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ORIGINAL PAPER

Functional traits of *Lactobacillus plantarum* from fermented *Brassica oleracea* var. *capitata* L. in view of multivariate statistical analysis

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Abstract
In the present study, *Lactobacillus plantarum* strain was isolated and identified from spontaneous fermentation of *Brassica oleracea* var. *capitata* L. We used the Unweighted Pair Group Method with Arithmetic Mean Analysis (UPGMA) and Principal Component Analysis (PCA) to examine the variations in the functional properties of the isolates. Six functional traits were analyzed, i.e., viability at low pH, resistance to lysozyme and to SIF, auto- and coaggregation, and ß-glucosidase activity. The present work is the first study in which the PCA and UPGMA statistical methods were used together to analyze data obtained from the same microbiological experiments. This provided information about the similarity of the examined isolates in terms of their functional traits. Additionally, the level of the analyzed functional traits within the particular groups of isolates was shown. The presented approach is the basis for choosing isolates that are most closely related to the reference strain isolated from pickled cabbage.

Keywords *Lactobacillus plantarum* · *Brassica oleracea* L. · Functional traits · UPGMA · PCA

Introduction

Sauerkraut is one of the best-known traditional fermented vegetables in Central and Eastern Europe and the United States. It is typically produced by spontaneous fermentation by lactic acid bacteria (LAB), which naturally occur in white cabbage (*Brassica oleracea* var. *capitata*). During preparation, cabbage without central cores is shredded and mixed with sodium chloride (0.7–2.5%), which contributes to maintenance of an anaerobic conditions and inhibits the growth of spoilage microorganisms, thus influencing the quality and microbial composition of the final product [1, 2].

Fermentation can be defined as an intended process of raw material modification caused by microorganisms and their enzymes. Lactic acid fermentation of *Brassica* vegetables is a preservation method used in the food industry, which has received a lot of attention, as it improves safety, shelf life, and organoleptic properties of food [3, 4]. When cabbage is subjected to fermentation, the microbial composition dynamically changes, starting from the occurrence of heterofermentative species, which are replaced by homofermentative LAB species due to the modification of ecological conditions. Therefore, in the first phase, the process is dominated mainly by microaerophilic *Leuconostoc* species, especially *L. mesenteroides*, which produce significant amounts of carbon dioxide and acids (acetic and lactic). This leads to a drop in the pH value and replacement of *Leuconostoc* species by more acid-tolerant strains belonging to the genera *Lactobacillus* [2, 5].

Lactobacillus plantarum is a universal species, highly versatile and found in many different niches. In line with this ability, *Lb. plantarum* is widely used in diverse food and health applications. This bacterium displays numerous technological and functional properties improving the fermentation process and yielding healthier and safer food thus bringing additional value to the final product. For this reason, *Lb. plantarum* is commonly used as a component

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of functional starter cultures for fermentation of vegetables as well as milk products [6, 7]. Selected probiotic *Lb. plantarum* strains are also used to develop functional foods and live oral vaccine [8]. Diversity between strains belonging to *Lb. plantarum* is seen within phenotypic properties and metabolic capacity. This variability has huge impact on their further applications. For example, some of the features, which are beneficial for industrial use of LAB species, i.e., heat and oxidative stress susceptibility may vary depending on the type of metabolism, namely production of starter culture or probiotic in aerobic conditions may improve the survival of bacteria during treatments such as spay-drying and cold storage [9, 10]. There is an accumulating evidence that plant fermentation, storage conditions and, especially, chemical composition of plant matrices markedly affect the functional features of *Lb. plantarum* strains [11]. Many studies have shown that *Lb. plantarum* displays resistance to gastrointestinal conditions (acid and bile salts) and bile salt hydrolase activity [4, 12]. Additionally, adherence to host mucosal surfaces as well as β-glucosidase and β-galactosidase activities were also confirmed [13, 14]. Furthermore, this species protects the host from pathogenic infections through production of antimicrobial substances, competitive exclusion, and stimulation the host immune system [15, 16].

In this work, multivariate statistical techniques, PCA and UPGMA, were applied to the same set of data consisting of the functional characteristics of *Lb. plantarum* isolates.

