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ORIGINAL PAPER

Production of organic acids by *Lactobacillus* strains in three different media

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Abstract Ten strains of *Lactobacillus* (*Lb*). *casei*, *Lb. rhamnosus*, *Lb. plantarum*, *Lb. paracasei* and *Lb. curvatus* species were chosen to determine the production of organic acids after cultivation in skimmed milk, MRS broth and Jerusalem artichoke (JA) medium. The highest acidity was obtained in MRS broth and the weakest acidification was found in skimmed milk. *Lb. casei* Shirota produced the highest amount and *Lb. rhamnosus* VT1 the lowest amount of substances being estimated as titratable acidity. All strains produced lactic acid in the investigated broth and most of the strains produced acetic acid in MRS broth except *Lb. curvatus* 2768 and *Lb. casei* Shirota, in JA broth except *Lb. paracasei* SF1 and in skimmed milk except *Lb. casei* 2750, *Lb. curvatus* 2768, *Lb. curvatus* 2775 and *Lb. casei* Shirota. All strains, except *Lb. plantarum* 01, produced butyric acid in MRS broth. Beside the lactic and acetic acids, formic, citric, succinic and glutamic acids were also produced in MRS broth; formic and succinic acids were produced in skimmed milk and succinic acid in JA broth. Some strains showed change in their fermentation profile from homofermentative to mix-acid fermentation in milk. The antifungal efficiency of the lactic and acetic acid in the amount produced by lactobacilli was investigated. None of the investigated aspergilli were inhibited. The inhibitory effect of acids against *Fusarium* increased unequivocally with the increasing concentration. The study pointed at the dissimilarity of organic acid production of *Lactobacillus* strains, which was considerably influenced by the media.

Keywords Antifungal activity · Jerusalem artichoke broth · Lactobacillus · MRS broth · Organic acid · Reconstituted skimmed milk

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Introduction

The lactic acid bacteria (LAB) are used since ancient times for food formation and preservation. In our days, LAB are used for production of fermented dairy products, cheeses, sourdough bread and some special lacto-fermented vegetables also [1, 2] and some species are also used as protective culture in the meat industry [3, 4]. One of the most important and as a consequence, one of the most investigated genus of the LAB is the *Lactobacillus* genus. The habitats of lactobacilli in the nature show great diversity, they can be found, e.g. on plants and plant materials, soil, water, animal and human intestinal tract and spoilt food [5]. The preservation effect as a function of lactobacilli rises from the reduction of the amount of available carbohydrates and the formation of a range organic molecules, which in turn exhibit antimicrobial activity [6]. This antimicrobial activity of lactobacilli is mainly caused by production of lactic, acetic, formic, caproic, propionic, butyric and valeric acids [7]. Lactobacilli also produce other inhibitory substances such as H_2O_2 [8, 9], CO_2, diacethyl [6, 10] and bacteriocins—thermostable low molecular weight compounds [11, 12]. The organic acids effect on one hand by the acidification of the environment, and on the other hand by the antimicrobial effect of their non-dissociated molecule form, which is pH dependent. In point

of the metabolite products of lactobacilli, the lactic and acetic acids are regarded as the main organic acids, which posses antimicrobial behaviour. The growth of lactobacilli, the production and the activity of their antifungal substances depend on the composition of the media in which they are cultivated [13–15]. The main reason of differences is the presence of nutritional substances in the different media. However, the influence of growth conditions (time and temperature of incubation, pH) are also not negligible factors [16]. Nevertheless, the produced metabolites and their amounts principally depend on the strain, and as a consequence the different strains result in products with various taste due to the specific metabolites [17]. The aim of our study was to investigate the acid production of ten authentic *Lactobacillus* strains in different media and to measure the mould growth inhibition of the main organic acids (lactic and acetic) produced.

Materials and methods

Investigated strains

Ten *Lactobacillus* strains isolated from different sources have been chosen: [*Lactobacillus* (*Lb.*) *plantarum* 01, *Lb. paracasei* 05, *Lb. casei* 154, *Lb. paracasei* SF1, *Lb. rhamnosus* VT1] from the collection of the Department of Dairy and Fat Technology (DDFT), Prague, Czech Republic and the others (*Lb. plantarum* 2142, *Lb. paracasei* subsp. *paracasei* 2750, *Lb. curvatus* 2768, *Lb. curvatus* 2775, *Lb. casei* Shirota) from the collection of the Central Food Research Institute (CFRI), Budapest, Hungary. The inocula from the investigated strains were prepared by growing the cultures at 37 °C for 24 h in MRS broth at semi-anaerob condition (4% CO_2).

