd</sup>  | 0.515±0.001 <sup>Db</sup>   | 1.094±0.011 <sup>KLMa</sup>  | 0.454±0.008 <sup>Lc</sup>   | 0.000 <sup>Ac</sup>         |
|              | MF107                      | 0.000 <sup>Dd</sup>          | 0.029±0.019 <sup>BCDd</sup>  | 0.377±0.003 <sup>Fc</sup>   | 1.457±0.006 <sup>Da</sup>    | 1.288±0.045 <sup>Bb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF115                      | 0.010±0.014 <sup>CDd</sup>   | 0.000 <sup>Dd</sup>          | 0.238±0.003 <sup>HIc</sup>  | 1.328±0.032 <sup>EFa</sup>   | 0.796±0.027 <sup>Hb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF136                      | 0.009±0.012 <sup>CDd</sup>   | 0.007±0.010 <sup>CDd</sup>   | 0.323±0.004 <sup>Gc</sup>   | 1.554±0.013 <sup>Ba</sup>    | 1.112±0.023 <sup>Cb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF167                      | 0.000 <sup>Dd</sup>          | 0.026±0.013 <sup>BCDd</sup>  | 0.359±0.018 <sup>FGc</sup>  | 1.257±0.013 <sup>GIa</sup>   | 1.140±0.006 <sup>Cb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF179                      | 0.069±0.021 <sup>Ad</sup>    | 0.000 <sup>Dc</sup>          | 0.144±0.005 <sup>Kc</sup>   | 1.326±0.021 <sup>Fa</sup>    | 0.858±0.021 <sup>Gb</sup>   | 0.000 <sup>Ac</sup>         |
|              | MF180                      | 0.037±0.013 <sup>ABCDd</sup> | 0.000 <sup>Dd</sup>          | 0.661±0.036 <sup>Bc</sup>   | 1.232±0.030 <sup>Ha</sup>    | 0.985±0.064 <sup>Fb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF183                      | 0.000 <sup>Dd</sup>          | 0.000 <sup>Dd</sup>          | 0.218±0.011 <sup>HJc</sup>  | 1.086±0.032 <sup>KLMa</sup>  | 0.632±0.027 <sup>Jb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF185                      | 0.000 <sup>Dd</sup>          | 0.000 <sup>Dd</sup>          | 0.102±0.028 <sup>Lc</sup>   | 1.070±0.018 <sup>LMa</sup>   | 0.704±0.095 <sup>Ib</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF187                      | 0.000 <sup>Dc</sup>          | 0.081±0.011 <sup>Ad</sup>    | 0.491±0.002 <sup>Dc</sup>   | 1.298±0.018 <sup>FGa</sup>   | 0.573±0.007 <sup>Kb</sup>   | 0.000 <sup>Ac</sup>         |
|              | MF194                      | 0.000 <sup>Dd</sup>          | 0.000 <sup>Dd</sup>          | 0.256±0.015 <sup>Hc</sup>   | 1.440±0.002 <sup>Da</sup>    | 1.144±0.054 <sup>Cb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF196                      | 0.022±0.001 <sup>BCDd</sup>  | 0.034±0.001 <sup>BCDd</sup>  | 0.579±0.016 <sup>Cb</sup>   | 1.306±0.018 <sup>Fa</sup>    | 0.464±0.072 <sup>Lc</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF214                      | 0.040±0.008 <sup>ABCDc</sup> | 0.039±0.012 <sup>ABCDc</sup> | 0.946±0.027 <sup>Ab</sup>   | 1.095±0.010 <sup>KLa</sup>   | 1.103±0.009 <sup>Ca</sup>   | 0.000 <sup>Ac</sup>         |
|              | MF229                      | 0.053±0.001 <sup>ABCc</sup>  | 0.038±0.053 <sup>ABCDc</sup> | 0.558±0.001 <sup>Cb</sup>   | 1.920±0.004 <sup>Aa</sup>    | 1.886±0.003 <sup>Aa</sup>   | 0.000 <sup>Ad</sup>         |
| MF230        | 0.000 <sup>Dd</sup>        | 0.000 <sup>Dd</sup>          | 0.205±0.009 <sup>Ic</sup>    | 1.203±0.016 <sup>Ia</sup>   | 0.800±0.028 <sup>Fb</sup>    | 0.000 <sup>Ad</sup>         |                             |
| MF251        | 0.064±0.010 <sup>ABd</sup> | 0.000 <sup>Dc</sup>          | 0.211±0.013 <sup>Jc</sup>    | 1.505±0.006 <sup>Ca</sup>   | 1.036±0.002 <sup>Db</sup>    | 0.000 <sup>Ac</sup>         |                             |
| MF269        | 0.012±0.017 <sup>CDd</sup> | 0.000 <sup>Dd</sup>          | 0.341±0.021 <sup>FGc</sup>   | 1.533±0.005 <sup>BCa</sup>  | 1.137±0.020 <sup>Cb</sup>    | 0.000 <sup>Ad</sup>         |                             |
| <i>P. pa</i> | MF152                      | 0.000 <sup>Ad</sup>          | 0.012±0.017 <sup>Ad</sup>    | 0.058±0.005 <sup>Ec</sup>   | 0.470±0.006 <sup>Ea</sup>    | 0.286±0.006 <sup>Db</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF233                      | 0.021±0.030 <sup>Ad</sup>    | 0.021±0.001 <sup>Ad</sup>    | 0.766±0.011 <sup>Ac</sup>   | 1.272±0.006 <sup>Cb</sup>    | 1.299±0.013 <sup>Ba</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF244                      | 0.000 <sup>Ad</sup>          | 0.000 <sup>Ad</sup>          | 0.259±0.035 <sup>Dc</sup>   | 1.510±0.001 <sup>Aa</sup>    | 1.383±0.023 <sup>Ab</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF245                      | 0.015±0.021 <sup>Ad</sup>    | 0.000 <sup>Ad</sup>          | 0.569±0.004 <sup>Bc</sup>   | 1.051±0.005 <sup>Da</sup>    | 0.991±0.013 <sup>Cb</sup>   | 0.000 <sup>Ad</sup>         |
|              | MF249                      | 0.009±0.012 <sup>Ad</sup>    | 0.000 <sup>Ad</sup>          | 0.303±0.001 <sup>Cc</sup>   | 1.444±0.013 <sup>Ba</sup>    | 1.306±0.011 <sup>Bb</sup>   | 0.000 <sup>Ad</sup>         |

Values are expressed in mean ± standard deviation.