Principal component analysis (PCA) is a multivariate analysis reducing the number of studied features to a smaller number of independent principal components. The new variables preserve a relatively large part of the information contained in the original data. Each component is a linear combination of observed variables and delivers some substantive content [17]. This fact allowed us to relate the new variables obtained as a result of PCA with some characteristics of the isolates.

Cluster analysis in the form of Unweighted Pair Group Method with Arithmetic Mean Analysis (UPGMA) [18] is an agglomerative hierarchical clustering method building a dendrogram on the basis of the distance matrix [19]. The distance between two clusters was calculated as the average distance between all pairs of objects belonging to two different clusters. Each diagram node represents a combination of two or more clusters. The position of the nodes on the axis represents the distance at which the clusters are connected.

The aim of this work was to point out the phenotypic traits of bacteria correlated with important functional traits by examination of the variations of functional properties of *Lb. plantarum* isolates from fermented cabbage.

Materials and methods

Fermentation trials and isolation of lactic acid bacteria from sauerkraut

The cabbage heads were cleaned by removing the outer leaves. The remaining leaves were washed, dried, and then homogenized/cut into small pieces (2–4 cm × 4–8 cm). Spontaneous fermentations were carried out in a 5-L glass jar with 2.5% (w/v) sterile NaCl solution for 7 days. The fermentation jars were kept at ambient temperature (18–24 °C) during the experiments. Samples were collected to Eppendorf tubes after mixing the contents of the jar each day of the fermentation process. Samples from self-fermenting cabbage were taken and then transferred to saline. The Eppendorf tubes with the samples were shaken for 1 h at 30 °C on a ThermoMixer HLC (DITABIS AG, 37 °C). When the colonies were visible, those emitting surrounding clearings (zones) were selected. To obtain pure colonies/cultures, the isolation carried out by streaking onto MRS agar plates was repeated three times. Eighty-three colonies were selected for identification.

Identification by MALDI-TOF bio-typer

After 48-h incubation, a single colony was selected and transferred to 150 µL of sterile deionized water (Milli-Q water, Millipore Corp, Bedford, USA). The samples were homogenized by pipetting and vortexing for a minute. Appropriate homogenization of the sample influences the quality of the results obtained; therefore, vortexing is applied after the addition of each reagent. Then, 450 µL of pure ethanol (96%, POCH, Poland) was added to the tube, centrifugation was carried out for 5 min at 13,000 rpm, and the supernatant was removed. A volume of 40 µL of 70% (v/v) formic acid and 40 µL of 99% (v/v) acetonitrile was added. The samples were centrifuged for 5 min at 13,000 rpm; next, 1 µL of the supernatant was applied in triplicate onto an MTP AnchorChip stainless steel MALDI plate (Bruker, Germany). When the spots dried, they were covered with the same amount of matrix of 10 mg of HCCA-α-cyano-4-hydroxycinnamic acid/mL (Sigma-Aldrich, Poland). The plate was introduced to an UltrafleXtreme MALDI-TOF mass spectrometer (Bruker, Germany) with a 1000 Hz neodymium-doped yttrium–aluminum–garnet nitrogen laser (Nd–YAG). The mass spectra were analyzed and compared to the reference spectra using MALDI Bio-typer 3.0 software package (Bruker, Germany). The report presented a list of strains, and those above 1.7 log (score) were considered to be a reliable match to the genus. The log (score) above 2 means a high

probability of correct identification of the genus as well as the species. Only those isolates above 2 log (score) were selected for further analysis.

Genetic identification and species-specific PCR

The DNA of the selected strains was extracted using Genomic Mini AX Bacteria Spin (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions. For genetic identification, universal primers (27f) 5′-AGAGTTTGATCCTGGCTCAG-3′ and (1495r) 5′-CTACGGCTACCTTGTTACGA-3′ (Genomed S.A., Warszawa, Poland) specific for the 16S rRNA gene were used. The final volume of the reaction was 20 µL. Using PCR Master Mix(2x) (Thermo Fisher Scientific, Bremen, Germany), the amount of the reaction components were adjusted to the recommendations from the attached protocol. The PCR conditions were as follows: initial denaturation 95 °C—5 min, 30 cycles of denaturation 95 °C—1 min, annealing 48 °C—30 s, elongation 72 °C—2 min, and final extension 72 °C for 10 min. The PCR was performed in a Labcycler (SensoQuest Göttingen, Germany). The amplification products were separated on 1.5% (w/v) agarose gel (Eurx, Gdańsk, Poland) with (0.5 µg/mL) ethidium bromide (Sigma Chemical Co., MO, USA). For all strains tested, a 1.5 kbp PCR single product was obtained. The resulting PCR products were sequenced using the commercial sequencing service provider.

