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ISOLATION AND SCREENING OF LACTIC ACID BACTERIA FROM NATURALLY FERMENTED SOURCES WITH HIGH BIOTECHNOLOGICAL POTENTIAL

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ABSTRACT. A total number of 246 lactic acid bacteria were isolated from naturally fermented traditional foods and feed. These isolates were phenotypically characterized, classified and identified using 16S ribosomal DNA sequencing. 13 different species were detected from cheeses and from fermented plant materials. The isolates belonged to four genera: *Lactobacillus* genera 87%, *Pediococcus* 2%, *Enterococcus* 7%, *Leuconostoc* 4%. The fermentation capacity of the selected bacterial strains were tested in the presence of three different carbon sources. According to the results the most promising strains were *Lactobacillus plantarum* subsp. *plantarum A5, Lactobacillus plantarum* subsp. *plantarum C5, Lactobacillus paracasei* subsp. *Tolerans N16* and *Lb. acidipiscis H9*. These strains were able to produce high amount of L-lactic acid, contributing to the pH-decrease of the medium. This result indicates that the selected bacterial strains shows potential for biotechnological application as starter cultures for silage fermentation**.**

Keywords: *lactic acid bacteria, isolation, bacteria selection, lactic acid fermentation, silage*

INTRODUCTION

Lactic acid bacteria (LAB) play a significant role in lactic acid fermentation processes. These bacteria are industrially important microorganisms with functional properties. They are involved in different applications as probiotics, dairy starters, silage inoculants and microbial cell factories. They inhabit in

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different ecological niches containing rich sugar and organic nitrogen sources [1–3]. Genera of LAB include, among others, *Lactococcus, Enterococcus, Oenococcus, Pediococcus, Streptococcus, Leuconostoc*and *Lactobacillus.* They are Gram-positive organisms, that produce lactic acid by fermentation. The genus *Lactobacillus* is the largest group with over 100 species and subspecies [4]. The most common criteria for the selection of bacteria include rapid growth, intensive lactic acid production, and robustness to tolerate variable stress conditions in manufacturing processes where are involved [5,6]. Also the assessment of LAB used for feed additives for behavior in the presence of antibiotics is a requirement [7].

The LAB are associated with food and feed production due to their preservative action as acidification, flavor enhancement, texture and nutritive value. In forage preservation both homo- and heterofermentative LAB have potential advantages with positive effects.

Due to the production of lactic acid by homofermentative LAB, the pH is reduced faster, that inhibits the growth of undesirable microorganisms and improve the quality of fermentation. The heterofermentative LAB contribute to the good aerobic stability of the silages. Lactic acid and volatile fatty acids as acetic acid, propionic acid produced as the result of fermentation contribute to the energy supply of ruminant's [8]. The above mentioned and other well-known characteristics (e.g. bacteriocin production) expand the spectrum of biotechnological applicability of these microorganisms [4,9].

Natural fermented foods are often rich sources of beneficial LAB, whereas these are still potentially beneficial strains. The search of new LAB strains is (based on the identification of LABs with favorable combination of functional properties) an important issue, since a number of important LAB strains with beneficial properties still need to be characterized [4]. The most common criteria is the intensive lactic acid production that can be described also by fermentation capacity in different conditions.

Our aim was to realise a preliminary study for identification and characterization of LAB strains from naturally fermented sources with beneficial properties for biotechnological applications.

RESULT AND DISCUSSION

A number of 99 bacterial isolates with different colony size and morphology were screened for the most representative characteristics of LAB's as Gram staining, catalase absence, growth in the presence of NaCl (4% and 6.5%) microscopic morphology. The results showed that the majority of the isolates have a rod-like morphology, are catalase negative (85 isolate), and are Gram-positive (72

isolate). A number of 93 isolate showed growth in the presence of 4% NaCl and a number of 63 isolate in the presence of 6.5% NaCl. Bacterial isolates those characteristics suggested similarity with LAB strains were further studied.