The test strains for the investigation of antifungal activity of organic acids were from the DDFT [*Fusarium* (*F.*) *proliferatum* M5689], from the Research Institute of Crop Production, Prague, CR (*F. culmorum* 301, *F. culmorum* 302, *F. graminearum* 608, *F. graminearum* 821), and from the CFRI [*Aspergillus* (*A.*) *flavus*, *A flavus* 31, *A. parasiticus*, *A. parasiticus* 1039]. These strains were selected because strains from the *Fusarium* and *Aspergillus* genera can occur and cause food spoilage; furthermore, produce mycotoxin in several food, among others on cheeses and in juices [18, 19].

Fermentation

For the study, three different media have been chosen with respect to the usage of *Lactobacillus* strains in the production of fermented dairy and vegetable products. For the fermentation, reconstituted skimmed milk (prepared from milk powder, 10 m/v%) and Jerusalem artichoke vegetable juice (prepared from Jerusalem artichoke powder, 10 m/w%) and as control MRS (Oxoid Ltd, UK) broth were used. Fermentation experiments were conducted in glass flasks, each containing 100 ml sterile media. All samples were inoculated with a 24-h-old culture (1%, v/v) and were incubated at 37 °C under semi-anaerob conditions (4% CO_2). After 18 h, when the fermentation completely pass off, the active (pH) and the titratable acidity were measured, and organic acids were estimated quantitatively and qualitatively.

Determination of acidity and organic acids

After incubation the pH was determined by direct measuring with a pH meter, the titratable acidity was determined by titrating the fermented samples with 0.25 mol l^{-1} NaOH to pH 8.3 and the results were expressed as degree Soxhlet–Henkel (°SH/100 ml).

The quality and quantity of the produced acids were measured by isotachophoretic analysis from the supernatants. The samples were filtered and 1 ml was placed in a volumetric flask and filled with distilled water up to 100 ml. The samples were subjected to isotachophoretic analysis using the Isotachophoretic Analyser ZKI-01 (Slovakia) under the following conditions: leading electrolyte 0.01 mol l^{-1} HCl, co-ion = 6-aminobutyric acid (Lachema, Czech Republic), pH 4.25 and as terminating electrolyte 0.005 mol l^{-1} caproic acid and 0.005 mol l^{-1} histidine, pH 5.0 (Lachema, Czech Republic) were used. An electric current in the pre-separative column of 25 μA and in the analytical column of 50 μA was applied. The qualitative and quantitative evaluations were done by an analysis of model mixtures of selected organic acids (formic, citric, phosphoric, lactic, succinic, aspartic, acetic, glutamic, propionic, butyric and valeric acids) with different concentrations. The amounts of organic acids produced by the *Lactobacillus* strains were in terms of difference in the amount of organic acid in the supernatants after and before the fermentation and the concentration of each acid was expressed in mmol l^{-1}. The pooled standard error of the measurement is 2.284. Results are the average from three trials.

Investigation of the antifungal efficiency

The test mould strains were grown on Potato Dextrose Agar (PDA) (Oxoid Ltd., UK) slants. After sporulation, distilled water was poured on the culture, it was shaked and the spore suspension was diluted to approx 10^6 spore ml^{-1}. The antifungal activity of lactic and acetic acids was investigated by agar diffusion method. Briefly, the acids (lactic and acetic acid separately) concentrations were set to the same concentration that was produced by the strains

on the different media. Bacteriological agar (Oxoid Ltd, UK) plates were prepared and wells were made with cork borer (Ø10 mm) in the agar, one well per dish. Into the wells, 450 µl of acid samples was distributed, respectively. After the diffusion of acid, 9 ml soft PDA agar (agar 0.7%) containing the test organisms in 10^5 spore ml^{-1} concentration was poured onto the acid-containing agar plate. The plates were incubated at 30 °C for 2 days. The inhibitory zones were evaluated visually. Results are the average from two plates.

Results

Active and titratable acidity

The results of the active (pH) and titratable acidity measured after 18 h of incubation and compared to the control sample, which was not inoculated, revealed that the best fermentation was obtained in MRS broth and the faintest in reconstituted skimmed milk (Figs. 1, 2). The highest titratable acidity was observed for the strain *Lb. casei* Shirota and the lowest for the strain *Lb. rhamnosus* VT1 in all tested media. The pooled standard error of the measurement for pH is 0.08 and titratable acidity is 0.51. Results are the average from two trials.