The DNA sequence was determined by a commercial DNA sequencing service provider (Genomed, Warsaw, Poland) with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and a capillary sequencing system, i.e., a 3730xl DNA Analyzer (Applied Biosystems, USA). Contigs were submitted using the DNA Baser Assembler and then subjected to search using the BLAST algorithm (NCBI GenBank).

Due to the specificity of the species, additional identification was made—multiplex PCR for the *recA* gene described by Torriani et al. [20]. The amplicons were separated on 1.5% agarose gel (Eurx, Gdańsk, Poland) with 1 kb Ladder Perfect Plus (Eurx, Gdańsk, Poland).

Evaluation of functional properties

In vitro resistance to lysozyme

Cells of *Lb. plantarum* strains grown overnight in 10 mL MRS broth at 30 °C were centrifuged, washed twice with PBS (0.1 M, pH 7.0), and resuspended in 2 mL of saline solution. Bacterial suspensions were inoculated in a sterile electrolyte solution (SES; 0.22 g/L $CaCl_2$, 6.2 g/L NaCl, 2.2 g/L KCl, 1.2 g/L $NaHCO_3$) in the presence of 100 mg/L of lysozyme (Sigma-Aldrich) according to [21]. Bacterial suspensions in SES without lysozyme were included as controls. Samples were incubated at 37 °C and counted after 30 and 120 min on MRS agar (48 h; 30 °C). The survival rate was calculated as a percentage of the CFU/mL after 30 and 120 min compared to the CFU/mL at time 0.

Resistance to simulated intestinal fluid (SIF)

After 24-h incubation at 37 °C in MRS broth, 1 mL of each strain cell suspension was centrifuged (8000×*g*, 5 min at 4 °C). The pellets were washed twice in phosphate-buffered saline (pH 7.2) and resuspended to the initial volume. Washed cell suspensions (0.1 mL) were added to 0.9 mL of fresh simulated intestinal juice (SIF) at 37 °C. The simulated intestinal juice was composed of 0.136 g KH_2PO_4 and 0.018 g NaOH, resuspended with 20 mL DDI, and supplemented with pancreatin 1% (w/v), pH 6.8 ± 0.1. Bacterial cells in SIF were incubated for 2 h at 37 °C in anaerobic conditions with periodical shaking. 0.9 mL of sterile MRS broth inoculated with the same amount of the cell strain suspension as in the sample with SIF and cultured for the same period and under the same incubation conditions were the control samples for each strain. Surviving bacteria were enumerated with the pour plating method. All enumerations were carried out using the standard serial dilution method in a physiological solution, plated on MRS agar with 0.05% cysteine, and incubated at 37 °C for 48 h in anaerobic conditions.

Auto- and coaggregation

The autoaggregation assay was done according to the method of [22] with a slight modification. Briefly, lactobacilli were harvested in the stationary phase, collected by centrifugation (10,000×*g* for 10 min), washed twice, and resuspended in PBS (pH 7.2). In all experiments, the bacterial suspension was standardized to $OD_{600} = 1.0$ (2×10^8 CFU/mL). Optical density was measured in a spectrophotometer (Biorad, Germany) at regular intervals (2, 3, 4, and 5 h) without disturbing the microbial suspension, and the kinetics of sedimentation was obtained. The autoaggregation coefficient (AC_t) was calculated at time *t* as:

$$AC_t = 1 - \frac{OD_t}{OD_i} \times 100$$

where OD_i is the initial optical density at 600 nm of the microbial suspension and OD_t is the optical density at time *t*.

