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**BIOCHEMICAL AND MOLECULAR-GENETIC IDENTIFICATION OF
LACTOBACILLUS STRAINS OF HUMAN ORIGIN**

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Biochemical and molecular-genetic identification of *Lactobacillus* strains of human origin: *The morphological and physiological characteristics of two newly isolated Lactobacillus strains (Lactobacillus Pr9 and Lactobacillus Pr10) of human origin were determined. The strains were identified as representatives of the species Lactobacillus acidophilus by the application of biochemical (API 50 CHL) and molecular-genetic methods (ARDRA-analysis and sequencing of the 16S rRNA gene). After software processing with CLC Sequence Viewer it has been found that Lactobacillus acidophilus Pr9 and Lactobacillus acidophilus Pr10 were identical.*

Key words: *Lactobacillus, API 50 CHL, ARDRA, sequencing, 16S rRNA, CLC sequence viewer.*

INTRODUCTION

Lactobacilli are similar in phenotypic and physiological characteristics, probably due to their coevolution in the same ecological niche. The *Lactobacillus* genus includes more than 140 species. Horizontal transfer of plasmid - associated traits is characteristic for *Lactobacillus* species. Therefore, molecular-genetic methods are applied along with biochemical methods for their species differentiation. Molecular-genetic methods allow for the more accurate, rapid, and reproducible differentiation between closely related species, that are difficult to differentiate only on the basis of their phenotypic characteristics [4]. The similarity in the biochemical profiles of phylogenetically closely related species, as well as the influence of some factors on the metabolic activities of lactic acid bacteria require additional characterization by applying molecular-genetic methods [5]. In fact, many species of the genus *Lactobacillus* have been reclassified based on new information from molecular-genetic analyses and their taxonomic status has been determined, for example *L. cellobiosus*, *L. pastorianus*, *L. arizonensis* are allocated appropriately to the species *L. fermentum* [1], *L. paracollinoides* [2], and *L. plantarum*, respectively.

The purpose of the present work was to identify two newly isolated *Lactobacillus* strains using biochemical (API 50 CHL) and molecular-genetic methods (ARDRA-analysis and sequencing of the 16S rRNA gene).

MATERIALS AND METHODS

Microorganisms

Lactobacillus Pr9, *Lactobacillus* Pr10 of human origin.

Reference microorganisms: *Lactobacillus acidophilus* DSM 20079, *Lactobacillus delbrueckii* ssp. *bulgaricus* DSM 20081, *Lactobacillus casei* ssp. *casei* DSM 20011, *Lactobacillus helveticus* DSM 20075, *Lactobacillus plantarum* ssp. *plantarum* DSM 20174.

Determination of the biochemical profile - API 50 CHL (BioMerieux SA, France) according to manufacturer's instructions.

Molecular-genetic methods

Isolation of total DNA - E.Z.N.A.[®] kit according to manufacturer's instructions

PCR reactions and visualization. All PCR reactions were performed using PCR kit - Ready To Go[™] PCR beads (Amersham Biosciences), in a volume of 25 µl in Progene cyclor (Techne, UK). The resulting products were visualized on a 2% agarose gel, stained with ethidium bromide solution (0.5 µg/ml), using a UVP Documentation System (U.K.).

16S rDNA amplification and ARDRA-analysis (Amplified Ribosomal DNA Restriction Analysis). All PCR reactions were performed using PCR kit - PCR VWR, in a volume of 25 µl in Progene cyclor (Techne, UK) according to the manufacturer's instructions. 50 ng of total DNA of the studied strain and 10 pmol primers were used in each reaction. The DNA of the studied strain was amplified using universal primers for 16S rDNA - 27f (5'AGAGTTTGATCMTGGCTCAG3') [3] and 1492r (5'ACCTTGTTACGACTT3') [3]. The amplification program included: denaturation - 95 °C for 3 min, 40 cycles - 93 °C for 30 s, 55 °C for 30 s, 72 °C for 2 min, final elongation - 72 °C for 7 min.