Organic acid production

After the fermentation process the organic acids were qualitatively and quantitatively determined from the media. The quantity of acids produced was depended on the media and strains, as well. All strains produced lactic acid in the largest concentration in MRS broth (400–851 mmol l^{-1}). They also produced acetic acid (25–150 mmol l^{-1}) except strains *Lb. curvatus* 2768 and *Lb. casei* Shirota, and butyric acid was also produced by the strains except *Lb. plantarum* 01, but formic acid was measured in case of strain *Lb. plantarum* 01 only. Citric acid was measured from supernatants of three strains (*Lb. plantarum* 01, *Lb. paracasei* subsp. *paracasei* 05, *Lb. rhamnosus* VT1), succinic acid was produced by two (*Lb. casei* subsp. *casei* 154, *Lb. plantarum* 2142) and glutamic acid by two strains (*Lb. paracase*i subsp. *paracasei* 05, *Lb. paracase*i subsp. *casei* SF1), respectively (Fig. 3). Lactic acid was produced in skimmed milk in the concentration range 13–127 mmol l^{-1}, and only six strains produced acetic acid (8–100 mmol l^{-1}); however, in case of five strains its amount exceeded the concentration of lactic acid (Fig. 4), as much as the concentration of the formic acid produced by four strains (*Lb. plantarum* 01, *Lb. paracasei* subsp. *paracasei* 05, *Lb. paracasei* subsp. *casei* SF1, *Lb. rhamnosus* VT1). Succinic acid was also measured in case of four strains *(Lb. casei* subsp. *casei* 154, *Lb. rhamnosus* VT1, *Lb. paracasei* subsp. *paracasei* 2750, *Lb. curvatus* 2768) in milk. Lactic acid was measured in the concentration range 110–337 mmol l^{-1} in Jerusalem artichoke broth, the acetic acid, which was produced by all strains (except *Lb. paracasei* subsp. *casei* SF1), was in the range of 9–180 mmol l^{-1} (Fig. 5). Only in case of strain *Lb. paracasei* subsp. *paracasei* 2750 was the acetic acid concentration greater than the lactic acid concentration, and

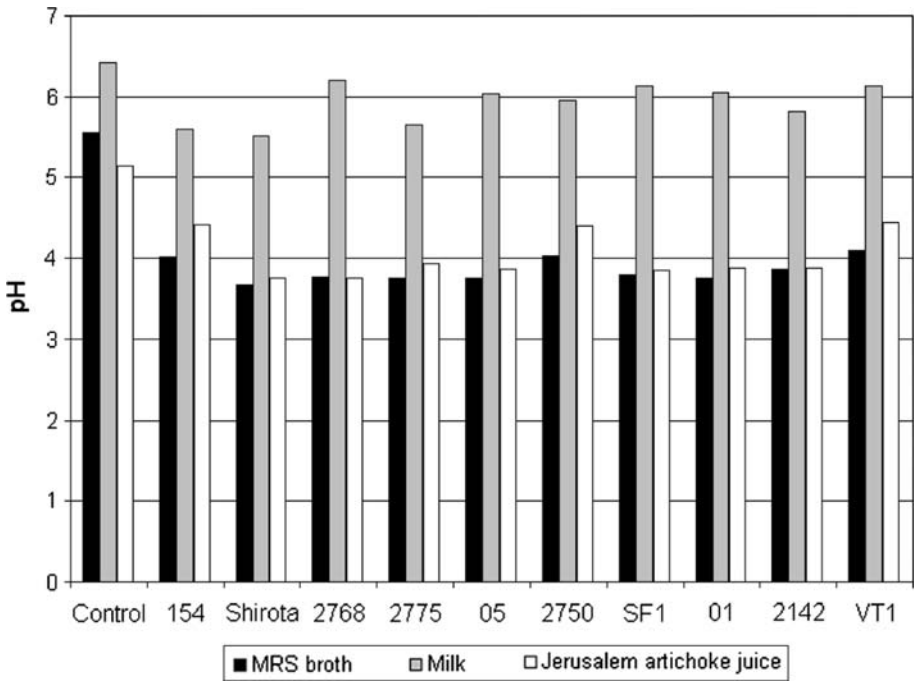

Fig. 1 The active acidity of media after fermentation (18 h, 37 °C)