In the coaggregation assay, suspensions of lactobacilli were obtained as described previously. Bacteria (*Bacillus cereus* and *Listeria monocytogenes*) were harvested in the stationary phase by 4 min centrifugation at 5000×*g* and resuspended in PBS (pH 7.2). One milliliter of the *Lactobacillus* suspension and 1 ml of the pathogenic bacterial suspension at the same optical density ($OD_{600} = 1.0$) were

mixed. Optical density was measured at regular intervals (2, 3, 4 and 5 h) to obtain the kinetics of sedimentation. The coaggregation coefficient (CC_t) was calculated as

$$CC_t = \frac{\left[\left(A_x + A_y\right)/2\right] - A_t(x+y)}{A_x + A_y/2} \times 100$$

Tolerance to low pH

To determine the acid tolerance of the examined strains, MRS medium with pH adjusted (using HCl) to 2.0, 3.0, 4.0, 5.0, and 6.3 (control) was prepared. The growth rate of each bacterial strain was monitored using Bioscreen C (LabSystem, Finland). Briefly, OD_{600} was measured every 2 h by 48 h at 37 °C, starting from OD_{600} 0.1. The growth curves were determined three times and the results are shown as a mean.

ß-Glucosidase activity

The activity of β-glucosidase was determined as follows: a crude extract of sonicated cells (15 min in a VC-130 sonifier 130 W, Sonics and Materials Inc., USA) was diluted with distilled water to 0.5 mL and then mixed with 0.5 mL of twofold citrate phosphate buffer containing 5 mM/L *p*-nitrophenyl-β-*D*-glucopyranoside (pNPG) (Sigma, USA) in a final volume of 1 mL. The reaction mixture was incubated at 30 °C and pH 5.0 for 1 h and was stopped by adding 2 mL of 1 M Na_2CO_3. The absorbance of the supernatants at 400 nm was measured using a spectrophotometer (Beckman DU-800 spectrophotometer).

Statistical analysis

The similarity between the bacterial strains was determined on the basis of the results of bottom-up hierarchical cluster analysis, using average linkage clustering as a linkage criterion (UPGMA). This method produces a dendrogram from a distance matrix by applying a sequential clustering algorithm, in which local topological relationships are inferred in order of decreasing similarity. The data were standardized before analysis to avoid the effect of the differences in measurement units between the parameters on the values of Euclidean distances [23].

Principal component analysis (PCA) was used to present the patterns of bacterial strains in the reduced dimensions of the studied functional properties. The results are presented in the form of a table of factor loadings showing correlations between these features and the obtained factors (PC1, PC2, and PC3) as well as in the form of a scatter plot of bacterial strain factor scores in the PC1–PC2, PC1–PC3 and PC2–PC3 coordinate systems. The factor loadings were obtained after VARIMAX rotation maximizing the sum of the variances of the squared loadings [24].

Results and discussion

Among the results obtained with MALDI-TOF, strains with score above 2.1 and (also) those whose second or third match indicated the same species were selected for further studies. All these nine isolates indicated the assignment to the species *Lactobacillus plantarum*. The tenth isolate, i.e., reference *Lb. plantarum* strain ATCC 14917 used in the study, was also identified correctly, which confirms the other results.

The nine isolates selected using MALDI-TOF were subjected to identification by BLAST alignment. All sequences were found to be the most similar to *Lb. plantarum* WCFS1. Due to the extremely high similarity in the genotype and phenotype, they were distinguished among three species: *Lb. plantarum, Lb. pentosus*, and *Lb. paraplantarum* by a specific multiplex PCR. The assignment to these individual species is based on the *recA* gene sequence properties. The multiplex reaction yielded amplicons of various lengths: *Lb. paraplantarum* has a gene about 107 bp long, *Lb. pentosus* around 218 bp, and *Lb. plantarum* 318 bp (Fig. 1). The identification of nine isolates was performed by analyzing sequences of the 16S rRNA gene. The DNA sequences obtained were aligned by BLAST with the nucleotide gene bank; it was revealed that all strains are > 99% similar to *Lb. plantarum* (Fig. 2). The 16S rRNA gene sequences from examined isolates were deposited in gene bank under the following numbers: *Lb. plantarum* BOC1—MG966275, *Lb. plantarum* BOC2—MG966276, *Lb. plantarum* BOC3 MG966277, *Lb. plantarum* BOC4—MG966278, *Lb. plantarum* BOC5—MG963281, *Lb. plantarum* BOC6—MG963282, *Lb. plantarum* BOC7—MG963283, *Lb. plantarum* BOC8—MG963284, *Lb. plantarum* BOC9—MG963285. In this article, *Lb. plantarum* species were