Values with different capital letters within a column indicate significant differences between LAB strains ( $P < 0.05$ ).

Values with different lower case letters within a row indicate significant differences between pH values ( $P < 0.05$ ).

Sp: Species, *E. ca*: *E. casseliflavus*, *L. br*: *L. brevis*, *L. bu*: *L. buchneri*, *L. na*: *L. namurensis*, *L. pa*: *L. parabrevis*, *L. pl*: *L. plantarum*, *P. et*: *P. ethanolidurans*, *P. pa*: *P. parvulus*

### Production of biogenic amines

Biogenic amines are organic, basic, nitrogenous compounds which occur in a wide range of foods, including sauerkraut, fishery products, cheese, wine, beer, dry sausages and other fermented fruits and vegetables (Tamang et al., 2009; Spano et al., 2010; Sonar and Halami, 2014). In fermented foods, these compounds are mainly formed by decarboxylation of the corresponding amino acids through substrate specific enzymes of the microorganisms present in foods (Belgacem et al., 2010). The biogenic amines commonly found in fermented vegetables (e.g. sauerkraut, kimchi, and fermented olives) include histamine (from histidine), tyramine (from

tyrosine), cadaverine (from lysine) and putrescine (from arginine) (Hutkins, 2006).

The production of biogenic amines is an undesirable trait for LAB strains to be selected as starter cultures (Buckenhüskes, 1993) since the consumption of foods containing large amounts of biogenic amines may cause toxicological problems (Spano et al., 2010). None of the tested strains showed biogenic amine production from histidine, lysine or tyrosine in the method applied. These results support the data that LAB of vegetable origin have weak capability to decarboxylate aminoacids, as reported by Daeschel et al. (1987). Similarly, the disability to



produce biogenic amines was also observed previously for *Lactobacillus* strains isolated from fermented vegetables (Seseña et al., 2005; Tamang et al., 2009; Bevilacqua et al., 2010). As a result, the inability of LAB strains from the fermented pickles to produce biogenic amines is a good indication of their potential for the possible

development as starter culture. However, it is important to point out that the non-production of biogenic amines by LAB strains needs to be confirmed by qualitative and quantitative analysis of biogenic amines in the fermented vegetable products, rather than in synthetic medium.