The PCR product obtained was subjected to restriction with FastDigest endonucleases *Eco* RI, *Hae* III and *Alu* I (ThermoFisher Scientific) at a concentration of 10 units/µl.

The products were visualized on a 2% agarose gel stained with ethidium bromide solution (0.5 µg/ml) using a UVP Documentation System (U.K.).

Purification of the PCR product (16S rDNA) from TAE-agarose gel

The purification of the 16S rDNA was performed with a kit for DNA purification (GFX Microspin[™]) according to the manufacturer's instructions.

Sequencing of the 16S rDNA

The sequencing of the 16S rDNA was performed by the Sanger method by "Macrogen Europe Laboratory", the Netherlands.

The sequencing results for the forward and reverse partial sequences of each strain were assembled using software *CLC Sequence Viewer*. The assembled sequences of the 16S rRNA gene were compared with the sequences available in the online GenBank database through online software BLASTn and the species identification of the strains with the corresponding percentage of similarity between the sequence of the studied strain and the reference strain from the online database was determined.

RESULTS AND DISCUSSION

Phenotypic characteristics of the newly isolated lactobacilli strains

In cultivation on MRS-agar *Lactobacillus* Pr9 and *Lactobacillus* Pr10 formed small, milky white colonies with star shape and uneven edges, which could easily be separated from the medium. The cells were long and rod-shaped, with rounded edges, arranged singly and in short chains.

Biochemical characteristics of the newly isolated *Lactobacillus* strains

The biochemical profiles of *Lactobacillus* Pr9 and *Lactobacillus* Pr10 were examined using the system for rapid lactobacilli identification API 50 CHL (Biomerieux, France). Both strains utilized galactose, D-glucose, D-fructose, D-mannose, manitol, sorbitol, N-acetyl-glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, melezitose, D-raffinose, β -gentiobiose, D-turanose, gluconate. *Lactobacillus* Pr9 utilized D-xylose as well, while *Lactobacillus* Pr10 utilized amidon. The results from the API 50 CHL for the strains' ability to utilize the 49 carbon sources included in the system API 50 CHL were processed with software apiweb[®] and the two strains were identified as representatives of the species *Lactobacillus plantarum* with the corresponding percentage of reliability - for *Lactobacillus* Pr9 - 87,9%, for *Lactobacillus* Pr10 - 99,9%.

The analyses using the API 50 CH systems provide rapid and reproducible identification of certain lactobacilli types, but sometimes the percentage of discrimination is not high enough. The use of classic phenotypic and biochemical characteristics alone does not always allow to reliably distinguish between lactobacilli types, especially considering that in the *Lactobacillus* genus there is phenotypic variability. Therefore, in accordance with modern concepts of taxonomic lactobacilli identification are applied both phenotypic and molecular-genetic methods, especially in cases with a relatively low percentage of discrimination by the use of classical methods alone [5].

Molecular - taxonomic characterization

ARDRA-analysis of *Lactobacillus* Pr9 and *Lactobacillus* Pr10 with the FastDigest endonucleases *Hae* III, *Alu* I and *Eco* RI was performed to confirm the results for their species identification obtained by the conventional identification methods. The results of the molecular-genetic experiments are shown in Fig. 1, Fig. 2 and Fig. 3.

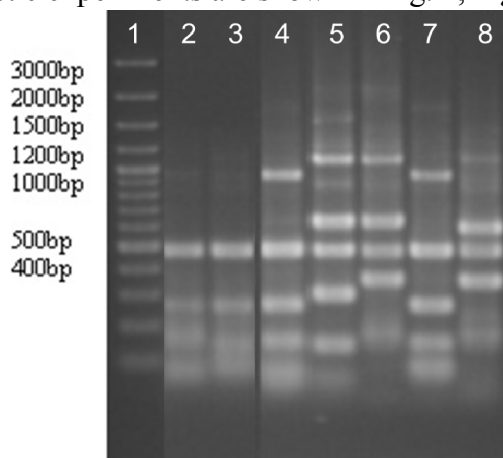


Fig. 1. Restriction profile with *Hae* III

1. M 100 bp plus DNA Ladder
2. *Lactobacillus* Pr9
3. *Lactobacillus* Pr10
4. *Lactobacillus acidophilus* DSM 20079
5. *Lactobacillus delbrueckii* ssp. *bulgaricus* DSM 20081
6. *Lactobacillus casei* ssp. *casei* DSM 20011
7. *Lactobacillus helveticus* DSM 20075
8. *Lactobacillus plantarum* ssp. *plantarum* DSM 20174