A total number of 54 LAB isolates originated from different sources were identified by partial 16SrDNA, sequencing. Our results show that the LAB isolates belonged to 4 genera; 7 species from *Lactobacillus* genera (87%), 1 species from *Pediococcus (*2%), 4 species from *Enterococcus* (7%), 1 species from *Leuconostoc* (4%). 13 different species were detected from the cheeses and the fermented plant materials: *Lactobacillus pentosus, Lb. plantarum* subsp*. plantarum, Lb. gasseri, Lb. paracasei* subsp*. tolerans, Lb. buchneri, Lb. acidipiscis, Lb. brevis, Enterococcus faecalis, E. faecium, E. durans, E. italicus, Leuconostoclactis* and *Pediococcusparvulus.* Among lactobacilli, *Lb. pentosus* and *Lb. plantarum* subsp*. plantarum* were the most commonly isolated from different traditional cheeses originated from different regions.

Results of antibiotic susceptibility of the bacterial strains tested according to the guidelines reported by European Food Safety Authority [7] are shown in table 1.

Table 1. Minimal Inhibitory Concentration (MIC) of selected LAB strains

	Amp.	**Tetr.**	**Chlo.**	**Kan.**	**Str.**	**Pen.**	**Gent.**
Lb. plantarum subsp. *plantarum A5*	4	32	16	> 128	> 128	16	128
Lb. paracasei subsp*. tolerans N16*	1	2	16	> 128	128	1	64
Lb. plantarum subsp. *plantarum C5*	1	16	16	> 128	128	1	64
Lb. pentosus C10	4	64	4	> 128	> 128	1	128
Lb. pentosus C2	4	64	4	> 128	4	1	> 128
Leuconostoclactis N19	8	4	4	128	> 128	1	16
Lb. buchneri H1	4	> 128	64	> 128	32	1	64
Lb. acidipiscis H9	4	16	16	> 128	128	1	32
Lb. brevis H15	4	16	16	> 128	> 128	8	32

(**Amp.**: ampicillin, **Tetr.**: tetracycline, **Chlo**.: chloramphenicol, **Kan**.: kanamycin, **Str.**: streptomycin, **Pen.**: penicillin, **Gent**.: gentamicin)

The MIC test results against seven antibiotics (ampicillin, tetracycline, chloramphenicol, kanamycin, streptomycin, penicillin, gentamicin) are shown in table 1. For ampicillin with the exception of one strain (*Leuconostoclactis N19,* MIC=8 $\mu g.ml^{-1}$) all the others had a MIC ≤4 $\mu g.ml^{-1}$. In case of tetracycline with the exception of *Lb. buchneri H1* (MIC>128 $\mu g.ml^{-1}$), the MIC values varied between 2-64 $\mu g.ml^{-1}$. For chloramphenicol the MIC values were between 4-16

μg.ml^{-1}, with exception of *Lb. buchneri H1* (MIC=64 μg.ml^{-1}). For kanamycin the MIC value with the assayed concentration was not detected exactly, but was higher than 128μg.ml^{-1}. For streptomycin, the MIC value were 128 μg.ml^{-1} for three of the studied LBA strains (*Lb. acidipiscis H9*, *Lb. plantarum* subsp*. plantarum C5* and *Lb. Paracasei* subsp*. Tolerans N16).* In case of four strains the MIC value was higher than 128 μg.ml^{-1}. For *Lb. pentosus C2* strain the MIC value was 4 μg.ml^{-1}and *Lb. buchneri H1* strain was 32 μg.ml^{-1}.

The lowest MICs where observed for penicillin, where only two strains has MIC>1 μg.ml^{-1} (*Lb. plantarum* subsp*. plantarum,* MIC= 16 μg.ml^{-1} and *Lb. brevis* H15 MIC=8 μg.ml^{-1}). For gentamicin the MIC values varied between 16-128 μg.ml^{-1} with the exception of *Lb. pentosus C2* (MIC>128 μg.ml^{-1}).

Phenotypic characterization of the selected LAB strains was realized with Biolog system using GEN III Microplates. The fermentation patterns of the selected strains are shown in table 2. The nine selected LAB showed differences in metabolic patterns.

