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# Production of phenyllactic acid by lactic acid bacteria: an approach to the selection of strains contributing to food quality and preservation

Francesca Valerio, Paola Lavermicocca *, Michelangelo Pascale, Angelo Visconti

*Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Viale Einaudi 51, 70125 Bari, Italy*

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**Abstract**

The ability of lactic acid bacteria (LAB) to produce phenyllactic (PLA) and 4-hydroxy-phenyllactic (OH-PLA) acids, metabolites involved in food quality and preservation, has been evaluated by HPLC analysis in 29 LAB strains belonging to 12 species widely used in the production of fermented foods. Metabolite production was demonstrated for all strains of the species *Lactobacillus plantarum*, *Lactobacillus alimentarius*, *Lactobacillus rhamnosus*, *Lactobacillus sanfranciscensis*, *Lactobacillus hilgardii*, *Leuconostoc citreum*, and for some strains of *Lactobacillus brevis*, *Lactobacillus acidophilus* and *Leuconostoc mesenteroides* subsp. *mesenteroides*. Strains were distinguished by analysis of variance in three groups including 15 strains that produced both metabolites (0.16–0.46 mM PLA and 0.07–0.29 mM OH-PLA), five strains accumulating in culture only PLA (0.17–0.57 mM) and nine non-producer strains ($\leqslant 0.10$ mM PLA and $\leqslant 0.02$ mM OH-PLA). Improvement of phenyllactic acid production was obtained in a selected *L. plantarum* strain by increasing the concentration of phenylalanine in culture and using low amounts of tyrosine.


*Keywords:* Screening of LABs; Antimicrobial compounds; Flavour compounds; Fermented foods; Bioactive molecules; Phenyllactic acid

## 1. Introduction

Lactic acid bacteria (LAB) have many potential uses in fermented foods because they greatly influence the nutritional, sensory and shelf-life characteristics of products. They are known to produce bioactive molecules (such as organic acids, fatty acids, hydrogen peroxide, diacetyl and bacteriocins) that show antimicrobial activity against spoilage organisms and pathogens. Because of its antifungal activity, *Lactobacillus plantarum* has been investigated mainly in relation to the production of organic acids and/or cyclic dipeptides [1–5].

A selected *L. plantarum* strain (ITM21B), used as a starter in sourdough bread, has been shown to delay the growth of *Aspergillus niger* and *Penicillium roqueforti* for up to 7 days and significantly prolong the shelf-life of bread [3,6]. Phenyllactic acid (PLA) and its 4-hydroxy derivative (OH-PLA) have been found in the culture filtrate of the starter, and PLA has been recognized as the major factor responsible for such antifungal activity and prolonged shelf-life [3]. The inhibitory properties of PLA have been demonstrated against several fungal species isolated from bakery products, flour and cereals, including some mycotoxigenic species such as *Aspergillus ochraceus*, *Penicillium verrucosum* and *Penicillium citrinum*, and against some bacterial contaminants, namely *Listeria* spp., *Staphylococcus aureus* and *Enterococcus faecalis* [3,6–9].

Phenyllactic and hydroxy-phenyllactic acids have also been found as metabolites involved in the formation of cheese flavour and produced by LAB strains through phenylalanine (Phe) and tyrosine (Tyr) degradation, respectively [10–12]. In particular, Kieronczyk et al. [10]

* Corresponding author. Tel.: +39-080-5912837; fax: +39-080-5486063.

*E-mail address:* paola.lavermicocca@ispa.cnr.it (P. Lavermicocca).

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studied the contribution of two lactobacilli (*Lactobacillus paracasei* and *Lactobacillus casei*) in the production of carboxylic acids by a *Lactococcus lactis* subsp. *cremoris* strain in cheese: in lactobacilli Phe was transformed by transamination into phenylpyruvate and phenyllactate, which were subsequently converted to the potent aroma compounds, carboxylic acids.

Considering that LAB species are used as starters or non-starter LAB in fermented foods, the assessment of the production of PLA and OH-PLA by strains representative of almost all species involved in food transformation is of importance for the technological application of these metabolites as antimicrobials and/or flavour precursor compounds. Therefore, in our study, several strains of LAB belonging to 12 species were screened in order to assess their ability to produce PLA and OH-PLA and identify high producers. Additionally, the influence of phenylalanine and tyrosine metabolism on PLA formation in a *L. plantarum* producer strain was investigated.