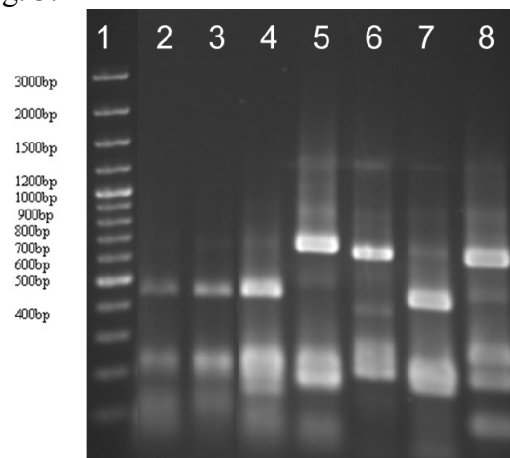


Fig. 2. Restriction profile with *Alu* I

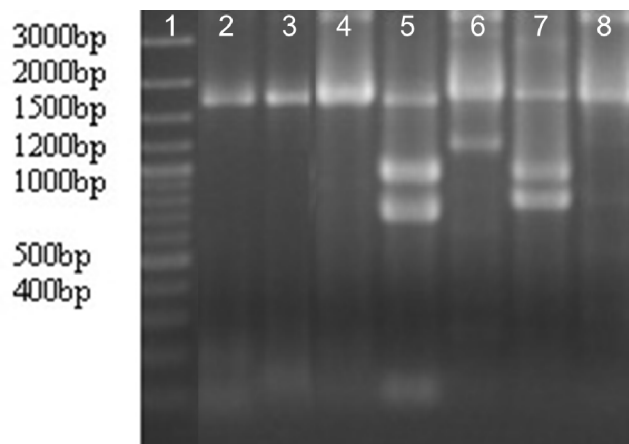


Fig. 3. Restriction profile with *Eco* RI

When comparing the restriction profiles obtained with *Hae* III it was found that the profiles of *Lactobacillus* Pr9 and *Lactobacillus* Pr10 were similar to these of *Lactobacillus acidophilus* and *Lactobacillus helveticus*, but in order to determine the species identification of *Lactobacillus* Pr9 and *Lactobacillus* Pr10 it was necessary to conduct ARDRA-analysis with two more restriction enzymes - *Alu* I and *Eco* RI.

The ARDRA results obtained with *Alu* I (Fig. 2) and *Eco* RI (FIG. 3) identified *Lactobacillus* Pr9 and *Lactobacillus* Pr10 as belonging to the species *Lactobacillus acidophilus*.

1.3.2. Sequencing of the 16S rRNA gene

For the complete species identification of the studied strains a second molecular-genetic method was used - sequencing of the 16S rRNA gene. The results of the sequence analysis of the 16S rDNA identified *Lactobacillus* Pr9 and *Lactobacillus* Pr10 as representatives of the species *Lactobacillus acidophilus* with 98% of similarity between the sequence of the 16S rDNA of *Lactobacillus* Pr9 and the partial sequence of the 16S rDNA of *Lactobacillus acidophilus* VPI 6032 (Fig. 4); and 98% of similarity between the sequence of the 16S rDNA of *Lactobacillus* Pr10 and the partial sequence of the 16S rDNA of *Lactobacillus acidophilus* NBRC 13951 (Fig. 5).

The results of the conducted comparative sequence analysis of the 16S rRNA genes of the strains *Lactobacillus acidophilus* Pr9 and *Lactobacillus acidophilus* Pr10 with the software *CLC Sequence Viewer* showed that the two strains were identical (Fig. 6).