All strains of LAB were able to ferment α-D-glucose. With the exception of *Lb. paracasei* subsp*. Tolerans N16* the strain were able to ferment D-maltose and with the exception of *Lb. buchneri H1* they were able to utilize N-acetyl-D-glucoseamine. Five strain were able to utilize D-cellobiose (*Lb. plantarum* subsp*. plantarum C5, Lb. pentosus C10, Lb. pentosus C2, Leuconostoclactis N19, Lb. acidipiscis H9, Lb. brevis H1*5) D-fructose (*Lb. paracasei* subsp. *Tolerans N16 Lb. plantarum* subsp*. Plantarum C5*, *Leuconostoclactis N19, Lb. acidipiscis H9, Lb. brevis H15*), D-mannitol (*Lb. plantarum* subsp*. Plantarum A5*, *Lb. paracasei* subsp*. Tolerans N16*, *Lb. plantarum* subsp*. Plantarum C5*, *Lb. pentosus C10*, *Lb. pentosus C2*), and gentibiose (*Lb. plantarum* subsp*. Plantarum C5*, *Lb. pentosus C10*, *Lb. pentosus C2*, *Leuconostoclactis N19*, *Lb. acidipiscis H9*).

Only three of the strains degraded sucrose (*Lb. plantarum* subsp*. plantarum C5, Lb. pentosus C2, Leuconostoclactis N19*) and β-methyl-D-glucoside (*Lb. plantarum subsp. plantarum C5, Lb. pentosus C2, Lb. acidipiscis H9*). A single strain fermented D-raffinose (*Lb. buchneri H1*) and glycerol (*Lb. acidipiscis H9*). Two of the tested bacterial strains were able to decompose inosine (*Lb. buchneri H1* and *Lb. acidipiscis H9*), D- sorbitol (*Lb. plantarum* subsp*. plantarum* C5*, Lb. pentosus* C2), D-melibiose (*Lb. buchneri H1*, *Lb. brevis H15*), and D-salicin (*Lb. plantarum* subsp*. plantarum C5*, *Lb. pentosus C2*). Four LAB strains were able to utilize D-galactose (*Lb. paracasei* subsp*. Tolerans N16*, *Lb. plantarum* subsp*. plantarum C5*, *Lb. buchneri H1*, *Lb. acidipiscis H9*) and D-turanose (*Lb. plantarum* subsp*. plantarum C5*, *Lb. pentosus C10, Lb. pentosus C2*, *Leuconostoclactis N19*). With the exception of two LAB strains (*Lb. buchneri H1* and *Lb. brevis H15*) the strains were able to ferment D-mannose. Carbohydrate fermentation results of the assayed strains showed a variability in the enzymatic activities. Chemical sensitivity showed that *Lb. brevis H15* is able to grow in the presence of rifamycin SV whereas *Lb. buchneri H1* showed