Table 3. Acid production of LAB strains isolated from pickles

| Spc          | Strain no.               | % Acidity-24h            | % Acidity-48h              | Spc                      | Strain no.               | % Acidity-24 h           | % Acidity-48 h             |
|--------------|--------------------------|--------------------------|----------------------------|--------------------------|--------------------------|--------------------------|----------------------------|
| <i>E. ca</i> | MF535                    | 0.02±0.00                | 0.41±0.22                  | <i>L. pl</i>             | MF357                    | 1.17±0.01 <sup>CDE</sup> | 1.51±0.02 <sup>DEFGH</sup> |
|              | MF105                    | 0.34±0.01 <sup>BC</sup>  | 0.67±0.02 <sup>D</sup>     |                          | MF376                    | 1.08±0.02 <sup>FG</sup>  | 1.53±0.02 <sup>CDEF</sup>  |
|              | MF158                    | 0.27±0.01 <sup>D</sup>   | 0.70±0.02 <sup>C</sup>     |                          | MF377                    | 1.29±0.03 <sup>A</sup>   | 1.48±0.02 <sup>GHI</sup>   |
|              | MF243                    | 0.16±0.02 <sup>E</sup>   | 0.59±0.01 <sup>F</sup>     |                          | MF380                    | 1.22±0.01 <sup>BC</sup>  | 1.47±0.02 <sup>HI</sup>    |
|              | MF250                    | 0.20±0.12 <sup>E</sup>   | 0.81±0.02 <sup>A</sup>     |                          | MF404                    | 1.20±0.02 <sup>BC</sup>  | 1.53±0.01 <sup>CDEF</sup>  |
|              | MF314                    | 0.38±0.01 <sup>B</sup>   | 0.73±0.02 <sup>B</sup>     |                          | MF513                    | 1.20±0.06 <sup>BC</sup>  | 1.52±0.01 <sup>CDEFG</sup> |
|              | MF343                    | 0.46±0.04 <sup>A</sup>   | 0.75±0.03 <sup>B</sup>     |                          | MF548                    | 1.25±0.05 <sup>AB</sup>  | 1.56±0.03 <sup>BC</sup>    |
|              | MF354                    | 0.39±0.03 <sup>B</sup>   | 0.63±0.01 <sup>E</sup>     |                          | MF556                    | 1.10±0.01 <sup>EFG</sup> | 1.41±0.03 <sup>J</sup>     |
|              | MF493                    | 0.29±0.01 <sup>CD</sup>  | 0.65±0.00 <sup>DE</sup>    |                          | MF11                     | 0.34±0.01 <sup>BC</sup>  | 0.94±0.02 <sup>F</sup>     |
| MF494        | 0.35±0.03 <sup>BC</sup>  | 0.67±0.01 <sup>D</sup>   | MF14                       | 0.30±0.02 <sup>CDE</sup> | 1.09±0.02 <sup>C</sup>   |                          |                            |
| MF531        | 0.35±0.02 <sup>BC</sup>  | 0.67±0.02 <sup>D</sup>   | MF48                       | 0.33±0.01 <sup>BCD</sup> | 1.00±0.02 <sup>D</sup>   |                          |                            |
| <i>L. br</i> | MF12                     | 0.12±0.01 <sup>A</sup>   | 0.51±0.04                  | MF50                     | 0.36±0.02 <sup>B</sup>   | 0.95±0.03 <sup>EF</sup>  |                            |
|              | MF102                    | 0.06±0.02 <sup>C</sup>   | 0.48±0.18                  | MF78                     | 0.24±0.02 <sup>FGH</sup> | 0.98±0.04 <sup>DE</sup>  |                            |
|              | MF114                    | 0.08±0.01 <sup>B</sup>   | 0.51±0.02                  | MF82                     | 0.28±0.03 <sup>EF</sup>  | 0.95±0.02 <sup>EF</sup>  |                            |
|              | MF117                    | 0.09±0.02 <sup>B</sup>   | 0.57±0.03                  | MF83                     | 0.29±0.02 <sup>DE</sup>  | 0.92±0.03 <sup>FG</sup>  |                            |
|              | MF271                    | 0.02±0.00 <sup>D</sup>   | 0.42±0.04                  | MF86                     | 0.27±0.02 <sup>EFG</sup> | 0.94±0.01 <sup>F</sup>   |                            |
|              | MF272                    | 0.05±0.01 <sup>C</sup>   | 0.48±0.01                  | MF107                    | 0.33±0.01 <sup>BCD</sup> | 0.80±0.01 <sup>I</sup>   |                            |
|              | MF192                    | 0.21±0.01                | 0.55±0.02                  | MF115                    | 0.34±0.01 <sup>BC</sup>  | 1.09±0.01 <sup>C</sup>   |                            |
| <i>L. bu</i> | MF275                    | 0.18±0.01                | 0.50±0.01                  | MF136                    | 0.44±0.01 <sup>A</sup>   | 1.18±0.01 <sup>A</sup>   |                            |
|              | MF192                    | 0.21±0.01                | 0.55±0.02                  | MF167                    | 0.41±0.06 <sup>A</sup>   | 1.00±0.03 <sup>D</sup>   |                            |
| <i>L. na</i> | MF231                    | 0.16±0.02                | 0.44±0.03                  | MF179                    | 0.29±0.02 <sup>DE</sup>  | 1.07±0.03 <sup>C</sup>   |                            |
|              | MF4                      | 1.31±0.02 <sup>A</sup>   | 1.60±0.01 <sup>A</sup>     | MF180                    | 0.33±0.01 <sup>BCD</sup> | 1.00±0.02 <sup>D</sup>   |                            |
| <i>L. pa</i> | MF33                     | 1.25±0.02 <sup>AB</sup>  | 1.52±0.02 <sup>CDEFG</sup> | MF183                    | 0.21±0.02 <sup>H</sup>   | 0.89±0.03 <sup>GH</sup>  |                            |
|              | MF99                     | 1.10±0.02 <sup>FG</sup>  | 1.48±0.02 <sup>GHI</sup>   | MF185                    | 0.28±0.01 <sup>EF</sup>  | 0.92±0.02 <sup>FG</sup>  |                            |
|              | MF118                    | 1.00±0.15 <sup>HI</sup>  | 1.41±0.02 <sup>J</sup>     | MF187                    | 0.27±0.02 <sup>EFG</sup> | 1.18±0.01 <sup>A</sup>   |                            |
|              | MF143                    | 1.11±0.10 <sup>EFG</sup> | 1.43±0.02 <sup>J</sup>     | MF194                    | 0.33±0.03 <sup>BCD</sup> | 0.95±0.00 <sup>EF</sup>  |                            |
|              | MF150                    | 1.13±0.03 <sup>DEF</sup> | 1.54±0.01 <sup>CDE</sup>   | MF196                    | 0.30±0.02 <sup>CDE</sup> | 1.13±0.00 <sup>B</sup>   |                            |
|              | MF169                    | 1.21±0.01 <sup>BC</sup>  | 1.50±0.02 <sup>EFGH</sup>  | MF214                    | 0.12±0.07 <sup>I</sup>   | 0.55±0.04 <sup>J</sup>   |                            |
|              | MF178                    | 0.95±0.06 <sup>I</sup>   | 1.52±0.03 <sup>CDEFG</sup> | MF229                    | 0.35±0.03 <sup>B</sup>   | 0.77±0.02 <sup>I</sup>   |                            |
|              | MF205                    | 1.07±0.02 <sup>FG</sup>  | 1.59±0.03 <sup>AB</sup>    | MF230                    | 0.23±0.01 <sup>GH</sup>  | 0.87±0.02 <sup>H</sup>   |                            |
|              | MF213                    | 0.96±0.01 <sup>I</sup>   | 1.47±0.02 <sup>HI</sup>    | MF251                    | 0.26±0.01 <sup>EFG</sup> | 1.17±0.05 <sup>A</sup>   |                            |
|              | MF219                    | 0.29±0.00 <sup>J</sup>   | 0.69±0.03 <sup>K</sup>     | MF269                    | 0.26±0.04 <sup>EFG</sup> | 0.92±0.04 <sup>FG</sup>  |                            |
|              | MF232                    | 1.11±0.03 <sup>EFG</sup> | 1.55±0.03 <sup>CD</sup>    | MF152                    | 0.06±0.02 <sup>D</sup>   | 0.40±0.02 <sup>D</sup>   |                            |
|              | MF239                    | 1.05±0.01 <sup>GH</sup>  | 1.50±0.02 <sup>EFGH</sup>  | MF233                    | 0.34±0.01 <sup>A</sup>   | 1.21±0.03 <sup>A</sup>   |                            |
|              | MF265                    | 1.12±0.02 <sup>EFG</sup> | 1.56±0.05 <sup>BC</sup>    | MF244                    | 0.32±0.01 <sup>B</sup>   | 1.06±0.06 <sup>B</sup>   |                            |
|              | MF303                    | 1.08±0.02 <sup>FG</sup>  | 1.47±0.03 <sup>HI</sup>    | MF245                    | 0.26±0.01 <sup>C</sup>   | 0.97±0.03 <sup>C</sup>   |                            |
|              | MF305                    | 1.17±0.01 <sup>CDE</sup> | 1.44±0.05 <sup>J</sup>     |                          |                          |                          |                            |
| MF322        | 1.19±0.02 <sup>BCD</sup> | 1.49±0.02 <sup>FGH</sup> |                            |                          |                          |                          |                            |

Values are expressed in mean ± standard deviation.