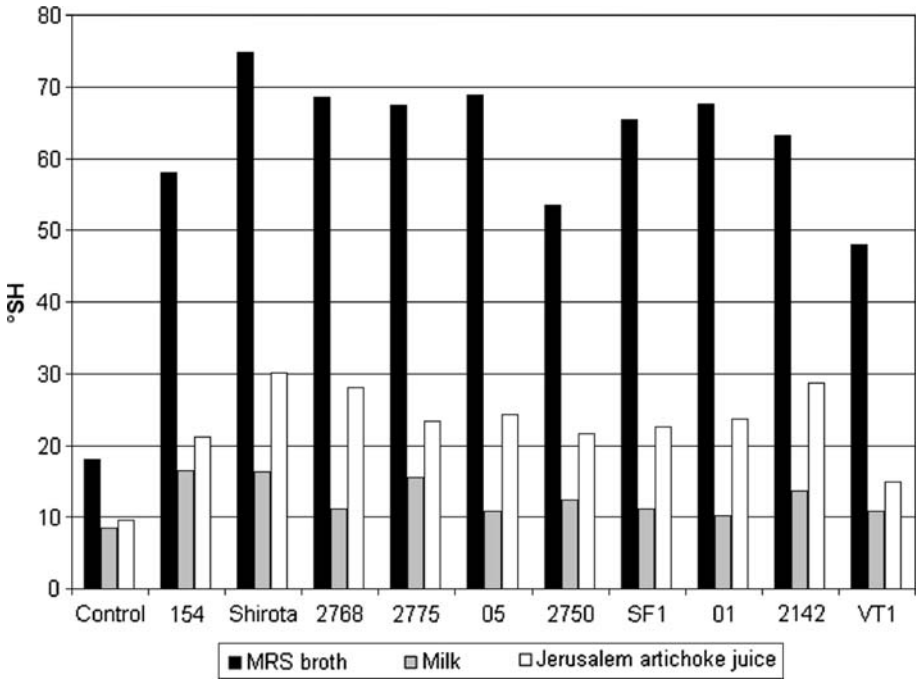

Fig. 2 The titratable acidity of media after fermentation (18 h, 37 °C)

besides this strain only two others (*Lb. casei* subsp. *casei* 154, *Lb. plantarum* 2142) produced succinic acid in the JA broth.

Antifungal efficiency of lactic and acetic acids

The antifungal effect of the lactic and acetic acids in the concentration produced by the lactobacilli in the different media was investigated on the test mould strains by agar diffusion method. In this way, the effect of other metabolites was eliminated and the unalloyed antifungal effect of the produced lactic and acetic acids was measured (Table 1). All *Fusarium* strains were inhibited, however, in different rate, but none of the *Aspergillus* strains were inhibited by the organic acids. The most sensitive test strains were *Fusarium culmorum* 301 and *F. graminearum* 608. According to the inhibitory effect of produced organic acids by lactic acid bacteria, the strains *Lb. casei* 154, *Lb. plantarum* 2142 and *Lb. paracasei* 05 could inhibit the *Fusarium* species the most efficiently.

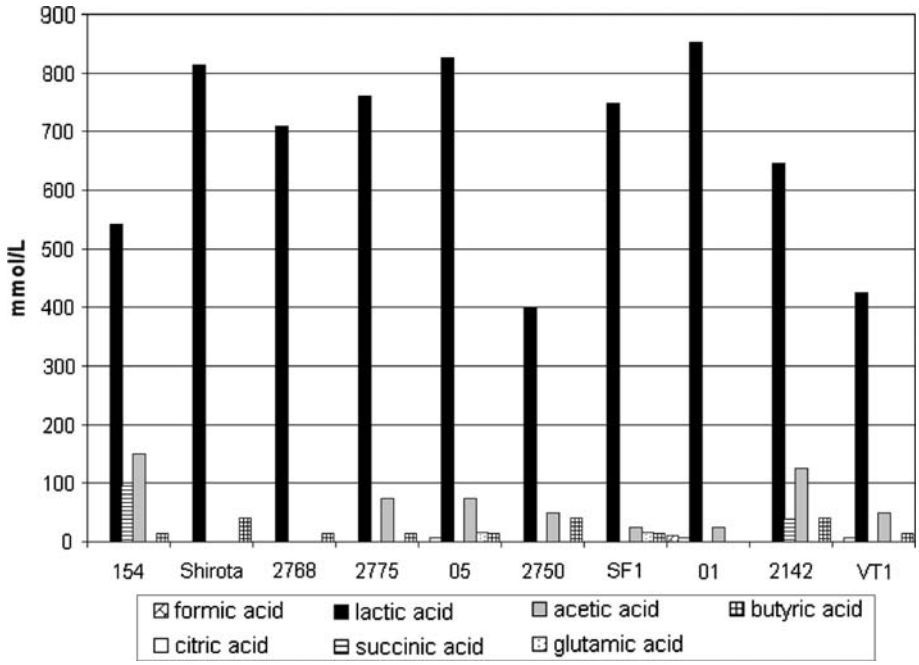

Fig. 3 Organic acid production of lactobacilli in MRS broth (18 h, 37 °C)