Table 2. Fermentation patterns and chemical sensitivity of the LBA strains

	A5	***N16***	***C5***	***C10***	***C2***	***N19***	***H1***	***H9***	***H15***
D-maltose	+	-	+	+	+	+	+	+	+
D-trehalose	-	+	+	-	-	-	-	+	-
D-cellobiose	-	-	+	+	+	+	-	+	-
Gentiobiose	-	-	+	+	+	+	-	+	-
Sucrose	-	-	+	-	+	+	-	-	-
D-turanose	-	-	+	+	+	+	-	-	-
pH 6	-	-	-	-	-	-	+	-	+
pH 5	-	+	-	-	-	-	+	-	-
D-raffinose	-	-	-	-	-	-	+	-	-
α-D-lactose	+	+	+	+	+	+	-	-	-
D-melibiose	-	-	-	-	-	-	+	-	+
β-methyl-D-glucoside	-	-	+	-	+	-	-	+	-
D-salicin	-	-	+	-	+	-	-	-	-
N-acetyl-glucosamine	+	+	+	+	+	+	-	+	+
NaCl, 1%	-	+	-	-	-	-	+	-	-
α-D-glucose	+	+	+	+	+	+	+	+	+
D-mannose	+	+	+	+	+	+	-	+	-
D-fructose	-	+	+	-	-	+	-	+	+
D-galactose	-	+	+	-	-	-	+	+	-
Inosine	-	-	-	-	-	-	+	+	-
Sodium lactate, 1%	-	+	+	-	-	-	+	-	+
D-serine	-	+	-	-	-	-	+	-	-
D-sorbitol	-	-	+	-	+	-	-	-	-
D-mannitol	+	+	+	+	+	-	-	-	-
Glycerol	-	-	-	-	-	-	-	+	-
Rifamycin SV	-	-	-	-	-	-	-	-	+
Minocycline	-	-	-	-	-	-	+	-	-
D-galacturonic acid	-	-	-	-	-	-	-	-	+
D-gluconic acid	-	-	+	-	-	-	-	-	+
D-glucuronic acid	-	-	-	-	-	-	-	-	+
Vancomycin	-	+	-	-	-	-	+	-	+
L-alanine	-	-	-	-	-	-	-	-	+
L-lactic acid	-	-	-	-	-	-	+	-	-
Nalidixic acid	-	+	-	-	-	-	+	-	+
Potassium tellurite	-	-	-	-	-	-	+	-	+
Aztreonam	-	+	-	-	-	-	+	-	+

A5 Lb. plantarum subsp. *plantarum A5, N16 Lb. paracasei* subsp. *tolerans N16 Lb. plantarum* subsp. *plantarum C5 Lb. pentosus C10 Lb. pentosus C2 Leuconostoclactis N19 Lb. buchneri H1 Lb. acidipiscis H9 Lb. brevis H15*

activity in the presence of minocyclin. Three bacterial strains showed activity in the presence of vancomycin (*Lb. paracasei* subsp. *Tolerans N16, Lb. brevis H15*, *Lb. buchneri H1*), nalidixic acid (*Lb. paracasei* subsp. *Tolerans N16*, *Lb. brevis H15*, *Lb. buchneri H1*) and aztreonam (*Lb. paracasei* subsp. *Tolerans N16, Lb. Brevis H15*, *Lb. Buchneri H1*). Two strains showed growth in the presence of potassium tellurite (*Lb. brevis H15*, *Lb. buchneri H1*). The nine LAB strains also showed differences in the chemical sensitivity of the strains.

Fermentation capacity of the nine selected LAB strains was determined in the presence of three different carbon sources (glucose, mixture of glucose and xylose and distiller's dried grains with solubles (DDGS)). The cumulative L-lactic acid amounts after 72 h and the Y_{PX} are shown in table 3.

Table 3. The produced L-lactic acid (LA), acetic acid (AA) and the product yield coefficient after 72 h fermentation in the three different media.

A5	**M_1**	**M_2**	**M_3**	**A5**	**M_1**	**M_2**	**M_3**
LA g/l	39.09	28.10	5.80	**AA g/l**	7.84	3.12	2.10
Y_{PX}mgP/CFU X	1.27	0.23	0.37	**Y_{PX}mgP/CFU X**	0.02	0.001	0.18
N16				**N16**			
LA g/l	31.51	33.40	10.29	**AA g/l**	1.92	2.24	3.25
Y_{PX}mgP/CFU X	0.44	1.46	0.34	**Y_{PX}mgP/CFU X**	0.04	0.17	0.18
C5				**C5**			
LA g/l	29.80	29.39	6.53	**AA g/l**	2.33	3.75	3.50
Y_{PX}mgP/CFU X	0.28	0.88	0.22	**Y_{PX}mgP/CFU X**	0.03	0.08	0.14
C2				**C2**			
LA g/l	29.88	23.34	5.67	**AA g/l**	2.58	3.90	3.46
Y_{PX}mgP/CFU X	0.001	0.02	0.012	**Y_{PX}mgP/CFU X**	0.0006	0.004	0.02
N19				**N19**			
LA g/l	13.50	13.39	7.64	**AA g/l**	1.15	1.71	3.08
Y_{PX}mgP/CFU X	0.04	0.39	0.21	**Y_{PX}mgP/CFU X**	0.007	0.06	0.11
H1				**H1**			
LA g/l	20.30	19.78	5.91	**AA g/l**	2.25	4.11	2.45
Y_{PX}mgP/CFU X	0.08	0.07	0.16	**Y_{PX}mgP/CFU X**	0.01	0.01	0.08
H9				**H9**			
LA g/l	33.07	30.39	6.20	**AA g/l**	3.46	2.11	2.95
Y_{PX}mgP/CFU X	0.35	0.54	0.03	**Y_{PX}mgP/CFU X**	0.05	0.07	0.02
H15				**H15**			
LA g/l	20.10	21.46	4.23	**AA g/l**	2.56	4.26	2.12
Y_{PX}mgP/CFU X	0.029	0.02	0.006	**Y_{PX}mgP/CFU X**	0.005	0.007	0.005
C10				**C10**			
LA g/l	36.37	25.63	6.32	**AA g/l**	3.08	3.91	3.06
Y_{PX}mgP/CFU X	0.03	0.19	0.26	**Y_{PX}mgP/CFU X**	0.005	0.05	0.07