Values with different capital letters within a column indicate significant differences between LAB strains ( $P < 0.05$ ). Spc: Species, *E. ca*: *E. casseliflavus*, *L. br*: *L. brevis*, *L. bu*: *L. buchneri*, *L. na*: *L. namurensis*, *L. pa*: *L. parabrevis*, *L. pl*: *L. plantarum*, *P. et*: *P. ethanolidurans*, *P. pa*: *P. parvulus*

### Enzymatic profile

The enzymatic activities of the LAB strains, as evaluated by the semiquantitative API-ZYM system, are shown in Table 4. High or at least intermediate leucine and valine aminopeptidase activities were observed for all tested strains except for *E. casseliflavus* MF535, which showed no activity. However, cystine aminopeptidase activity was low or absent. Only two strains of *P. ethanolidurans* (MF136, MF229) showed definite cystine aminopeptidase activity (around 20 nanomole hydrolyzed substrate). Similar results with regard to aminopeptidase activities were also observed by Boulares et al. (2012) for *L. plantarum*, *L. paracasei* and *L. brevis*. The tested strains showed higher peptidase activities than proteinases (trypsin, chymotrypsin), as also reported by other authors (Georgieva et al., 2009; Tamang et al., 2009; Boulares et al., 2012; Taboada et al., 2014). Lipolytic activities of esterase (C4), esterase lipase (C8) and lipase (C14) were weak or absent, in the range of 0-2, for the majority of tested strains. In contrast, *L. brevis* and *L. namurensis* strains exhibited intermediate or high esterase activity. Our results support the evidence that *Lactobacillus* species are weakly lipolytic (Montel et al., 1998). The presence of lipolytic activity is often desired, since it can improve the flavour of foods (e.g. fermented olives) through the formation of volatile compounds that can be generated by the catabolism of free fatty acids (Rodríguez-Gómez et al., 2012; Bleve et al., 2015). The degradation of amino acids, such as leucine, isoleucine, valine, phenylalanine, methionine, into volatile molecules, such as aldehydes, alcohols and acids, plays an important role in flavour development of food products (Montel et al., 1998; Ammor et al., 2005). However, highly proteolytic strains are not eligible starter cultures since excessive proteolysis may lead to uncontrolled production of bitter peptides and other undesirable compounds, or may result in an over-soft final product (Zeng et al., 2014). Overall, the absence of proteinases (trypsin and chymotrypsin) and presence of strong peptidase (leucine-, valine-, and cystine-arylamidase) and esterase-lipase (C4 and C8) activities produced by the LAB strains are

desirable properties for their use in the production of typical flavour (Thapa et al., 2006; Dewan and Tamang, 2007; Tamang et al., 2009). Tamang et al. (2009) reported that high phosphatase activity of LAB strains showed their possible role in phytic acid degradation in fermented vegetables. In this study, acid phosphatase and phosphohydrolase activities were shown for most strains whereas weak alkaline phosphatase activities were detected in some strains, as similarly reported by Papamanoli et al. (2003) for *L. plantarum* and Taboada et al. (2014) for pediococci. Corroborating with the results of Taboada et al. (2014),  $\beta$ -galactosidase activities of the tested lactobacilli (particularly *L. brevis* and *L. buchneri*) were generally higher than in pediococci.  $\alpha$ -galactosidase activity was generally absent or low in the tested strains, apart from some strains of *L. brevis*, *L. buchneri* and *L. plantarum*. With a few rare exceptions (*E. casseliflavus* MF535, *L. namurensis* strains, a few strains of *P. ethanolidurans*), the tested LAB strains exhibited  $\alpha$ -glucosidase activity, albeit to different extent. Similar results were also observed for the  $\beta$ -glucosidase activities. With regard to  $\beta$ -glucoaminidase, the most significant activity was observed for *L. plantarum*, followed by *P. ethanolidurans*. Most of the tested strains did not show  $\beta$ -glucuronidase,  $\alpha$ -fucosidase and  $\alpha$ -mannosidase activities, with the exception of *L. buchneri* MF12 with high  $\beta$ -glucuronidase activity, as well as a few strains of *L. brevis* with moderate  $\beta$ -glucuronidase activity. The presence of the enzyme activities correlated with carbohydrate catabolism such as glucosidase and galactosidase is of vital importance for strains with proper utilization of sugars found in foods (Belgacem et al., 2010). The high glucosidase and galactosidase activities, and low activities toward other carbon sources (mannose, fructose and glucuronides) suggest that most of the tested LAB strains prefer glucose and lactose as their carbon and energy sources. In addition, the high  $\beta$ -galactosidase activity of LAB strains makes them suitable as starter cultures for food products intended to be used for lactose intolerant people.

Table 4 Enzymatic profiles of LAB strains isolated from pickles using API-zym system