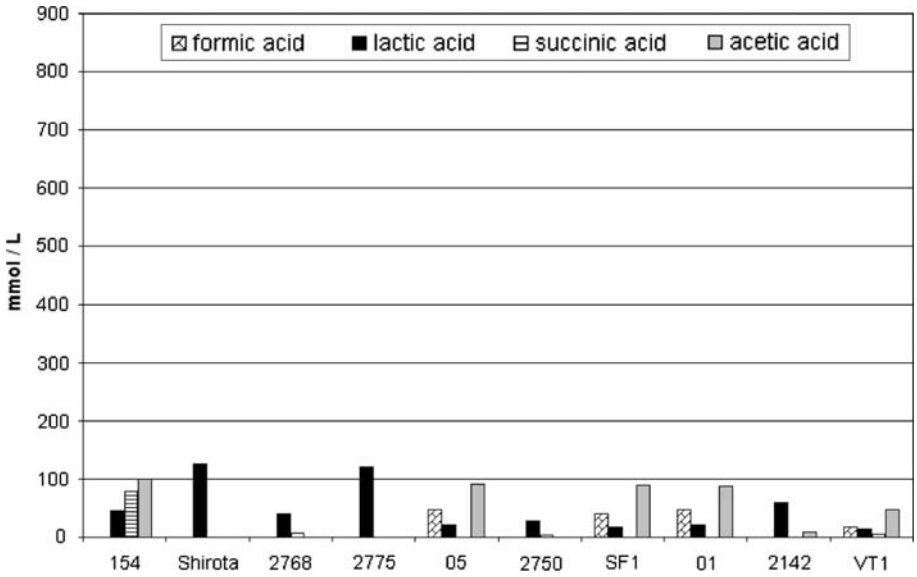

Fig. 4 Organic acid production of lactobacilli in milk (18 h, 37 °C)

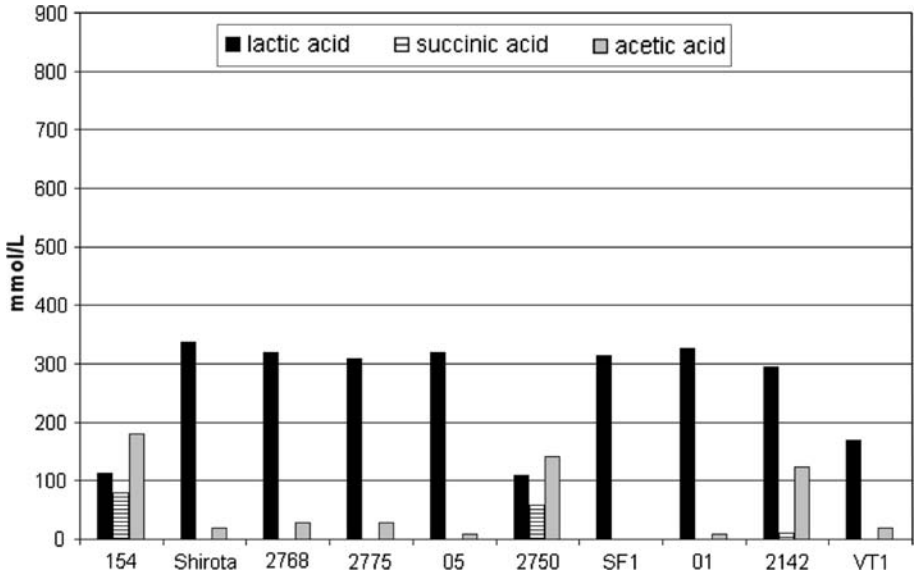

Fig. 5 Organic acid production of lactobacilli in Jerusalem artichoke juice (18 h, 37 °C)

Discussion

In this study, metabolism of lactobacilli was compared after cultivation of strains in three different media, MRS broth, reconstituted skimmed milk and Jerusalem artichoke medium. Reconstituted skimmed milk was selected as a most common food substrate for lactobacilli [20–22]. The lactobacilli can metabolize the oligosaccharides and the long-chain inulin with their fructofuranosidase enzymes [23–25], but not much information on the ability of bacteria to ferment Jerusalem artichoke medium is available. In most microbiological studies, both Jerusalem artichoke tubers and inulin as a potential substrate in the fermentation industry have been used [26–30]. For this reason, Jerusalem artichoke medium was selected as one of the investigated medium. MRS broth served as control complex medium, which was developed for the lactobacilli [31].