In case of *LB. plantarum* subsp*. plantarum* A5 was detected the highest LA concentration and yield in the M_1 medium (39.092 $g.l^{-1}$, Y_{PX}=1.26 mg P/CFU X). In the case of growing in M_3 medium the cumulative LA was slightly smaller but the Y_{PX} was higher.

Bacterial isolate *Lb. paracasei* subsp*. Tolerans* N16 produced larger amount of LA in M_2 medium, where the Y_{PX} was also the highest (Y_{PX}=1.46 mg P/CFU X).

In the case of the two *Lb. pentosus C10* and *C2* the amount of accumulated lactic acid was high, but the Y_{PX} reached very small values (Y_{PX} values were 0.001 and 0.02 P/CFU X). In the case of the M_3 medium the cumulative amount of the LA was small but the Y_{PX} was higher compared to M_1 and M_2 media. The Y_{PX} in the case of *Lactobacillus plantarum* subsp*. plantarum C5* fermentation process in M_2 medium reached the 0.883 value, even though the accumulated LA was same in M_1 and M_2 medium.

Leuconostoclactis N19 produced small amount of LA compared with other strains, the maximum value of Y_{PX} was 0.39 mg P/CFU X in the case of the M_2 medium.

The L-LA concentration exceeded 30 g/l in the case of the isolated bacterium *Lb. acidipiscis H9* in both glucose containing media, whereas the Y_{PX} was higher than 0.5 (Y_{PX}=0.54 mg P/ CFU X) in medium containing xylose beside glucose.

Bacterial strains *Lb. buchneri H1* and *Lb. brevis H15* produced similar amount of L-lactic acid in both glucose containing media. In these media the accumulated L-lactic acid were 20.295 $g.l^{-1}$ and 20.102 $g.l^{-1}$ with small yield coefficient (Y_{PX}=0.08 mg P/ CFU X and 0.0288 mg P/ CFU X).

Meanwhile the amount of the acetic acid was small, didn't not exceeded the 8g/l. But in the most cases the concentration varied between 2-4 $g.l^{-1}$. The amount of accumulated LA was always higher than AA.

The selection of LAB as suitable starters for biotechnological processes as silage production is a complex process involving the evaluation of some fermentation performances and desired metabolic traits as well as the identification.

There are reports about microbial inoculants that are used to preserve the nutritive value of the crops [15,16]. However, few data are available for the fermentation characteristics in function of available carbohydrate source for natural lactic acid bacteria to enhance the silage fermentation.

Several authors characterised LAB from the natural traditionally produced dairy products, silages, and fermented vegetables [17–19].

In our study the majority of the isolated bacterial strains belong to *Lactobacillus* genera. The predominant strains were the *Lb. plantarum* subsp*. plantarum* and *Lb. pentosus.* The first one possess diverse metabolic activities,

due to the different environmental provenience. Bringel, F. et al., compared the genome of the two LAB species and observed a high similarity that makes difficult the differentiation between the two species [20].