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НАУЧНИ ТРУДОВЕ НА РУСЕНСКИЯ УНИВЕРСИТЕТ - 2016, том 55, серия 10.2

Query 19	ATACATGCA-GTCAGCCGAGCAGACAGCAGATTTACTTCGGTAAATGACGCTGGGGACG	77
Sbjct 26	ATACATGCAAGTCCAGCCGAGCTGAACCAACAGATTCACCTCGGTGATGACGCTGGGAACG	85
Query 78	CGAGCGCGGATGGGTGATTAACACGTTGGGGAACCTGCCCATAGTCTAGGATACCACCT	137
Sbjct 86	CGAGCGCGGATGGGTGATTAACACGTTGGGGAACCTGCCCATAGTCTGGGATACCACCT	145
Query 138	GGAAACAGTGTCTAATACCCGATTAAGAACAGATCGCATGTCAGCTTATAAAGGGCGG	197
Sbjct 146	GGAAACAGTGTCTAATACCCGATTAAGAACAGATCGCATGTCAGCTTATAAAGGGCGG	205
Query 198	CGTAAGCTGTGCTATGGGATGCCCGCGGTGCATTAGCTAGTTGGTAAGTAAACGGCT	257
Sbjct 206	CGTAAGCTGTGCTATGGGATGCCCGCGGTGCATTAGCTAGTTGGTAAGTAAACGGCC	265
Query 258	TACCAGGCAATGATGCATAGCCAGTTGAGAGATGAACGGCCACATTTGGGACTGAGAC	317
Sbjct 266	TACCAGGCAATGATGCATAGCCAGTTGAGAGATGAACGGCCACATTTGGGACTGAGAC	325
Query 318	ACGGCCAACTCCTACGGGAGCAGCAGTAGGGAATCTCCACAATGGACGCAAGCTCG	377
Sbjct 326	ACGGCCAACTCCTACGGGAGCAGCAGTAGGGAATCTCCACAATGGACGCAAGCTCG	385
Query 378	ATGGAGCAACCGCCGCTGAGTGAAGAAGTTTTCGGATCGTAAGCTCTGTGTGGTGA	437
Sbjct 386	ATGGAGCAACCGCCGCTGAGTGAAGAAGTTTTCGGATCGTAAGCTCTGTGTGGTGA	445
Query 438	AGAAGTAGAGGTAAGTACCTGGCTTTATTTGACGGTAATCAACCAGAAATCAGCGCT	497
Sbjct 446	AGAAGTAGAGGTAAGTACCTGGCTTTATTTGACGGTAATCAACCAGAAATCAGCGCT	505
Query 498	AACTAGCTGCAGCAGCCGGGTAATACGTAGTGGCAAGCGTTGTCGGATTTATTGGG	557
Sbjct 506	AACTAGCTGCAGCAGCCGGGTAATACGTAGTGGCAAGCGTTGTCGGATTTATTGGG	565
Query 558	CGTAAGCGAGCCAGCGGGAAGATTAATCTGATGTGAAGCCCTCGCTTAACCGAGG	617
Sbjct 566	CGTAAGCGAGCCAGCGGGAAGATTAATCTGATGTGAAGCCCTCGCTTAACCGAGG	625
Query 618	AATTGCAATCGAAACTGTTTTCTTGAGTGCAGAAAGAGAGAGTGAACCTCATGTGTAG	677
Sbjct 626	AATTGCAATCGAAACTGTTTTCTTGAGTGCAGAAAGAGAGAGTGAACCTCATGTGTAG	685
Query 678	CGGTGAATCGCTAGATATATGGAAGAACCACAGTGGCGAAGCGGCTCTCTGTCTGCA	737
Sbjct 686	CGGTGAATCGCTAGATATATGGAAGAACCACAGTGGCGAAGCGGCTCTCTGTCTGCA	745