Active and titratable acidity

The organic acid production of the LAB strains can be tracked unambiguously in the change of active and titratable acidity. The pH decrease and the titratable acidity increase were greater in case of the better acid producer strains in a given media. These changes of acidity just slightly correlate with the amount of produced total acids, but more correlate with the lactic acid production. This is not only because this acid was produced generally in the greatest amount, but also from the two most significant acids the lactic acid is stronger acid ($pKa = 3.86$) than the acetic acid ($pKa = 4.73$). The titratable acidity in the

Table 1 Antifungal activity of lactic and acetic acids in the concentration produced by LAB strains during incubation at 37 °C for 18 h in different media

Mould strains	Media	MRS																			
	LAB strains	154		Shirota		2768		2775		05		2750		SF1		01		2142		VT1	
		Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic
301		+	+++	++	No production	++	No production	++	+	+++	++	+	+	++	−	++	−	+	++	+	+
302		+	++	++		++		++	+	+++	+	+	−	++	−	++	−	++	++	+	−
M5689		+	++	++		++		++	+	++	+	+	−	++	−	+	−	++	++	+	−
608		++	++	++		++		++	+	+++	++	+	−	+++	−	+++	−	++	++	+	−
821		++	++	++		++		++	+	++	−	+	−	++	−	++	−	++	++	+	+
A.f.		−	−	−		−		−	−	−	−	−	−	−	−	−	−	−	−	−	−
A.p.		−	−	−		−		−	−	−	−	−	−	−	−	−	−	−	−	−	−
31		−	−	−		−		−	−	−	−	−	−	−	−	−	−	−	−	−	−
1039		−	−	−		−		−	−	−	−	−	−	−	−	−	−	−	−	−	−

	Media	Milk																			
		Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic
301		−	+++	+	No production	−	No production	+	No production	−	+++	−	No production	−	++	−	++	−	−	−	+
302		−	+	−		−		−		−	+	−		−	+	−	+	−	−	−	−
M5689		−	++	−		−		+		−	−	−		−	++	−	−	−	−	−	−
608		−	+	+		−		+		−	−	+		−	+	−	+	−	−	−	−
821		+	+	+		−		−		−	++	−		−	−	−	+	−	−	+	+
A.f.		−	−	−		−		−		−	−	−		−	−	−	−	−	−	−	−
A.p.		−	−	−		−		−		−	−	−		−	−	−	−	−	−	−	−
31		−	−	−		−		−		−	−	−		−	−	−	−	−	−	−	−
1039		−	−	−		−		−		−	−	−		−	−	−	−	−	−	−	−

	Media	Jerusalem artichoke																			
		Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic	Lactic	Acetic
301		+	+++	+	−	−	−	−	−	++	−	−	++	++	No production	++	−	+	++	+	−
302		−	++	+	−	+	−	+	−	+	−	−	+	+		+	−	+	+	−	−
M5689		−	++	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−
608		−	+++	+	−	+	−	+	−	−	−	−	++	+		−	−	−	++	−	−
821		−	+++	+	−	+	−	−	−	+	−	−	+	+		+	−	−	−	+	−
A.f.		−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−
A.p.		−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−
31		−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−
1039		−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	−

− No inhibitory effect, + weak inhibitory effect, ++ strong inhibitory effect, +++ great inhibitory effect

different media was changed according to the acid production of strains in the given media, naturally. We obtained the highest titratable acidity in the MRS broth, where the acid production of investigated strains was also the greatest, and the lowest in milk media, where the acid production was the weakest. The decrease of the pH values correlated with the increase of the titratable acidity values; however, in the MRS media the pH values were higher than we can expect according to the increasing of titratable acidity, but this can be explained with the greater buffer capacity of the MRS media (it is two and three times more than the buffer capacity of milk and JA broth, respectively).

Organic acid production

The results show that the greatest lactic acid production (400–851 mmol l^{-1}) was in the complex MRS broth medium. It is natural due to the optimal composition of this media, which was developed exactly for this group of bacteria [31]. We obtained differences in the lactic acid quantity between species, and also between the strains of the same subspecies (*Lb. paracasei* subsp. *paracasei* 05 and 2750). In this medium, the sole carbohydrate source is the glucose, which can be fermented by the *Lactobacillus* strains through two major pathways: the glycolysis (Embden–Meyerhof pathway) is used by the homofermentative LAB,

$$1\ \text{glucose} + 2\ \text{Pi} \rightarrow\ 2\ \text{lactate} + 2\ \text{ATP} + 1\ \text{H}_2\text{O}$$

and the 6-phosphogluconate/phosphoketolase (6-PG/PK) pathway,

$$1\,\text{glucose} + \text{Pi} + \text{ADP} \rightarrow 1\,\text{lactate} + 1\,\text{acetate} + \text{ATP} + 1\,\text{CO}_2,$$

is used by heterofermentative LAB [32]. On the glucose substrate, the lactic acid is the main end product in both metabolic pathways, as our results show, but while in the glycolysis the fermentation of 1 mol glucose results in 2 mol lactic acid, in the 6-PG/PK pathway gives only 1 mol lactic acid. According to this and our results it seems that the strain *Lb. paracasei* subsp. *paracasei* 2750 behaves heterofermentatively under certain conditions, but several other factors may also influence the process.