## 2. Materials and methods

### 2.1. Chemicals

DL-Phenylalanine (Phe), L-tyrosine (Tyr), DL-phenyllactic acid (PLA) and DL-4-hydroxy-phenyllactic acid (OH-PLA) were purchased from Sigma–Aldrich (Milan, Italy). Methanol HPLC grade, ethyl acetate, 98% formic acid and $Na_2SO_4$ anhydrous were provided by Mallinckrodt Baker (Milan, Italy). Ultra-pure water was produced using a Millipore Milli-Q System (Millipore, Bedford, MA). Trifluoroacetic anhydride (TFA, 99%) was supplied by Pierce Chemical Company (Illinois, USA).

### 2.2. Cultures and media

Strains listed in Table 1 were grown in Man-Rogosa-Sharpe (MRS) broth (Difco Laboratories, Detroit, USA) at 30 °C for 24 h; cell suspensions were stored at

Table 1
Phenyllactic (PLA) and 4-hydroxy-phenyllactic (OH-PLA) acid production by lactic acid bacterial strains grown in MRS broth for 72 h at 30 °C

| Code | Strain[a] | Metabolite concentrations[b] | | Source |
|---|---|---|---|---|
| | | PLA (mM) | OH-PLA (mM) | |
| A | *Lactobacillus plantarum* ITM21B | 0.34 ± 0.02* | 0.29 ± 0.02* | Sourdough |
| B | *L. plantarum* ITM20B | 0.33 ± 0.02* | 0.26 ± 0.01* | Sourdough |
| C | *L. plantarum* ITM21A | 0.35 ± 0.01* | 0.28 ± 0.01* | Sourdough |
| D | *L. plantarum* ATCC4008 | 0.26 ± 0.02* | 0.22 ± 0.01* | Not reported |
| E | *L. plantarum* ICMP5826 | 0.27 ± 0.01* | 0.24 ± 0.01* | Plants |
| F | *Lactobacillus alimentarius* ITM8D | 0.16 ± 0.01* | 0.09 ± 0.01* | Sourdough |
| G | *L. alimentarius* ITM5Q | 0.16 ± 0.02* | 0.07 ± 0.01* | Sourdough |
| H | *L. alimentarius* ATCC29643 | 0.37 ± 0.02* | 0.19 ± 0.01* | Fish products |
| I | *Lactobacillus rhamnosus* GG ATCC53103 | 0.23 ± 0.02* | 0.08 ± 0.01* | Human origin |
| J | *L. rhamnosus* IMPC11 | 0.21 ± 0.03* | 0.08 ± 0.02* | Human origin |
| K | *L. rhamnosus* IMPC19 | 0.17 ± 0.02* | 0.05 ± 0.01 | Human origin |
| L | *Lactobacillus fermentum* ITM18B | 0.08 ± 0.03 | 0.02 ± 0.01 | Sourdough |
| M | *L. fermentum* ITM6E | 0.02 ± 0.01 | n.d[c] | Sourdough |
| N | *L. fermentum* ITM18C | 0.02 ± 0.01 | n.d. | Sourdough |
| O | *Lactobacillus sanfranciscensis* IDMCB1 | 0.22 ± 0.03* | 0.04 ± 0.03 | Sourdough |
| P | *L. sanfranciscensis* IDMC57 | 0.35 ± 0.07* | 0.18 ± 0.03* | Sourdough |
| Q | *Lactobacillus acidophilus* IDMA2 | 0.20 ± 0.04* | 0.04 ± 0.03 | Sourdough |
| R | *L. acidophilus* ATCC4356 | 0.03 ± 0.01 | n.d. | Human origin |
| **S** | ***Lactobacillus brevis*** ITM1D | **n.d.** | **n.d.** | Sourdough |
| T | *L. brevis* ITM1F | 0.22 ± 0.01* | 0.03 ± 0.02 | Sourdough |
| U | *L. brevis* ATCC14869 | 0.46 ± 0.06* | 0.25 ± 0.01* | Human origin |
| V | *Lactobacillus hilgardii* IDM51B | 0.33 ± 0.03* | 0.17 ± 0.01* | Sourdough |
| W | *Weissella confusa* ITM14A | 0.06 ± 0.01 | n.d. | Sourdough |
| X | *Enterococcus faecium* ATCC882 | 0.09 ± 0.08 | n.d. | Cheese |
| Y | *Leuconostoc mesenteroides* subsp. *mesenteroides* ITM12K | 0.09 ± 0.03 | n.d. | Sourdough |
| Z | *Leuconostoc mesenteroides* subsp. *mesenteroides* ITMY30 | 0.57 ± 0.04* | 0.05 ± 0.01 | Olive phylloplane |
| A1 | *Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC8293 | 0.10 ± 0.006 | n.d. | Fermenting olives |
| B1 | *Leuconostoc citreum* ITM10M | 0.26 ± 0.03* | 0.11 ± 0.02* | Sourdough |
| C1 | *Leuconostoc citreum* ITM22A | 0.43 ± 0.1* | 0.17 ± 0.04* | Sourdough |