Query 738	ACTGACGCTGAGGCTCGAAGCATGGTAGCGAACAGGATTAGATACCTGGTAGTCCAT	797
Sbjct 746	ACTGACGCTGAGGCTCGAAGCATGGTAGCGAACAGGATTAGATACCTGGTAGTCCAT	805
Query 798	GCCGTAACAGTGAAGTCTAAGTGTGGGAGTTTCCGCCCTCTCAGTCTGACGCTAACG	857
Sbjct 806	GCCGTAACAGTGAAGTCTAAGTGTGGGAGTTTCCGCCCTCTCAGTCTGACGCTAACG	865
Query 858	CATTAGCACTCCGCTGGGAGTACGACCCGAGGTTGAACTCAAGGAATGACGGG	917
Sbjct 866	CATTAGCACTCCGCTGGGAGTACGACCCGAGGTTGAACTCAAGGAATGACGGG	925
Query 918	GGCCCGCACAAGCGTGGGAGCATGGTTAATTCGAAGCAACCGAAGAACCCTACCAG	977
Sbjct 926	GGCCCGCACAAGCGTGGGAGCATGGTTAATTCGAAGCAACCGAAGAACCCTACCAG	985
Query 978	GTCTTACATCTAGTGCATACCGTAGAGATACCGAGTCCCTTCGGGCACTAAGACAG	1037
Sbjct 986	GTCTTACATCTAGTGCATACCGTAGAGATACCGAGTCCCTTCGGGCACTAAGACAG	1045
Query 1038	GTGGTGCATGGCTCTGTCAGCTGCTGCTGAGATGTTGGGTTAAGTCCCGCAAGAGCC	1097
Sbjct 1046	GTGGTGCATGGCTCTGTCAGCTGCTGCTGAGATGTTGGGTTAAGTCCCGCAAGAGCC	1105
Query 1098	GCACCCCTTATTTAGTGCAGCATTAACTGGGCACTTAATGAGACTCCGCGGTGAC	1157
Sbjct 1106	GCACCCCTTATTTAGTGCAGCATTAACTGGGCACTTAATGAGACTCCGCGGTGAC	1165
Query 1158	AAACCGGAGGAGGTTGGGATGACCTCAAGTCATCATGCCCTTATGACCTGGGCTACAC	1217
Sbjct 1166	AAACCGGAGGAGGTTGGGATGACCTCAAGTCATCATGCCCTTATGACCTGGGCTACAC	1225
Query 1218	ACGTGCTACAAATGGCACTACAAAGGAGCAAGCCTGGGAGGCAAGCCGAATCTTTAA	1277
Sbjct 1226	ACGTGCTACAAATGGCACTACAAAGGAGCAAGCCTGGGAGGCAAGCCGAATCTTTAA	1285
Query 1278	AGCTGTTCTCAGTTCGGACTGCACTGCAACTCGACTGCACGAAGCTGGAATCGTAGT	1337
Sbjct 1286	AGCTGTTCTCAGTTCGGACTGCACTGCAACTCGACTGCACGAAGCTGGAATCGTAGT	1345
Query 1338	AATCGCGGATCAGAACCGCCGGTGAATACTCCCGGCCCTGTACACACCGCCGCTCA	1397
Sbjct 1346	AATCGCGGATCAGAACCGCCGGTGAATACTCCCGGCCCTGTACACACCGCCGCTCA	1405
Query 1398	CACCATGGAAGTCTGCAATGCCAAAGCCGGTGGCTAACCTTCGGGAAGGACCGCTCA	1457
Sbjct 1406	CACCATGGAAGTCTGCAATGCCAAAGCCGGTGGCTAACCTTCGGGAAGGACCGCTCA	1465
Query 1458	AGGCATGTCAGATG 1471	
Sbjct 1466	AGGCAGGCGAGATG 1479	