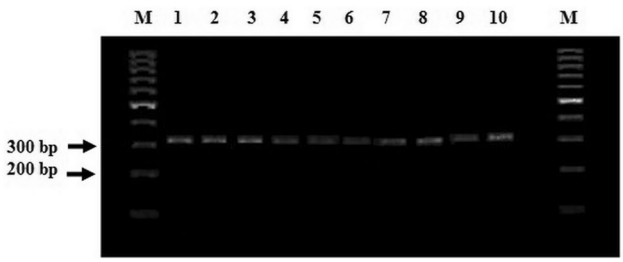

Fig. 1 Multiplex PCR amplification products obtained for *recA* gene. Lane M contains a 1 kb Ladder Perfect Plus (Eurx, Gdańsk, Poland). Lane 1–9 contains the amplification product of isolates of *Lb. plantarum* from this study. Lane 10 contains reaction product for the reference strain *Lb. plantarum* ATCC 14917

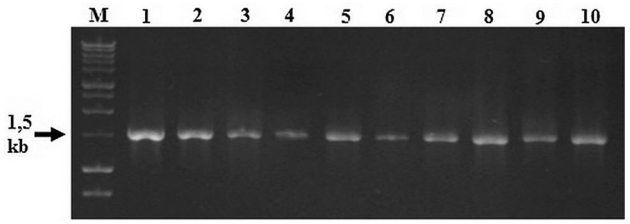

Fig. 2 PCR amplification products obtained for 16S rRNA gene. Lane M contains a 1 kb Ladder Perfect Plus (Eurx, Gdańsk, Poland). Lane 1–9 contains the amplification product of isolates of *Lb. plantarum* from this study. Lane 10 contains reaction product for the reference strain *Lb. plantarum* ATCC 14917

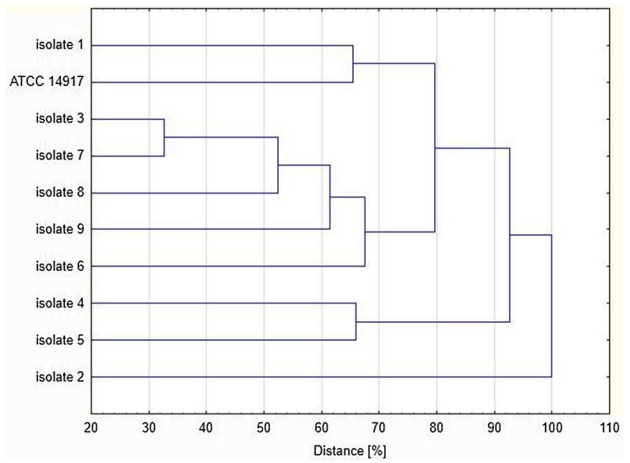

Fig. 3 Unweighted pair group method with arithmetic means (UPGMA) clustering of the *Lb. plantarum* isolates

chosen for evaluation because of their versatility. The genome of *Lb. plantarum* has a relatively large number of genes responsible for regulation, transport, outer surface proteins, and utilization of various sugars, which contribute to the incredible flexibility of the species to adapt to diverse environments [25].

Six functional traits of *Lb. plantarum* isolates were analyzed, i.e., viability at low pH, resistance to lysozyme and to SIF, auto- and coaggregation, and ß-glucosidase activity. To examine these data, the multivariate analyses such as Unweighted Pair Group Method with Arithmetic Mean (UPGMA) as well as Principal Component Analysis (PCA) were performed. These methods have powerful effect on the field of food technology such as proteolysis and aroma compounds of Cheddar cheese [26], antioxidant properties of wine lactic acid bacteria [27], sensory assessment tool for fermented food [28]. This approach provided foundation to understanding the interactions between the analyzed traits in the examined isolates and selection of the most promising functional isolates.