[a] ITM, culture collection of the Institute of Sciences of Food Production, CNR, Bari, Italy [13,14]; IDM, culture collection of the Department of Food Science, Section of Food Microbiology, University of Perugia, Italy; ATCC, American Type Culture Collection, Rockville, Maryland, USA; ICMP, International Collection of Microrganisms from Plants, Auckland, New Zealand; IMPC, culture collection of Microbiology Institute of Catholic University of Piacenza, Italy.

[b] Data expressed as means ± SE of three independent experiments. Metabolite concentrations scored with asterisk significantly differed ($P < 0.05$, analysis of variance followed by Dunnet's test) from those of the non-producer strain *Lactobacillus brevis* ITM1D (code S).

[c] Not detected, <0.02 mM.

−80 °C in MRS supplemented with 20% v/v glycerol. The following experiments were performed:

(i) *Assessment of PLA and OH-PLA production in cultures.* Twenty-four-hour-old cells of lactic acid bacteria were used to inoculate (2%, v/v) MRS broth. All strains were incubated at 30 °C for 72 h. After incubation under still conditions, cell-free supernatant was prepared by centrifugation (4000*g* for 10 min, 4 °C) and filtration (0.45 μm-pore-size filter; Millipore, Bedford, MA). Culture filtrates were produced in triplicate for each strain.

(ii) *Influence of phenylalanine and tyrosine on PLA and OH-PLA production*. In order to assess the kinetics of PLA and OH-PLA production by *L. plantarum*, the ITM21B strain was grown in a synthetic medium (SM), pH 6.7 ± 0.2, containing Phe and Tyr at the concentration of 0.1 $g l^{-1}$and prepared according to the commercial formulation of the amino acid assay medium [15]. The culture was grown for 2, 4, 6, 8, 10, 24 and 72 h at 30 °C, centrifuged (4000*g* for 10 min, 4 °C) and filter sterilized. In order to study the influence of Phe and Tyr on PLA and OH-PLA production, *L. plantarum* ITM21B was grown in two modified SM with different concentrations of Phe or Tyr: SM1, which contained 0, 0.1, 0.2 or 0.4 $g l^{-1}$ of Phe, and SM2 containing 0, 0.1, 0.2 or 0.4 $g l^{-1}$ of Tyr. Twenty-four-hour-old cells of *L. plantarum* ITM21B were used to inoculate (2%, v/v) the media. Cultures were incubated at 30 °C for 72 h, centrifuged (4000*g* for 10 min, 4 °C) and the supernatants were filter sterilized.

### 2.3. High performance liquid chromatographic analysis

Ten millilitres of cell-free supernatant was adjusted to pH 2.0 with 10 M formic acid and extracted four times with 30 ml of ethyl acetate. Anhydrous $Na_2SO_4$ was added to the combined organic extracts, which were then filtered through Whatman No. 4 filter paper (Whatman, Maidstone, UK) and evaporated using a vacuum rotary apparatus (Büchi RE 111, Switzerland). The dried residue was reconstituted to the initial concentration with 10 ml of water containing 0.05% TFA (v/v), filtered (0.22 μm, Millipore) and 100 μl was injected into the HPLC system (full loop injection). PLA and OH-PLA were separated on an HPLC system (AKTA Basic 10, P-900 series pump, Amersham Biosciences AB, Uppsala, Sweden) using a μRPC C2/C18 column (100 × 4.6 mm, 3 μm particles) equipped with a security guard filter (C18, 4.0 × 3.0 mm) (Phenomenex, Torrance, CA). The solvent system was: solvent A, methanol–0.05% TFA and solvent B, water–0.05% TFA. The mobile phase was a linear gradient from 10% to 50% A for 15 min with a flow rate of 1 $ml\,min^{-1}$. The sample was monitored using a three-channel UV detector (Amersham Biosciences 900) that allowed simultaneous measurements at 210 nm for PLA and at 220 nm for OH-PLA. The amount of metabolites produced by bacterial strains was determined by integrating calibration curves obtained from standards. Aliquots of standard solution (0.1 and 0.05 $g l^{-1}$; $n = 3$) were added to MRS or SM to determine recoveries of the extraction method. Recoveries of PLA were 97 ± 2.0% in MRS and 88 ± 1.8% in SM. Recoveries of OH-PLA were 98 ± 1.8% and 98 ± 1.5% in MRS and SM, respectively. Detection limits, based on a signal-to-noise ratio of 3:1, were 0.150 and 0.078 μg in SM for PLA and OH-PLA, respectively (corresponding to 0.003 mM for PLA and 0.001 mM for OH-PLA), and 0.320 μg for both compounds in MRS medium (corresponding to 0.019 mM for PLA and 0.017 mM for OH-PLA).