The selected LAB showed differences in the phenotypic patterns, antimicrobial susceptibility and fermentation capacity. The differences might be explained by the adaptation to microhabitats and by the fact, that the LAB isolates has origins from different ecological niches [21].

One important selection and characterization criteria is the safety of the LAB strains, characterized by the antibiotic resistance profile. The antibiotic resistance was determined according to the guidelines reported by European Food Safety Authority EFSA[7]. The selected strains showed almost similar MIC values, except for chloramphenicol and tetracycline.

For determination of the fermentation capacity an important issue is to describe the production in quantitative terms correlated with growth rate. The product formation rate is closely related to biomass formation rate and is influenced by different environmental factors. The productivity is a significant factor, that describe the fermentation [22]. The quantity of lactic and acetic acid resulted from experimental data was used for calculation of product formation yield. The results point out that *Lactobacillus plantarum* subsp*. plantarum* A5 and *Lactobacillus paracasei* subsp*. Tolerans N16* are the most promising strains from fermentation point of view. In both cases, the Y_{PX} was higher than 1 mg P/ CFU X. In case of *Lactobacillus plantarum* subsp*. plantarum A5* the highest quantity of LA was observed in M_1 and M_2 media. Some researchers [23] studied also the lactic acid production in media containing the same components as in the case of M_2 media. The maximal LA formation rate (by inoculation with single LAB strain) was slower [23] than observed in case of our experiment. The results showed that the bacterial origin influenced the LA production. It was reported that some of the heterofermentative LAB such as *Lb. brevis* and *Lb. pentosus* are able to convert xylose (released from wheat straw) to lactic acid [24]. There are no data available for the conversion of DDGS to lactic acid. Our results revealed that the assayed nine LAB strains were able to produce lactic acid from this substrate. Our data suggests that the isolated LAB strains converted different substrate as DDGS in LA.

During the fermentation the pH variation caused by metabolites influenced the bacterial growth in all cases. With the increase of the LA concentration a slight decline of CFU was observed. The fluctuation of the lactic acid concentration is probably caused by the fact that some LAB strains can use it as substrate.

In order to establish a ranking criteria for the nine selected LAB strain, we determined the beneficial value index (BVI) for each bacterial strain. This was calculated taking into consideration the fermentation capacity and the fermentation pattern of the strains. Taking into account of the LA production

capacity in the presence of three different substrates, the strains were scored. In case when the value of the product yield coefficient was higher than 1 the strain was scored with 3, with 2 when the product yield was between 0.5-1 and 1 if was between 0.2-0.5. No score was given for a product yield lower than 0.2. In the case of carbon source utilization, the LAB with the maximal utilization was scored with 3, and the next two strains were scored with 2 and 1 respectively. The BVI was defined as the sum of the individual scores.

On basis of BVI, the most beneficial four LABs from substrate utilization point of view, in descending order was: *Lb. plantarum* subsp. *plantarum C5* (BVI=7), *Lactobacillus paracasei* subsp. *Tolerans N16* (BVI=6), *Lactobacillus plantarum* subsp. *plantarum A5* (BVI=5), and *Lb. acidipiscis H9* (BVI=4). In the case of the other five LABs the index was low (BVI ≤2).

CONCLUSION

This work represents a preliminary study for the selection of beneficial LAB for biotechnological processes. Our results revealed that the four strains (*Lb. plantarum* subsp*. plantarum* A5*, Lb. plantarum* subsp*. Plantarum* C5 and *Lactobacillus paracasei* subsp*. Tolerans N16*) shows potential to be used as starter cultures for silage fermentation, due by their safety assessment and fermentation capacity. Based on this assays they can be further studied as single inoculums or as consortia, to optimise their beneficial effects on different silage fermentation, improving this way the quality of the resulted feedstock.

EXPERIMENTAL SECTION

Isolation and screening of bacterial isolates

A total number of 246 lactic acid bacteria were isolated on de Man, Rogosa, and Sharpe (MRS) and Rogosa agar plates in aerobic and anaerobic conditions from different naturally fermented traditional foods and feed on as follows: eight different traditional cheese, cheese whey, artisanal sauerkraut, corn and vetch silage, [10].