| Species      | Strain no.   | 1     | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |   |
|--------------|--------------|-------|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|---|
| <i>E. ca</i> | MF535        | 0     | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0  | 1  | 1  | 0  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |   |
|              | MF105        | 0     | 0 | 3 | 2 | 0 | 4 | 4 | 1 | 0 | 0  | 3  | 2  | 4  | 5  | 2  | 4  | 4  | 0  | 0  | 0  |   |
|              | MF158        | 0     | 1 | 3 | 2 | 0 | 4 | 5 | 2 | 0 | 0  | 3  | 2  | 4  | 5  | 0  | 4  | 3  | 1  | 0  | 0  |   |
|              | MF243        | 0     | 1 | 2 | 1 | 0 | 3 | 4 | 1 | 0 | 0  | 3  | 1  | 2  | 5  | 2  | 3  | 4  | 0  | 1  | 1  |   |
|              | MF250        | 0     | 0 | 2 | 0 | 1 | 4 | 3 | 1 | 0 | 0  | 2  | 1  | 3  | 5  | 1  | 2  | 3  | 0  | 0  | 1  |   |
|              | <i>L. br</i> | MF314 | 0 | 0 | 2 | 1 | 0 | 4 | 4 | 1 | 0  | 0  | 1  | 1  | 2  | 4  | 0  | 4  | 3  | 0  | 0  | 0 |
|              |              | MF343 | 0 | 0 | 3 | 1 | 0 | 5 | 5 | 2 | 0  | 0  | 2  | 1  | 3  | 5  | 0  | 3  | 3  | 0  | 0  | 0 |
|              |              | MF354 | 0 | 0 | 3 | 2 | 0 | 4 | 3 | 0 | 0  | 0  | 2  | 1  | 0  | 4  | 0  | 3  | 4  | 0  | 0  | 0 |
|              |              | MF493 | 0 | 0 | 4 | 3 | 0 | 5 | 4 | 2 | 0  | 0  | 3  | 1  | 0  | 5  | 2  | 3  | 4  | 0  | 0  | 0 |
|              |              | MF494 | 0 | 0 | 3 | 2 | 0 | 5 | 4 | 2 | 0  | 0  | 2  | 1  | 0  | 5  | 2  | 3  | 5  | 0  | 0  | 0 |
| MF531        |              | 0     | 1 | 3 | 2 | 0 | 5 | 5 | 2 | 0 | 0  | 2  | 1  | 0  | 5  | 0  | 3  | 4  | 0  | 0  | 0  |   |
| MF12         |              | 0     | 0 | 1 | 1 | 0 | 4 | 3 | 0 | 0 | 0  | 1  | 1  | 3  | 5  | 4  | 1  | 3  | 0  | 0  | 0  |   |
| MF102        | 0            | 0     | 0 | 2 | 1 | 3 | 2 | 0 | 0 | 0 | 2  | 2  | 4  | 5  | 0  | 2  | 3  | 0  | 0  | 0  |    |   |
| <i>L. bu</i> | MF114        | 0     | 0 | 1 | 1 | 0 | 4 | 3 | 1 | 0 | 0  | 1  | 1  | 3  | 5  | 1  | 1  | 2  | 0  | 0  | 0  |   |
|              | MF117        | 0     | 0 | 1 | 2 | 0 | 4 | 3 | 1 | 0 | 1  | 2  | 1  | 4  | 5  | 1  | 1  | 3  | 0  | 0  | 0  |   |
|              | MF271        | 0     | 1 | 1 | 1 | 0 | 4 | 2 | 1 | 0 | 0  | 2  | 1  | 3  | 5  | 0  | 3  | 4  | 0  | 0  | 0  |   |
|              | MF272        | 0     | 1 | 1 | 1 | 0 | 3 | 2 | 2 | 0 | 0  | 1  | 1  | 3  | 5  | 1  | 3  | 4  | 0  | 0  | 0  |   |
|              | MF192        | 0     | 0 | 3 | 2 | 0 | 4 | 3 | 2 | 0 | 0  | 2  | 3  | 1  | 4  | 0  | 0  | 2  | 0  | 1  | 0  |   |
| <i>L. na</i> | MF275        | 0     | 0 | 3 | 2 | 0 | 4 | 2 | 1 | 0 | 0  | 3  | 2  | 0  | 4  | 0  | 0  | 4  | 0  | 0  | 0  |   |
| <i>L. pa</i> | MF231        | 0     | 0 | 2 | 2 | 0 | 5 | 3 | 0 | 0 | 0  | 1  | 1  | 1  | 3  | 2  | 2  | 2  | 0  | 0  | 0  |   |
|              | MF4          | 0     | 0 | 0 | 1 | 0 | 5 | 5 | 1 | 0 | 0  | 1  | 2  | 0  | 3  | 0  | 1  | 2  | 2  | 0  | 0  |   |
|              | MF33         | 0     | 1 | 1 | 0 | 1 | 4 | 3 | 0 | 0 | 0  | 3  | 1  | 3  | 5  | 0  | 5  | 4  | 5  | 0  | 0  |   |
|              | MF99         | 0     | 2 | 1 | 1 | 2 | 4 | 3 | 0 | 0 | 0  | 2  | 2  | 0  | 3  | 0  | 1  | 3  | 4  | 0  | 0  |   |
|              | MF118        | 0     | 1 | 1 | 2 | 0 | 4 | 4 | 1 | 0 | 0  | 3  | 3  | 3  | 5  | 0  | 4  | 3  | 4  | 0  | 0  |   |
|              | MF143        | 0     | 2 | 2 | 1 | 0 | 4 | 3 | 2 | 0 | 0  | 2  | 3  | 0  | 5  | 0  | 2  | 4  | 4  | 1  | 0  |   |
|              | MF150        | 0     | 0 | 1 | 1 | 0 | 4 | 4 | 1 | 0 | 0  | 3  | 2  | 1  | 3  | 0  | 5  | 4  | 5  | 1  | 1  |   |
|              | MF169        | 0     | 1 | 1 | 1 | 1 | 4 | 4 | 2 | 1 | 0  | 2  | 2  | 0  | 5  | 0  | 3  | 4  | 4  | 0  | 0  |   |
|              | MF178        | 0     | 1 | 0 | 1 | 0 | 4 | 3 | 2 | 0 | 0  | 2  | 1  | 0  | 2  | 0  | 3  | 4  | 4  | 0  | 0  |   |
|              | MF205        | 0     | 1 | 0 | 1 | 0 | 3 | 3 | 2 | 0 | 0  | 2  | 2  | 0  | 4  | 0  | 3  | 3  | 1  | 0  | 0  |   |
| <i>L. pl</i> | MF213        | 0     | 1 | 1 | 2 | 1 | 4 | 3 | 2 | 0 | 0  | 2  | 2  | 0  | 4  | 0  | 4  | 3  | 4  | 0  | 0  |   |
|              | MF219        | 0     | 0 | 2 | 1 | 0 | 5 | 3 | 1 | 0 | 0  | 2  | 1  | 0  | 5  | 0  | 1  | 2  | 0  | 0  | 0  |   |
|              | MF232        | 0     | 1 | 1 | 1 | 0 | 3 | 2 | 2 | 0 | 0  | 2  | 3  | 0  | 5  | 0  | 4  | 4  | 4  | 0  | 0  |   |
|              | MF239        | 0     | 1 | 0 | 2 | 1 | 3 | 2 | 0 | 1 | 0  | 1  | 2  | 0  | 1  | 0  | 1  | 4  | 2  | 0  | 0  |   |
|              | MF265        | 0     | 1 | 1 | 1 | 0 | 4 | 2 | 1 | 0 | 0  | 2  | 2  | 0  | 3  | 0  | 3  | 4  | 4  | 0  | 0  |   |
|              | MF303        | 0     | 1 | 1 | 1 | 0 | 4 | 3 | 2 | 0 | 0  | 1  | 1  | 1  | 5  | 0  | 4  | 3  | 5  | 0  | 0  |   |
|              | MF305        | 0     | 0 | 0 | 0 | 0 | 4 | 4 | 1 | 0 | 0  | 2  | 1  | 0  | 3  | 0  | 2  | 3  | 3  | 0  | 0  |   |
|              | MF322        | 0     | 0 | 1 | 1 | 0 | 4 | 3 | 1 | 0 | 0  | 2  | 2  | 0  | 4  | 0  | 3  | 3  | 3  | 0  | 0  |   |
|              | MF352        | 0     | 0 | 1 | 1 | 0 | 4 | 3 | 1 | 0 | 0  | 1  | 1  | 0  | 3  | 0  | 2  | 2  | 3  | 0  | 1  |   |
|              | MF357        | 0     | 0 | 0 | 1 | 0 | 4 | 3 | 0 | 0 | 0  | 2  | 1  | 1  | 3  | 0  | 3  | 4  | 3  | 0  | 0  |   |
|              | MF376        | 0     | 1 | 1 | 1 | 1 | 3 | 3 | 1 | 0 | 0  | 2  | 1  | 0  | 4  | 0  | 3  | 3  | 4  | 0  | 0  |   |
|              | MF377        | 0     | 1 | 1 | 1 | 0 | 5 | 4 | 1 | 0 | 0  | 1  | 2  | 0  | 4  | 0  | 3  | 3  | 2  | 0  | 0  |   |
|              | MF380        | 0     | 0 | 1 | 1 | 0 | 4 | 4 | 1 | 0 | 0  | 2  | 2  | 1  | 4  | 0  | 4  | 4  | 5  | 0  | 0  |   |
|              | MF404        | 0     | 2 | 1 | 1 | 0 | 4 | 4 | 1 | 0 | 0  | 1  | 1  | 1  | 3  | 0  | 2  | 4  | 2  | 0  | 0  |   |
|              | MF513        | 0     | 0 | 0 | 0 | 0 | 4 | 3 | 1 | 0 | 0  | 2  | 1  | 0  | 3  | 0  | 3  | 3  | 4  | 0  | 0  |   |
|              | MF548        | 0     | 0 | 1 | 1 | 0 | 4 | 4 | 2 | 0 | 0  | 1  | 2  | 0  | 2  | 0  | 2  | 3  | 4  | 0  | 0  |   |
|              | MF556        | 0     | 1 | 1 | 1 | 1 | 4 | 2 | 1 | 0 | 0  | 2  | 2  | 0  | 4  | 0  | 4  | 3  | 4  | 0  | 1  |   |