### 2.4. Statistical analysis

Data related to metabolite production were analysed by one-way analysis of variance followed by Dunnet's multiple-comparison test using STATISTICA 6.0 software (StatSoft software package, Tulsa, OK). A $P$ value $<0.05$ was accepted as indicating statistical significance.

## 3. Results and discussion

### 3.1. PLA and OH-PLA production

The LAB strains investigated are representative of almost all species used in the production of fermented foods. In particular, *Leuconostoc*, *Weissella*, *Enterococcus* and *Lactobacillus* spp. are currently associated as starter and/or non-starter bacteria with a variety of foodstuffs such as vegetables, meat, dairy and bakery products, and are used as probiotics in many foods for their beneficial effects on human and animal health [16–18].

Results of HPLC analyses of PLA and OH-PLA in MRS culture filtrate extracts of 29 strains of LAB belonging to 12 species are reported in Table 1. All tested strains except *Lactobacillus brevis* ITM1D produced up to 0.57 mM PLA, while OH-PLA was produced by 21 strains up to 0.29 mM. The correlation between OH-PLA and PLA production is represented by the linear regression in Fig. 1 that shows a positive correlation coefficient ($R_2 = 0.5177$) between metabolites. Therefore, results obtained show that LAB generally formed more PLA than OH-PLA, although the production varies greatly among strains and species, and that concentrations of the metabolites was highly correlated for most of strains tested (20 out of 29) (Fig. 1).

In order to distinguish strains on the basis of metabolite levels (Table 1), analysis of variance followed by Dunnet's test was used to compare the data (PLA or OH-PLA concentrations) to those of the non-producer strain *L. brevis* ITM1D (code S) and three groups of

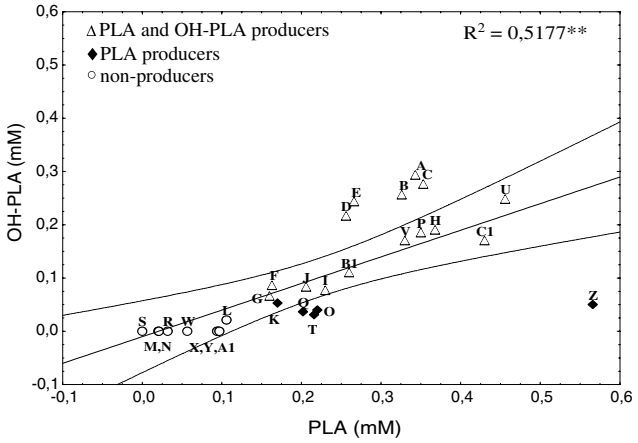
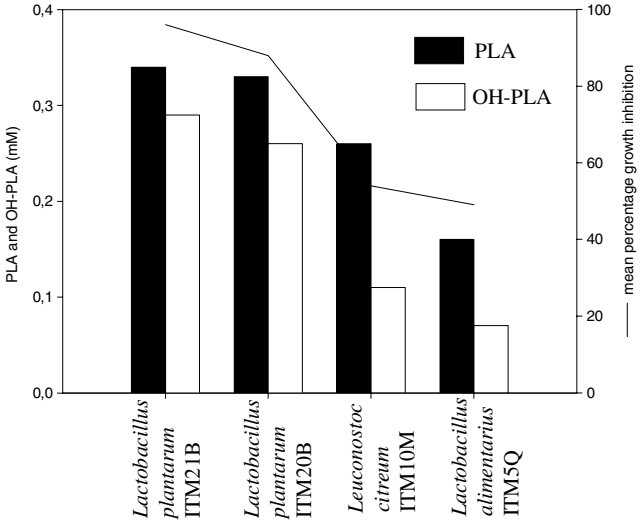



Fig. 1. Correlation between 4-hydroxy-phenyllactic (OH-PLA) and phenyllactic (PLA) acid concentrations (mM) in the culture filtrates of 29 LAB strains grown in MRS broth. Curved lines represents the 99% confidence limits. Strains were scored with different symbols on the basis of statistical analysis of data reported in Table 1 and are coded with letters as in the same table. Each point represents mean values of three experiments.