Based on colony morphology and provenience 99 bacterial isolates were further analysed for the most representative characteristics of LAB as: Gram staining, absence of catalase, growth in the presence of (4% and 6.5%) NaCl, gas production and microscopic morphology [11].

Identification of the selected bacterial strains

For the identification at the species level of 54 LAB isolates were selected based on physiological characteristics. The identification was realized using 16SrDNA gene sequence analysis. Genomic DNA was isolated using AccuPrep® Genomic DNA Extraction Kit from Bioneer Isolation Kit, according to the manufacturer's protocol. A part of the bacterial 16SrDNAgene was amplified with the universal oligonucleotides 27f 5' AGAGTTTGATCMTGGCTCAG 3' and 1492r5'TACGGYTACCTTGTTACGACTT3' primers flanking the bacterial 16SrDNA region. The amplification included an initial denaturation at 94 °C for 5 min, which was followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 7 min. Sequencing were realized by LGC Genomics (Germany). The sequences were edited and aligned with Chromas (Technelysium Pty. Ltd., South Brisbane, Australia); phylogenetic analyses were conducted using Molecular Evolutionary Genetics Analysis 4 system [12] (www.megasoftware.net). The isolates were identified trough comparison of the sequences using the EzTaxon server on the basis of 16SrDNA sequence data [13] (*www.ezbiocloud.net/eztaxon*;).

Biochemical and phenotypic characterization of lactic acid bacteria

Metabolic patterning of the selected LAB strains was realized by BiologMicrolog Gen III (Biolog, Inc., Hayward, USA), including utilization of 71 carbon source and 23 chemical sensitivity assay.

Antibiotic susceptibility

Determination of antimicrobial susceptibilities of LAB's was realized according to the guidelines reported in EFSA (2008) [7]. For the assessment of the susceptibility to ampicillin, tetracyclin, chloramphenicol, kanamycin, streptomycin, penicillin, gentamicin, serial two-fold dilutions were realized ranging from 0 up to 128 $\mu g.ml^{-1}$, in MRS broth.

Fermentation capacity of bacterial strains

The fermentation capacity of the nine beneficial bacterial strains were determined in three different fermentation medium containing diverse fermentation substrates: glucose 20 g/l (M_1), a mixture of glucose and xylose 15 and 5 g/L (M_2), and dried distiller's grain with soluble (DDGS) 20 g/l (M_3). All the fermentation media were supplemented with 5 g/L yeast extract, 2 g/L K_2HPO_4, 0.05 g/L $MnSO_4 \cdot H_2O$, and 0.1 g/L $MgSO_4 \cdot 7H_2O$. 2 mL of LAB inoculum was added into 45 mL vials containing 38 mL of fermentation medium [23].The fermentation tests were conducted at 37 ºC. For the determination of lactic acid and acetic

acid samples were taken at 0, 24, 36, 48, 60 and 72 h The amount of these acids were detected using high performance liquid chromatography (HPLC Varian Pro Star 210), equipped with TransgenomicCoregel87H3 column (Transgenomic, Inc., Omaha, USA) and UV detector at 50 ºC, mobile phase 0.8 mMH_2SO_4, at a flow rate of 0.6 mL.min^{-1}.

Product formation was assayed by the measured concentration data, on which a polynomial function was fitted and calculated the yield coefficient related to the product. The fermentation capacity was determined by product yield coefficient (eq. 1), defined as the ratio of product formation rate (v_{RP}) (eq. 2) and the growth rate (v_{RX}) (eq. 3) of the tested lactic acid bacteria [14].

$$YPX = \frac{vRP}{vRX} \tag{1}$$

where the rate of product formation (v_{RP}) is (eq. 2)

$$vRP = \frac{d\,CP}{dt} \tag{2}$$

and the growth rate (v_{RX}) (eq. 3)

$$vRX = \frac{d\,CX}{dt} \tag{3}$$

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