Table 4 continued

|              |       |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
|--------------|-------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|              | MF11  | 0 | 0 | 0 | 1 | 0 | 5 | 5 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 3 | 2 | 0 | 0 |
|              | MF14  | 0 | 0 | 0 | 1 | 0 | 5 | 5 | 2 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 4 | 4 | 2 | 0 | 0 |
|              | MF48  | 0 | 1 | 0 | 1 | 1 | 5 | 3 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 0 | 5 | 5 | 3 | 0 | 0 |
|              | MF50  | 0 | 0 | 0 | 0 | 1 | 5 | 5 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 3 | 2 | 0 | 0 |
|              | MF78  | 0 | 0 | 0 | 0 | 1 | 4 | 3 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 3 | 3 | 0 | 0 |
|              | MF82  | 0 | 0 | 0 | 1 | 1 | 4 | 4 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 3 | 4 | 3 | 0 | 0 |
|              | MF83  | 0 | 0 | 1 | 0 | 1 | 5 | 5 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 4 | 3 | 0 | 0 |
|              | MF86  | 0 | 0 | 1 | 1 | 1 | 4 | 4 | 1 | 0 | 0 | 1 | 2 | 2 | 0 | 0 | 4 | 4 | 3 | 0 | 0 |
|              | MF107 | 0 | 0 | 0 | 1 | 0 | 4 | 4 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 4 | 4 | 3 | 0 | 0 |
|              | MF115 | 0 | 0 | 1 | 1 | 1 | 4 | 4 | 1 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 3 | 4 | 2 | 0 | 1 |
|              | MF136 | 0 | 1 | 1 | 0 | 1 | 5 | 5 | 3 | 1 | 0 | 1 | 2 | 1 | 0 | 0 | 3 | 3 | 2 | 0 | 0 |
| <i>P. et</i> | MF167 | 0 | 0 | 2 | 1 | 1 | 4 | 4 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 4 | 1 | 0 | 0 |
|              | MF179 | 0 | 0 | 1 | 1 | 1 | 5 | 4 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 3 | 2 | 1 | 0 | 0 |
|              | MF180 | 0 | 0 | 2 | 0 | 0 | 5 | 4 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 |
|              | MF183 | 0 | 0 | 0 | 1 | 0 | 4 | 4 | 1 | 1 | 0 | 1 | 2 | 0 | 0 | 0 | 2 | 3 | 3 | 0 | 0 |
|              | MF185 | 0 | 0 | 1 | 1 | 0 | 4 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 2 | 1 | 0 | 0 |
|              | MF187 | 0 | 0 | 2 | 1 | 2 | 5 | 4 | 2 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 4 | 4 | 3 | 0 | 0 |
|              | MF194 | 0 | 0 | 0 | 0 | 1 | 5 | 5 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
|              | MF196 | 0 | 0 | 1 | 1 | 1 | 5 | 5 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 3 | 2 | 0 | 0 |
|              | MF214 | 0 | 0 | 2 | 2 | 1 | 3 | 4 | 0 | 0 | 0 | 2 | 1 | 1 | 4 | 0 | 3 | 2 | 0 | 1 | 1 |
|              | MF229 | 0 | 0 | 3 | 2 | 1 | 5 | 4 | 3 | 0 | 0 | 2 | 1 | 0 | 4 | 0 | 2 | 4 | 0 | 0 | 0 |
|              | MF230 | 0 | 0 | 1 | 1 | 1 | 5 | 4 | 1 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 3 | 2 | 2 | 0 | 0 |
|              | MF251 | 0 | 0 | 0 | 1 | 0 | 5 | 3 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 |
|              | MF269 | 0 | 0 | 1 | 1 | 1 | 4 | 4 | 1 | 0 | 0 | 2 | 3 | 0 | 0 | 0 | 1 | 4 | 2 | 0 | 0 |
|              | MF152 | 0 | 0 | 2 | 2 | 0 | 4 | 4 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 2 | 3 | 0 | 0 |
|              | MF233 | 0 | 0 | 0 | 1 | 0 | 4 | 3 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| <i>P. pa</i> | MF244 | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
|              | MF245 | 0 | 1 | 0 | 1 | 0 | 4 | 5 | 1 | 0 | 0 | 3 | 2 | 0 | 0 | 0 | 1 | 2 | 0 | 1 | 0 |
|              | MF249 | 0 | 0 | 0 | 1 | 0 | 5 | 3 | 1 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 |

1: control; 2: alkaline phosphatase; 3: esterase (C4); 4: esterase lipase (C8); 5: lipase (C14); 6: leucine aminopeptidase; 7: valine aminopeptidase; 8: cystine aminopeptidase; 9: trypsin; 10: a-chymotrypsin; 11: acid phosphatase; 12: naphthol-As-Bi-phosphohydrolase; 13:  $\alpha$ -galactosidase; 14:  $\beta$ -galactosidase; 15:  $\beta$ -glucuronidase; 16:  $\alpha$ -glucosidase; 17:  $\beta$ -glucosidase; 18: N-acetyl- $\beta$ -glucosaminidase; 19:  $\alpha$ -mannosidase; 20:  $\alpha$ -fucosidase.