LAB were identified. The producer group included all strains [namely *L. plantarum* (codes A–E), *Lactobacillus alimentarius* (F–H), *Lactobacillus rhamnosus* (I, J), *Lactobacillus sanfranciscensis* (P), *L. brevis* (U), *Lactobacillus hilgardii* (V), *Leuconostoc citreum* (B1, C1)] accumulating the highest amounts of both PLA and OH-PLA in culture. The production of metabolites by these strains was in the ranges 0.16–0.46 and 0.07–0.29 mM ($P < 0.05$), respectively (Table 1 and Fig. 1). In particular, all strains of *L. plantarum* (A–E) produced similar amounts of PLA and OH-PLA ($0.31 \pm 0.019$ mM and $0.26 \pm 0.013$ mM, respectively; $n = 5$). Moreover, strains *L. sanfranciscensis* (O), *L. brevis* (T), *L. acidophilus* (Q), *L. rhamnosus* (K) and *Leuconostoc mesenteroides* subsp. *mesenteroides* (Z) were grouped among the producers for PLA levels (0.17–0.57 mM), since they differed from *L. brevis* (S) ($P < 0.05$), while they produced insignificant OH-PLA amounts ($P > 0.05$) (Table 1 and Fig. 1). Within this group, *L. mesenteroides* subsp. *mesenteroides* strain ITMY30 (Z) behaved differently from the other strains since it accumulated the highest level of PLA in culture ($0.57 \pm 0.04$ mM) (Table 1 and Fig. 1). Finally, strains *Enterococcus faecium* (X), *L. fermentum* (L–N), *L. acidophilus* (R), *Weissella confusa* (W) and *L. mesenteroides* subsp. *mesenteroides* (Y, A1) ($\leqslant 0.10$ mM of PLA and $\leqslant 0.02$ mM of OH-PLA) were identified as non-producers since they were not significantly different ($P > 0.05$) from *L. brevis* ITM1D (Table 1 and Fig. 1).

The evaluation of metabolite production within species indicated that all strains of *L. plantarum* (5), *L. alimentarius* (3), *L. rhamnosus* (3), *L. sanfranciscensis* (2), *L. hilgardii* (1), *L. citreum* (2), and some strains of *Lactobacillus brevis* (2 out of 3), *L. acidophilus* (1 out of 2) and *L. mesenteroides* subsp. *mesenteroides* (1 out of 3) formed relevant amounts of PLA or both acids, while strains of *L. fermentum* (3), *W. confusa* (1) and *E. faecium* (1) were unable to produce metabolites (Table 1). However, a higher number of strains for each species should be screened before the relationship between metabolite production and strains or species can be ascertained definitively.

Information obtained on the metabolite production confirms the antimicrobial features of some LAB (*L. plantarum* ITM21B, ITM20B, *L. alimentarius* ITM5Q, *L. citreum* ITM10M) previously investigated by Lavermicocca et al. [3]. Fig. 2 shows the comparison of the




| Fungal strains | % growth inhibition[a] | | | |
|---|---|---|---|---|
| *A. niger* | 98.6 | 69 | 97.5 | 28.8 |
| *A. flavus* | 86.5 | 55 | 35 | 17 |
| *E. rubrum* | 99.7 | 99.5 | 98.3 | 97 |
| *E. repens* | 100 | 100 | 25 | 52.4 |
| *E fibuliger* | 100 | 100 | 60 | 77.5 |
| *P. corylophilum* | 100 | 100 | 15 | 26.2 |
| *P. roqueforti* | 86.3 | 80 | 0 | 0 |
| *M. sitophila* | 100 | 100 | 100 | 97.6 |
| **Mean of percentages** | **96** | **88** | **54** | **49** |

[a]Percentage of growth inhibition from Lavermicocca *et al.* [3]

Fig. 2. Comparison of phenyllactic (PLA) and 4-hydroxy-phenyllactic (OH-PLA) acid production with antifungal activity of some LAB strains. The antifungal activity is expressed as the mean percentage of the inhibition of spore germination of all fungal species (*Aspergillus niger* FTDC3227, *Aspergillus flavus* FTDC3226, *Eurotium rubrum* FTDC3228, *Eurotium rubrum* IBT18000, *Endomyces fibuliger* IBT605, *Penicillium corylophilum* IBT6978, *Penicillium roqueforti* IBT18687 and *Monilia sitophila* IDM/FS5).