Fig. 4. Comparison between the nucleotide sequence of the 16S rDNA of *Lactobacillus* Pr9 and the partial sequence of the 16S rDNA of *Lactobacillus acidophilus* VPI 6032

Query 7	TGGCGGGTGGCT--ATACATGCA-GTCAGCCGAGCAGAACAGCAGATTTACTTCGGTAA	63
Sbjct 10	TGGCGGGTGGCTAATACATGCAAGTCCAGCCGAGCTGAACCAACAGATTCACCTCGGTGA	69
Query 64	TGACGCTGGGGACGCGAGCGCGGATGGGTGATTAACACGTTGGGGAACCTGCCCATAGT	123
Sbjct 70	TGACGCTGGGGACGCGAGCGCGGATGGGTGATTAACACGTTGGGGAACCTGCCCATAGT	129
Query 124	CTAGGATACCCTTGAACAGGCTGCTAATACCCGATTAAGAACAGATCGCATGATCAG	183
Sbjct 130	CTGGGATACCCTTGAACAGGCTGCTAATACCCGATTAAGAACAGATCGCATGATCAG	189
Query 184	CTTATAAAGGCGGCTAAGCTGTCGCTATGGGATGCCCGCGGTGCATTAGCTAGTTG	243
Sbjct 190	CTTATAAAGGCGGCTAAGCTGTCGCTATGGGATGCCCGCGGTGCATTAGCTAGTTG	249
Query 244	GTAAAGTAAAGGCTTACCAGGCAATGATGCATAGCCAGTTGAGAGATGAACGGCCAC	303
Sbjct 250	GTAAAGTAAAGGCTTACCAGGCAATGATGCATAGCCAGTTGAGAGATGAACGGCCAC	309
Query 304	ATTGGGACTGAGACCGGCCAACTCCTACGGGAGCAGCAGTAGGGAATCTCCACAA	363
Sbjct 310	ATTGGGACTGAGACCGGCCAACTCCTACGGGAGCAGCAGTAGGGAATCTCCACAA	369
Query 364	TGGACGCAAGTCTGATGGAGCAACCGCCGCTGAGTGAAGAAGTTTTCGGATCGTAAGC	423
Sbjct 370	TGGACGCAAGTCTGATGGAGCAACCGCCGCTGAGTGAAGAAGTTTTCGGATCGTAAGC	429
Query 424	CTGTGTTGGTGAAGAAGGATAGAGGTAGTACTGGCTTTATTTGACGGTAATCAACC	483
Sbjct 430	CTGTGTTGGTGAAGAAGGATAGAGGTAGTACTGGCTTTATTTGACGGTAATCAACC	489
Query 484	AGAAAGTACGGCTAAGTACTGTCAGCAGCCCGGTAATACGTAGTGGCAAGCGCTGT	543
Sbjct 490	AGAAAGTACGGCTAAGTACTGTCAGCAGCCCGGTAATACGTAGTGGCAAGCGCTGT	549
Query 544	CCGGATTATTTGGGCTAAAGCGAGCCAGCGGGAAGATTAAGTCTGATGTGAAGCCCT	603
Sbjct 550	CCGGATTATTTGGGCTAAAGCGAGCCAGCGGGAAGATTAAGTCTGATGTGAAGCCCT	609
Query 604	CGGCTTAAACGAGGAATTCATCGGAACTGTTTTCTTGAGTGCAGAAAGGAGAGTGG	663
Sbjct 610	CGGCTTAAACGAGGAATTCATCGGAACTGTTTTCTTGAGTGCAGAAAGGAGAGTGG	669
Query 664	AACCTCATGTGTACCGGCTGGAATGCGTGAATATGGAAGAACCACAGTGGCGAAGCGCG	723
Sbjct 670	AACCTCATGTGTACCGGCTGGAATGCGTGAATATGGAAGAACCACAGTGGCGAAGCGCG	729