0: no activity, 1: low activity, 2-3: intermediate activity, 4-5: high activity

*E. ca*: *E. casseliflavus*, *L. br*: *L. brevis*, *L. bu*: *L. buchneri*, *L. na*: *L. namurensis*, *L. pa*: *L. parabrevis*, *L. pl*: *L. plantarum*, *P. et*: *P. ethanolidurans*, *P. pa*: *P. parvulus*

## CONCLUSIONS

Although significant intra-species differences in the primary technological characteristics within each set of 8 LAB species (*L. plantarum*, *L. brevis*, *L. buchneri*, *L. namurensis*, *L. parabrevis*, *P. ethanolidurans*, *P. parvulus* and *E. casseliflavus*), *L. plantarum* species were apparently distinguished by their high acidification rate, tolerance to low pH and salt. The investigated *L. plantarum* strains, except for one strain (MF219), could be considered as potential starter cultures because of their desirable properties of having a high rate and extent of acidification, high tolerance to pH 4.0

and 10% NaCl, and non-production of biogenic amines. In addition, the tested strains of *P. ethanolidurans* could also be considered as promising strains due to their high salt tolerance and acid production. With respect to enzymatic activities, most of the tested strains shared similar characteristics including absence of proteolytic and lipolytic activities, and presence of peptidase, glucosidase and galactosidase activities. Consequently, further studies with the selected 25 *L. plantarum* strains, either alone or in combination, should be carried out in model fermentation systems in order to assess their

effectiveness as starter cultures for pickles and other fermented vegetable products. This work was a preliminary study in the development of autochthonous starter cultures in order to standardize the manufacture of pickles, to preserve their typical sensory characteristics and to improve the quality of final product.

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### REFERENCES

- Ammor, S., Dufour, E., Zagorec, M., Chaillou, S., Chevallier, I. (2005). Characterization and selection of *Lactobacillus sakei* strains isolated from traditional dry sausage for their potential use as starter cultures. *Food Microbiol*, 22: 529-538.
- Aquilanti, L., Silvestri, G., Zannini, E., Osimani, A., Santarelli, S., Clementi, F. (2007). Phenotypic, genotypic and technological characterization of predominant lactic acid bacteria in Pecorino cheese from central Italy. *J Appl Microbiol*, 103: 948-960.
- Axelsson, L. (2004). Lactic Acid Bacteria: Classification and Physiology. In: *Lactic Acid Bacteria Microbiological and Functional Aspects*, Salminen, S., von Wright, A., Ouwehand, A. (eds), Marcel Dekker, New York, pp. 1-66.
- Bağder Elmacı, S., Tokatlı, M., Dursun, D., Özçelik, F., Şanlıbaba, P. (2015). Phenotypic and genotypic identification of lactic acid bacteria isolated from traditional pickles of the Çubuk region in Turkey. *Folia Microbiol*, 60: 241-251.
- Beganović, J., Kos, B., Pavunc, A.L., Uroić, K., Jokić, M., Šušković, J. (2014). Traditionally produced sauerkraut as source of autochthonous functional starter cultures. *Microbiol Res*, 169: 623-632.
- Belgacem, Z.B., Abriouel, H., Omar, N.B., Lucas, R., Martinez-Canamero, M., Galvez, A., Manai, M. (2010). Antimicrobial activity, safety aspects, and some technological properties of bacteriocinogenic *Enterococcus faecium* from artisanal Tunisian fermented meat. *Food Control*, 21: 462-470.
- Benito, M.J., Martín, A., Aranda, E., Pérez-Nevado, F., Ruiz-Moyano, S., Córdoba, M.G. (2007). Characterization and selection of autochthonous lactic acid bacteria isolated from traditional Iberian dry-fermented Salchichón and Chorizo sausages. *J Food Sci*, 72: M193-M201.
- Bevilacqua, A., Altieri, C., Corbo, M.R., Sinigaglia, M., Ouoba, L.I.I. (2010). Characterization of lactic acid bacteria isolated from Italian Bella di Cerignola table olives: selection of potential multifunctional starter cultures. *J Food Sci*, 75: M536-M544.
- Bleve, G., Tufariello, M., Durante, M., Grieco, F., Ramires, F.A., Mita, G., Tasioula-Margari, M., Logrieco, A.F. (2015). Physico-chemical characterization of natural fermentation process of Conservolea and Kalamàta table olives and development of a protocol for the pre-selection of fermentation starters. *Food Microbiol*, 46: 368-382.
- Bonomo, M.G., Ricciardi, A., Zotta, T., Parente, E., Salzano, G. (2008). Molecular and technological characterization of lactic acid bacteria from traditional fermented sausages of Basilicata region (Southern Italy). *Meat Sci*, 80: 1238-1248.
- Boulares, M., Aouadhi, C., Mankai, M., Moussa, O.B., Essid, I., Hassouna, M. (2012). Characterisation, identification and technological properties of psychrotrophic lactic acid bacteria originating from Tunisian fresh fish. *J Food Safety*, 32: 333-344.
- Bover-Cid, S., Holzapfel, W.H. (1999). Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int J Food Microbiol*, 53: 33-41.
- Buckenhüskes, H.J. (1993). Selection criteria for lactic acid bacteria to be used as starter cultures for various food commodities. *FEMS Microbiol Rev*, 12: 253-272.
- Çetin, B. (2011). Production of probiotic mixed pickles (Tursu) and microbiological properties. *Afr J Biotechnol*, 10: 14926-14931.