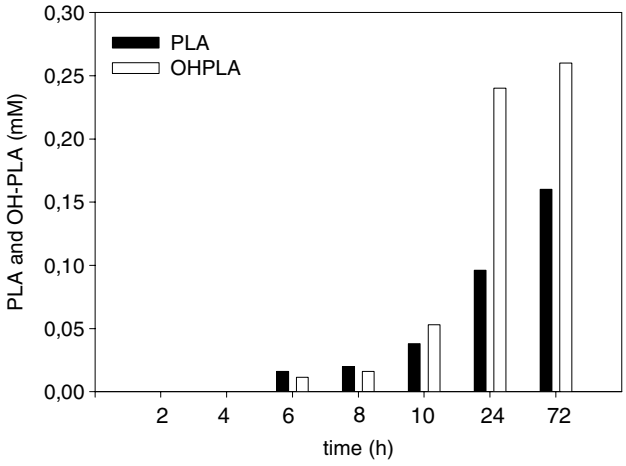



Fig. 3. Amounts of phenyllactic (PLA) and 4-hydroxy-phenyllactic (OH-PLA) acid produced by *Lactobacillus plantarum* ITM21B in synthetic medium (SM) over 72 h at 30 °C.

PLA and OH-PLA production by these strains with their ability to inhibit some fungal species in a conidial germination assay: the behaviour of the antifungal activity proved to be related to the metabolite content in the bacterial culture filtrate. In particular, the higher producer strains *L. plantarum* ITM21B and ITM20B inhibited all fungal strains tested, while *L. alimentarius* ITM5Q and *L. citreum* ITM10M weakly affected fungal growth. In addition, these results support previous data which showed that the PLA producing strain *L. plantarum* ITM21B used as a starter in sourdough bread, delayed the growth of *A. niger* FTDC3227 [3] and *P. roqueforti* IBT18687 [19] for up to 7 days at room temperature. This delaying effect was not observed in bread started with *L. brevis* ITM1D, a strain which was ineffective in inhibiting fungal growth and which does not actually produce PLA and OH-PLA in culture (Table 1).

### *3.2. Influence of phenylalanine and tyrosine on PLA or OH-PLA biosynthesis*

The producer strain *L. plantarum* ITM21B was grown in the presence of phenylalanine and tyrosine to investigate how amino acids affect the biosynthesis of PLA and OH-PLA. Results obtained during the 72-h observation of the metabolite kinetics in SM (Phe and Tyr 0.1 $g\,l^{-1}$) at 30 °C showed that the increase in the formation of compounds started after 6 h reaching a maximum level at 72 h (0.26 mM of OH-PLA and 0.165 mM of PLA) with a concentration of OH-PLA higher than PLA (Fig. 3).

Variations in the concentration of Phe in the presence of fixed amounts of Tyr (0.1 $g\,l^{-1}$) changed metabolite levels inversely. In fact, PLA was not produced at all in the absence of Phe, while increased amounts of this metabolite (from 0.17 to 0.33 mM) were observed using Phe concentrations ranging from 0.1 to 0.4 $g\,l^{-1}$ (Fig. 4(a)). At the same time, the concentration of OH-PLA dropped from 0.3 to 0.17 mM (Fig. 4(a)). Conversely, using a medium containing variable amounts of Tyr in the presence of fixed Phe levels (0.1 $g\,l^{-1}$), the growth of *L. plantarum* resulted in different PLA production (Fig. 4(b)). In particular, no OH-PLA was detected in the absence of Tyr, while increases in Tyr concentration enhanced production (from 0.26 to 0.645 mM) and decreased PLA concentration (from 0.19 to 0.07 mM) (Fig. 4(b)).