In case of milk medium, we have found two strains, the *Lb. casei* Shirota and the *Lb. curvatus* 2775, which produced from lactose, which is the sole carbohydrate source, only lactic acid and these two strains produced more than 100 mmol l^{-1}, exactly 127 and 120 mmol l^{-1}, respectively. The other strains produced between 13 and 60 mmol l^{-1}, in agreement with other authors [33, 34]. Although not the lactic acid was the main organic acid in case of some strains (*Lb. casei* subsp. *casei* 154, *Lb. paracasei* subsp. *paracasei* 05, *Lb. paracasei* subsp. *casei* SF1, *Lb. plantarum* 01, *Lb. rhamnosus* VT1). The different efficiency of the lactose-hydrolyzing/galactosidase enzyme activity may account for the great difference between the lactic acid production of the strains.

In the Jerusalem artichoke media the lactic acid concentration (110–337 mmol l^{-1}) was between the concentration, that we obtained in MRS and milk, but the trend of the production, namely the differences between the strains was the same like in the MRS media. In JA broth the carbohydrate sources were the inulin (polymers of fructose units, which have a terminal glucose) and its derivatives after hydrolysis (different fructooligosaccharides) and probably free fructose. The strains of *Lactobacillus* genera can perfectly metabolize the oligosaccharides with their fructofuranosidase enzymes [23, 25]. Makras et al. [24] reported that *Lactobacillus paracasei* subsp. *paracasei* 8700:2 degraded oligofructose and long-chain inulin and grew rapidly on both. We obtained the same results in case of JA juice that the lactic acid was the main end product of the *Lactobacillus* strains.

The presence of acetic acid in the media after fermentation by the *Lactobacillus* strains can be the result of the different biochemical pathway, e.g. the degradation product of the produced lactic acid [35], the result of the citrate metabolism [36] and/or it originates from the heterofermentative pathway [32]. In the MRS media great amount of acetic acid was produced compared to the lactic acid by the strains *Lb. casei* subsp. *casei* 154, *Lb. plantarum* 2142. Since the glucose inhibit the citrate utilization (which citrate in the MRS broth comes for the ammonium citrate ingredient) [37], the greater acetic acid production can be the result of the heterofermentative pathway, where the acetate is the one of the main end products.

In the milk media we had obtained great acetic acid production (near to 100 mmol l^{-1}) in case of four strains of six, which produce it at all (Fig. 4). This amount is greater than published by other author [34]. The acetate is important for the flavour development of many fermented dairy products, and this component is linked to the citrate metabolism [38–40]. We also observed in accord with this, that the initial citrate concentration (85,6 mmol l^{-1}), what we measured in the control milk sample, was greater than in the supernatant at the end of the fermentation in every case (0–77 mmol l^{-1}) and we obtained correlation between the citrate utilization and the acetate production (data not shown). While in the MRS and also in the JA juice, certain strains (*Lb. plantarum* 01, *Lb. paracasei* 05, *Lb. paracasei* SF1) showed unambiguous homofermentative acid profile, in milk the fermentation pattern of these strains changed from homolactic to a mixed-acid profile, which is explicable with the utilization of milk citrate [41].

In the Jerusalem artichoke juice the acetic acid production grew, however, in low concentration, in case of the

strains *Lb. casei* subsp. *casei* 154, *Lb. paracasei* subsp. *paracasei* 2750, *Lb. plantarum* 2142 and some strains (e.g. *Lb. curvatus* 2768, *Lb. casei* Shirota), which did not produce acetic acid in the MRS broth. Makras et al. [24] measured in case of *Lb. paracasei* subsp. *paracasei* 8700:2 on oligofructose and inulin energy sources significant amount of acetic acid. In the JA juice also the inulin and oligofructose are the sources of the higher amounts of acetic acid.

The presence of the succinic acid in the supernatant after the fermentation can also be explained with the citrate utilization of lactobacilli [41, 42]. In the absence of oxaloacetate decarboxylase the citrate utilization goes through the succinic acid pathway [32].