Query 724	CTCTGGTCTGCAACTGACGCTGAGGCTCGAAGCATGGTAGCAGCAACGATTAGATA	783
Sbjct 730	CTCTGGTCTGCAACTGACGCTGAGGCTCGAAGCATGGTAGCAGCAACGATTAGATA	789
Query 784	CCCTGGTAGTCCATCCGCTAAAGCATGAGTGTGGGAGTTTCCGCCCTCTCAG	843
Sbjct 790	CCCTGGTAGTCCATCCGCTAAAGCATGAGTGTGGGAGTTTCCGCCCTCTCAG	849
Query 844	TGCTGCAGCTAACGCATTAGCACTCCGCTGGGAGTACAGCCCGAAGTTGAACTCA	903
Sbjct 850	TGCTGCAGCTAACGCATTAGCACTCCGCTGGGAGTACAGCCCGAAGTTGAACTCA	909
Query 904	AAGGAATTGACGGGGCCCGCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACCGG	963
Sbjct 910	AAGGAATTGACGGGGCCCGCACAAGCGGTGGAGCATGTGGTTAATTCGAAGCAACCGG	969
Query 964	AAGAACCTTACCAAGGCTTGCATCTAGTGGCACTTAAAGATTAAGAGATTAGAGTTCCCTCGG	1023
Sbjct 970	AAGAACCTTACCAAGGCTTGCATCTAGTGGCACTTAAAGATTAAGAGATTAGAGTTCCCTCGG	1029
Query 1024	GGACGCTAAGACAGGTGGTGCATGGCTGCTGCTGACGCTGCTGCTGAGATGTTGGGTTAA	1083
Sbjct 1030	GGACGCTAAGACAGGTGGTGCATGGCTGCTGCTGACGCTGCTGCTGAGATGTTGGGTTAA	1089
Query 1084	GTCGCGCAAGGCGCAACCTTATTTAGTGCAGCATTAACTGGGCACTTAATG	1143
Sbjct 1090	GTCGCGCAAGGCGCAACCTTATTTAGTGCAGCATTAACTGGGCACTTAATG	1149
Query 1144	AGACTGCGGCTGACAAACCGGAGGAGGTTGGGATGACCTCAAGTCATCATGCCCTTAT	1203
Sbjct 1150	AGACTGCGGCTGACAAACCGGAGGAGGTTGGGATGACCTCAAGTCATCATGCCCTTAT	1209
Query 1204	GACCTGGGCTACACAGTGTCTACAATGGGCACTACACGAAAGGAGGCTCCGAGGCA	1263
Sbjct 1210	GACCTGGGCTACACAGTGTCTACAATGGGCACTACACGAAAGGAGGCTCCGAGGCA	1269
Query 1264	AGCGAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCACTGCAACTCGACTGCAAGG	1323
Sbjct 1270	AGCGAATCTCTGAAAGCTGTTCTCAGTTCGGACTGCACTGCAACTCGACTGCAAGG	1329
Query 1324	CTGGAACTGCTAGTAACTCGGGATCAGAACCGCCGGTGAATACCTCCCGGCCCTGTA	1383
Sbjct 1330	CTGGAACTGCTAGTAACTCGGGATCAGAACCGCCGGTGAATACCTCCCGGCCCTGTA	1389
Query 1384	CACACCGCCGCTCACACATGGAAGTCTGCAATGCCAAAGCCGGTGGCTAACCTTCGG	1443
Sbjct 1390	CACACCGCCGCTCACACATGGAAGTCTGCAATGCCAAAGCCGGTGGCTAACCTTCGG	1449
Query 1444	GAAGGAGCGCTTAAGGCATGGCA 1467	
Sbjct 1450	GAAGGAGCGCTTAAGGCAGGCA 1473	