These findings indicate that the direct correlation between Phe and PLA, Tyr and OH-PLA, previously reported for *L. lactis* subsp. *cremoris* [11,12], may also be established for *L. plantarum* ITM21B, since metabolites are produced only in the presence of these amino acids and neither PLA nor OH-PLA are detected in the absence of both amino acids (data not shown). Moreover, the reduction in OH-PLA concentration associated with the increase in PLA level supports the clear correlation observed between the two metabolites (Table

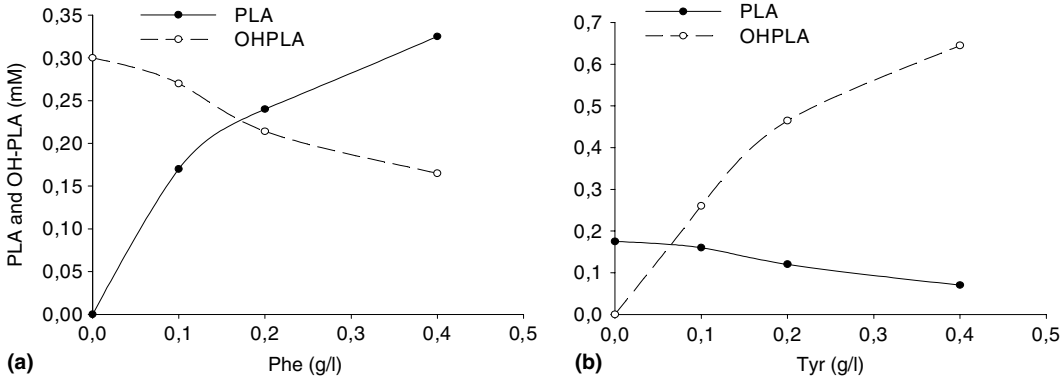



Fig. 4. Phenyllactic (PLA) (•) and 4-hydroxy-phenyllactic (OH-PLA) (○) acid production by *Lactobacillus plantarum* ITM21B grown for 72 h at 30 °C in synthetic media: SM1 supplemented with various phenylalanine concentrations (a) and SM2 supplemented with various tyrosine concentrations (b).

1 and Fig. 1). The more than 50% decrease in PLA with a concomitant increase in OH-PLA after adding Tyr to the medium suggests the indirect involvement of this amino acid in PLA production through the competition with Phe for the enzyme associated to PLA formation. A similar competition mechanism can be suggested in the case of OH-PLA reduction in the presence of Phe. This would also explain why Phe and Tyr appear as limiting factors in the formation of PLA and OH-PLA, respectively.

## 4. Conclusions

All LAB species analysed in this study have a pivotal role as starters and non-starters in food fermentation since they assure longer shelf-life as well as improved nutritional and flavour properties of the product.

Previously, very few LAB strains had been shown to produce phenyllactic and 4-hydroxy-phenyllactic acids. Our findings demonstrate that many species and/or strains are involved in the production of such metabolites that contribute to preserve food quality along with sensorial characteristic of fermented products. We found that 9 out of 12 tested species included strains able to produce relevant amounts of one metabolite, namely PLA, or both metabolites (PLA and OH-PLA). In particular, 11 strains produced comparable amounts of PLA to those of *L. plantarum* strain ITM21B, whose preserving properties have been demonstrated [3].

Furthermore, information gathered by supplying *L. plantarum* ITM21B with amino acid precursors indicates that PLA production is improved by using increased concentrations of Phe, while Tyr has to be maintained as low as possible because it limits PLA production.

In conclusion, the exploration of microbial diversity led us to select strains with interesting metabolic features (i.e., *L. plantarum* strains, *L. brevis* ATCC14869, *L. citreum* ITM22A and *L. mesenteroides* subsp. *mesenteroides* ITMY30), and the modification of bacterial growth conditions resulted in an enhancement of metabolite production. The application of these results, together with additional studies on the contribution of the metabolites to the quality and shelf-life of foods, as well as the knowledge of the enzymes involved in their formation will surely widen the use of lactic acid bacteria in fermentation strategies.