- Chao, S.H., Wu, R.J., Watanabe, K., Tsai, Y.C. (2009). Diversity of lactic acid bacteria in suan-tsai and fu-tsai, traditional fermented mustard products of Taiwan. *Int J Food Microbiol*, 135: 203-210.
- Çon, A.H., Karasu, N. (2009). Determination of antagonistic starter cultures for pickle and olive fermentation processes. *Czech J Food Sci*, 27: 185-193.
- Daeschel, M.A., Fleming, H.P. (1984). Selection of lactic acid bacteria for use in vegetable fermentations. *Food Microbiol*, 1: 303-313.
- Daeschel, M.A., Andersson, R.E., Fleming, H.P. (1987). Microbial ecology of fermenting plant materials. *FEMS Microbiol Rev*, 46: 357-367.
- Dewan, S., Tamang, J.P. (2007). Dominant lactic acid bacteria and their technological properties isolated from the Himalayan ethnic fermented milk products. *Antonie van Leeuwenhoek*, 92: 343-352.
- Di Cagno, R., Coda, R., De Angelis, M., Gobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol*, 33: 1-10.
- Essid, I., Medini, M., Hassouna, M. (2009). Technological and safety properties of *Lactobacillus plantarum* strains isolated from a Tunisian traditional salted meat. *Meat Sci*, 81: 203-208.
- Gardner, N.J., Savard, T., Obermeier, P., Caldwell, G., Champagne, C.P. (2001). Selection and characterization of mixed starter cultures for lactic acid fermentation of carrot, cabbage, beet and onion vegetable mixtures. *Int J Food Microbiol*, 64: 261-275.
- Georgieva, R., Iliev, I., Haertlé, T., Chobert, J.M., Ivanova, I., Danova, S. (2009). Technological properties of candidate probiotic *Lactobacillus plantarum* strains. *Int Dairy J*, 19: 696-702.
- Heperkan, D. (2013). Microbiota of table olive fermentations and criteria of selection for their use as starters. *Front Microbiol*, 4: Article no. 143, 1-11.
- Hutkins, R.W. (2006). Microbiology and Technology of Fermented Foods. Blackwell Publishing, USA, pp. 233-259.
- Josephsen, J., Jespersen, L. (2004). Starter Cultures and Fermented Products. In: *Handbook of Food and Beverage Fermentation Technology*, Hui, Y.H. (ed.), Marcel Dekker, New York, pp. 23-49.
- Kabak, B., Dobson, A.D.W. (2011). An introduction to the traditional fermented foods and beverages of Turkey. *Crit Rev Food Sci Nutr*, 51: 248-260.
- Karasu, N., Şimşek, Ö., Çon, A.H. (2010). Technological and probiotic characteristics of *Lactobacillus plantarum* strains isolated from traditionally produced fermented vegetables. *Ann Microbiol*, 60: 227-234.
- Mäkimattila, E., Kahala, M., Joutsjoki, V. (2011). Characterization and electrotransformation of *Lactobacillus plantarum* and *Lactobacillus paraplantarum* isolated from fermented vegetables. *World J Microbiol Biotechnol*, 27: 371-379.
- McDonald, L.C., Fleming, H.P., Hassan, H.M. (1990). Acid tolerance of *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. *Appl Environ Microbiol*, 56: 2120-2124.
- Montel, M.C., Masson, F., Talon, R. (1998). Bacterial role in flavour development. *Meat Sci*, 49: S111-S123.
- Papamanoli, E., Tzanetakis, N., Litopoulou-Tzanetaki, E., Kotzekidou, P. (2003). Characterization of lactic acid bacteria isolated from a Greek dry-fermented sausage in respect of their technological and probiotic properties. *Meat Sci*, 65: 859-867.
- Reina, L.D., Breidt, F., Fleming, H.P., Kathariou, S. (2005). Isolation and selection of lactic acid bacteria as biocontrol agents for nonacidified, refrigerated pickles. *J Food Sci*, 70: M7-M11.
- Rodríguez-Gómez, F., Romero-Gil, V., Bautista-Gallego, J., Garrido-Fernández, A., Arroyo-López, F.N. (2012). Multivariate analysis to discriminate yeast strains with technological applications in table olive processing. *World J Microbiol Biotechnol*, 28: 1761-1770.

- Seseña, S., Sánchez, I., Palop, L. (2005). Characterization of *Lactobacillus* strains and monitoring by RAPD-PCR in controlled fermentations of “Almagro” eggplants. *Int J Food Microbiol*, 104: 325-335.
- Sonar, N.R., Halami, P.M. (2014). Phenotypic identification and technological attributes of native lactic acid bacteria present in fermented bamboo shoot products from North-East India. *J Food Sci Technol*, 51: 4143-4148.
- Spano, G., Russo, P., Lonvaud-Funel, A., Lucas, P., Alexandre, H., Grandvalet, C., Coton, E., Coton, M., Barnavon, L., Bach, B., Rattray, F., Bunte, A., Magni, C., Ladero, V., Alvarez, M., Fernández, M., Lopez, P., de Palencia, P.F., Corbi, A., Trip, H., Lolkema, J.S. (2010). Biogenic amines in fermented foods. *Eur J Clin Nutr*, 64: S95-S100.
- Taboada, N., Núñez, M., Medina, R., Alzogaray, M.S.L. (2014). Characterization and technological properties of lactic acid bacteria isolated from traditional Argentinean goat's milk products. *Food Biotechnol*, 28: 123-141.
- Tamang, J.P., Tamang, B., Schillinger, U., Guigas, C., Holzapfel, W.H. (2009). Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas. *Int J Food Microbiol*, 135: 28-33.
- Thapa, N., Pal, J., Tamang, J.P. (2006). Phenotypic identification and technological properties of lactic acid bacteria isolated from traditionally processed fish products of the Eastern Himalayas. *Int J Food Microbiol*, 107: 33-38.
- Vinderola, C.G., Reinheimer, J.A. (2003). Lactic acid starter and probiotic bacteria: a comparative “in vitro” study of probiotic characteristics and biological barrier resistance. *Food Res Int*, 36: 895-904.
- Wouters, D., Bernaert, N., Anno, N., Van Droogenbroeck, B., De Loose, M., Van Bockstaele, E., De Vuyst, L. (2013a). Application and validation of autochthonous lactic acid bacteria starter cultures for controlled leek fermentations and their influence on the antioxidant properties of leek. *Int J Food Microbiol*, 165: 121-133.
- Wouters, D., Grosu-Tudor, S., Zamfir, M., De Vuyst, L. (2013b). Applicability of *Lactobacillus plantarum* IMDO 788 as a starter culture to control vegetable fermentations. *J Sci Food Agric*, 93: 3352-3361.
- Zeng, X., Xia, W., Wang, J., Jiang, Q., Xu, Y., Qiu, Y., Wang, H. (2014). Technological properties of *Lactobacillus plantarum* strains isolated from Chinese traditional low salt fermented whole fish. *Food Control*, 40: 351-358.