1 glucose + 1 citrate + 2 ADP + 2 Pi
→ 1 lactate + 2 acetate + 1 CO2 + 1 succinate + 2 ATP

According to Axelsson [32] and Kaneuchi et al. [40], the succinic acid production from citrate is fairly common among intestinal and plant-associated heterofermentative lactobacilli. From our results it is clear in case of the MRS and Jerusalem artichoke juice that the succinic acid production tightly attached to the extended acetic acid production, which in this way denote the heterofermentative character of the strains *Lb. casei* subsp. *casei* 154, *Lb. paracasei* subsp. *paracasei* 2750, *Lb. plantarum* 2142.

In milk we also obtained formic acid in low concentration in case of some strains (*Lb. plantarum* 01, *Lb. paracasei* subsp. *paracasei* 05, *Lb. paracasei* subsp. *casei* SF1, *Lb. rhamnosus* VT1) after fermentation; however, the amount of the produced formic acid was greater than the lactic acid (Fig. 4). Garrigues et al. [43] have investigated the growth and metabolism of the *Lactococcus lactis* subsp. *lactis* NCDO 2118 strain on various sugars and found that this lactic acid bacterium produces more formate on lactose than lactic and acetic acids, while on glucose the lactate was the major product. They explain it with the shift of metabolic pathway from homolactic to mix-acid fermentation. On the other hand, according to some authors, the formic acid production correlate with the citrate utilization [44], which is confirmed by our results since the four formic acid producers totally consumed the citrate from the milk, while the other, non-formic acid producer strains no or just partly consumed the citrate from the milk (data not showed).

The knowledge of the acidification and organic acid production is very important in the fermentation, because the strain selection is planned accordingly. From our results it is clear that for fermentation of different substrates different strains are optimal.

It can be found by the bald facts that for the milk fermentation the *Lb. casei* Shirota and *Lb. casei 154*, for the JA juice (and this kind of vegetable juices) fermentation the *Lb. casei Shirota*, *Lb. casei* 154 and *Lb. plantarum* 2142 strain can be the most appropriate of the investigated strains; however, also several other strains showed similar properties and just a small difference was obtained between them. In this way the most appropriate ones are the strains which produce more acetic acid, since this acid effect favourably influences the flavour and the antimicrobial property, but the ratio between the produced acids is not negligible either. According to these, the strain *Lb. plantarum* 2142 seemed to be the best for fermentation on Jerusalem artichoke media. Nevertheless, the sensory quality and the microbial safety of the end product is influenced by several other factors [e.g. the produced other acids, other antimicrobial metabolites (H_2O_2, bacteriocin, etc.), minor metabolites (diacetyl, acetoin, mannitol, etc.)].

Antifungal activity

As our results showed that all of the *Fusarium* test strains were inhibited by lactic or acetic acid; however, the rate of the inhibition was also influenced by test strains and the type and quantity of the acid, but in contrast with this, none of the *Aspergillus* strains were reactive to the acids in the investigated concentrations. This result corresponds with the observation of Higgins and Brinkhaus [45], who also found that the *Fusarium* was the most susceptible to organic acids, more than the *Aspergillus*. The inhibitory effects of acids against *Fusarium* strains increased unequivocally with the increasing acid concentration. According to the produced acid concentration the expected antifungal inhibitory effect is in the media of the order MRS > JA > milk. It is observable that in some cases (e.g. 154 MRS, 2142 MRS) the acetic acid was more effective in smaller concentrations, than the lactic acid; however, it is not strange since of the two acids, acetic acid is more inhibitory than lactic acid [20, 46]. Our results confirmed the key role of lactic and acetic acids in the antimicrobial activity of LAB strains and the influence of medium composition on the production of active metabolites.

Conclusion

In our study we have investigated the active and titratable acidity and organic acid production of ten selected *Lactobacillus* strains. Our results show unambiguously the effect of the media on the acidity and organic acid production. On one side the investigated three media have different energy sources and microelements for lactic acid bacteria, which have led to different organic acid production, and the other side the different strains use different pathways in the

metabolism, which causes the quantitative and qualitative variations in the end metabolites. As a consequence the antifungal activity of the strains is also influenced by the media and the strains too, as our results showed. We confirmed with our study that some strains can change their fermentative profile from homofermentative to mix-acid fermentation depending on the media. These results showed that circumspect selection is necessary for a starter culture, because the metabolite products, the acidification and consequently the antifungal activity are considerably influenced by the media and there are great differences between the strains even in the same species.

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