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## References

[1] Gourama, H. and Bullerman, L.B. (1995) Antimycotic and antiaflatoxigenic effect of lactic acid bacteria: a review. J. Food Prot. 57, 1275–1280.
[2] Laitila, A., Alakomi, H.-L., Raaska, L., Mattila-Sandholm, T. and Haikara, A. (2002) Antifungal activities of two *Lactobacillus plantarum* strains against *Fusarium* moulds in vitro and in malting of barley. J. Appl. Microbiol. 93, 566–576.
[3] Lavermicocca, P., Valerio, F., Evidente, A., Lazzaroni, S., Corsetti, A. and Gobbetti, M. (2000) Purification and characterization of novel antifungal compounds from the sourdough *Lactobacillus plantarum* strain 21B. Appl. Environ. Microbiol. 66, 4084–4090.
[4] Niku-Paavola, M.L., Laitila, A., Mattila-Sandholm, T. and Haikara, A. (1999) New types of antimicrobial compounds produced by *Lactobacillus plantarum*. J. Appl. Microbiol. 86, 29–35.
[5] Ström, K., Sjögren, J., Broberg, A. and Schnürer, J. (2002) *Lactobacillus plantarum* MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-*trans*-4-OH-L-Pro) and 3-phenyllactic acid. Appl. Environ. Microbiol. 68, 4322–4327.
[6] Lavermicocca, P., Valerio, F. and Visconti, A. (2003) Antifungal activity of phenyllactic acid against molds isolated from bakery products. Appl. Environ. Microbiol. 69, 634–640.
[7] Dieuleveux, V. and Gueguen, M. (1998) Antimicrobial effects of D-3-phenyllactic acid on *Listeria monocytogenes* in TSB-YE medium, milk, and cheese. J. Food Prot. 61, 1281–1285.
[8] Dieuleveux, V., Lemarinier, S. and Gueguen, M. (1998) Antimicrobial spectrum and target site of D-3-phenyllactic acid. Int. J. Food Microbiol. 40, 177–183.
[9] Dieuleveux, V., Van Der Pyl, D., Chataud, J. and Gueguen, M. (1998) Purification and characterization of anti-*Listeria* compounds produced by *Geotrichum candidum*. Appl. Environ. Microbiol. 64, 800–803.
[10] Kieronczyk, A., Skeie, S., Langsrud, T. and Yvon, M. (2003) Cooperation between *Lactococcus lactis* and nonstarter lactobacilli in the formation of cheese aroma from amino acids. Appl. Environ. Microbiol. 69, 734–739.
[11] Yvon, M., Thirouin, S., Rijnen, L., Fromentier, D. and Gripon, J.C. (1997) An aminotransferase from *Lactococcus lactis* initiates conversion of amino acids to cheese flavor compounds. Appl. Environ. Microbiol. 63, 414–419.
[12] Yvon, M., Berthelot, S. and Gripon, J.C. (1998) Adding α-ketoglutarate to semi-hard cheese curd highly enhances the conversion of amino acids to aroma compounds. Int. Dairy J. 8, 889–898.
[13] Corsetti, A., Lavermicocca, P., Morea, M., Baruzzi, F. and Gobbetti, M. (2001) Phenotypic and molecular identification and clustering of lactic acid bacteria and yeasts from wheat (varieties *Triticum durum* and *Triticum aestivum*) sourdoughs of the Southern Italy. Int. J. Food Microbiol. 64, 95–104.
[14] Lavermicocca, P., Gobbetti, M., Corsetti, A. and Caputo, L. (1998) Characterization of lactic acid bacteria isolated from olive phylloplane and table olive brines. Ital. J. Food Sci. 10, 27–39.
[15] Difco Manual (1984). 10th ed. Difco Laboratories, Detroit, MI.
[16] Axelsson, L. (1998) Lactic acid bacteria: classification and physiology. In: Lactic Acid Bacteria, Microbiology and Functional Aspects (Salmìnen, S. and von Wright, A., Eds.), second ed, pp. 1–72. Marcel Dekker, New York, NY.
[17] Buckenhüskes, H.J. (2001) Fermented vegetables. In: Food Microbiology, Fundamentals and frontiers (Doyle, M.P., Beuchat, L.R. and Montville, T.J., Eds.), second ed, pp.

665–679. American Society for Microbiology, Washington, DC.

[18] Johnson, M.E. and Steele, J.L. (2001) Fermented dairy products. In: Food Microbiology, Fundamentals and frontiers (Doyle, M.P., Beuchat, L.R. and Montville, T.J., Eds.), second ed, pp. 651–664. American Society for Microbiology, Washington, DC.

[19] Valerio, F., Lavermicocca, P., Corsetti, A., Gobbetti, M. and Visconti, A. (2004) Antifungal activity of phenyllactic acid produced by a sourdough *Lactobacillus plantarum* strain. In: Congresso Italiano di Scienza e Tecnologia degli alimenti (6°CISETA) Cernobbio, Como, Settembre 18–19, 2003. Sessione I. Chiriotti Editori (in press).