Fig. 5. Comparison between the nucleotide sequence of the 16S rDNA of *Lactobacillus* Pr10 and the partial sequence of the 16S rDNA of *Lactobacillus acidophilus* NBRC 13951

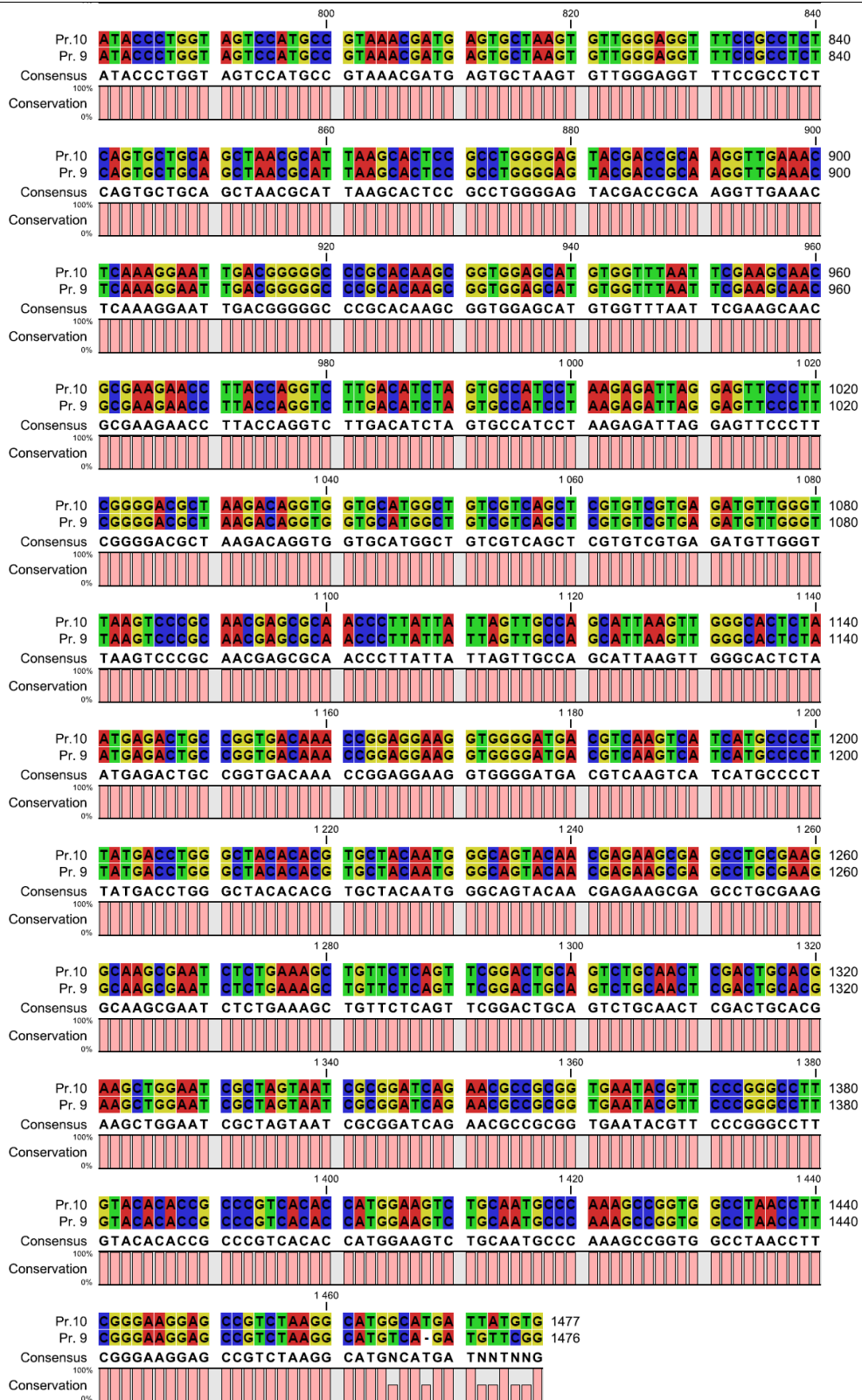


Fig. 6. Comparison between the sequences of the 16S rRNA genes of *Lactobacillus acidophilus* Pr9 and *Lactobacillus acidophilus* Pr10 with software *CLC Sequence Viewer*

CONCLUSION

The newly isolated strains *Lactobacillus* Pr9 and *Lactobacillus* Pr10 were identified using biochemical (API 50 CHL) and molecular-genetic methods (ARDRA-analysis and 16S rDNA sequencing). The results of the analysis with API 50 CHL and the consecutive software processing with *apiweb*[®] related the two strains to the species *Lactobacillus plantarum*. The conducted molecular-genetic (ARDRA-analysis and sequencing of the 16S rRNA gene) identified

Lactobacillus Pr9 and *Lactobacillus* Pr10 as representatives of the species *Lactobacillus acidophilus*. When comparing the nucleotide sequences of the 16S rDNA of the two newly isolated strains with the software *CLC Sequence Viewer* it was found that both strains were identical.

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