The results of the UPGMA analysis presented in Fig. 3 show the degree of similarity between the isolates. Taking into consideration the examined functional traits, it is evident that isolate 2 is the most different from the others. There is a high degree of similarity between isolates 3 and 7; 1 and 10 (ATCC 14917), as well as 4 and 5. Based on the distances presented in Fig. 3, we suggested division into the following groups of isolates with similar values of functional traits: Group 1 with reference strain 10 and the most similar isolate 1, Group 2 with isolates 4 and 5, Group 3 with isolate 2, and Group 4 with isolates 3, 6, 7, 8, and 9.

The principal component analysis revealed three principal components (PCs) accounting for over 78% of the total variance (25.5, 28.9, and 23.5% for the first, second, and third component, respectively). Table 1 shows factor loadings, which describe the degree of the correlation of principal components with the examined functional traits. Based on the values of factor loadings, we named three principal components designated in PCA: PC1 is connected with bacterial acid tolerance response (ATR) and synthesis of acid-shock proteins, PC2 is connected with the composition of the external cell envelope (cell surface proteins, teichoic and teichuronic acids), and PC3 is connected with the cell surface charge and external receptor proteins.

Two functional traits (tolerance to low pH and ß-glucosidase activity) were highly related to PC1. As a result of the production of lactic acid by bacteria, the pH of the external environment is reduced, which has a negative effect on bacterial growth and viability. During the logarithmic growth

Table 1 Correlation of variables with the factors of the PCA analysis based on factor loadings

Variables	PC1	PC2	PC3
Tolerance to low pH	−0.811059*	−0.098907	0.101234
Resistance to lysozyme	0.416761	−0.851017*	0.177171
Resistance to SIF	0.211145	0.742006*	0.258434
Autoaggregation	−0.559029	0.096072	−0.770006*
Coaggregation with *Bacillus cereus*	0.075746	0.060490	−0.877116*
Coaggregation with *Listeria monocytogenes*	0.055942	−0.827777*	0.330087
ß-Glucosidase activity	0.768558*	−0.215065	0.262830

*Factor loading > 0.7

phase, bacteria induce cell response connected with synthesis of acid-shock proteins such as superoxide dismutase LuxS and some chaperones like heat-shock proteins [29, 30]. ß-Glucosidase activity is necessary for carbohydrate metabolism in lactobacilli isolated from plants due to release a wide range of plant secondary metabolites from their ß-D-glucosylated precursors [31].

Three functional traits were highly related to PC2 (tolerance to lysozyme and SIF, coaggregation). *Lb. plantarum* belongs to Gram-positive bacteria characterized by a cell wall composed of a thick layer of peptidoglycan and a large amount of cell envelope proteins. Such external cell structure is responsible for resistance to lytic enzymes such as lysozyme and pancreatin. Specific external proteins play an important role in aggregation with bacteria from other species. In most lactobacilli, high cell surface hydrophobicity is determined by basic external proteins. Thus, the surface properties of *Lb. plantarum* isolates can be determined by covalently anchored proteins. The N-terminally anchored proteins represent the largest group of cell surface-anchored proteins in lactobacilli and are mainly involved in cell envelope metabolism, extracellular transport, and signal transduction [32]. Amino acids and amide groups on the cell surface determine the negative charge of *Lb. plantarum* cells, which facilitates coaggregation with *L. monocytogenes*.

Two functional traits were highly related to PC3, i.e., auto- and coaggregation with Gram-positive bacteria such as *B. cereus*. The cell surface charge and the presence of cell surface binding proteins are responsible for the bacterial ability to aggregate. Kainulainen et al. [33] observed that released external proteins were able to reassociate with different bacterial species, providing a mechanism of bacterium–bacterium interactions.

Using the component factors shown in Table 1, two-dimensional scatter plots of the isolates in new coordinates, designated by the principal components, were presented. These plots allow easy comparison of the traits for the examined isolates (Fig. 4). Graph PC1–PC2 (Fig. 4a) shows great differences between the isolates as regards tolerance to low pH—the lowest differences were noted in Group 1, which indicates that isolate 1 is similar in terms of sensitivity to low pH to reference *Lb. plantarum* ATCC 14917 isolated from pickled cabbage. The negative value of PC1 indicates

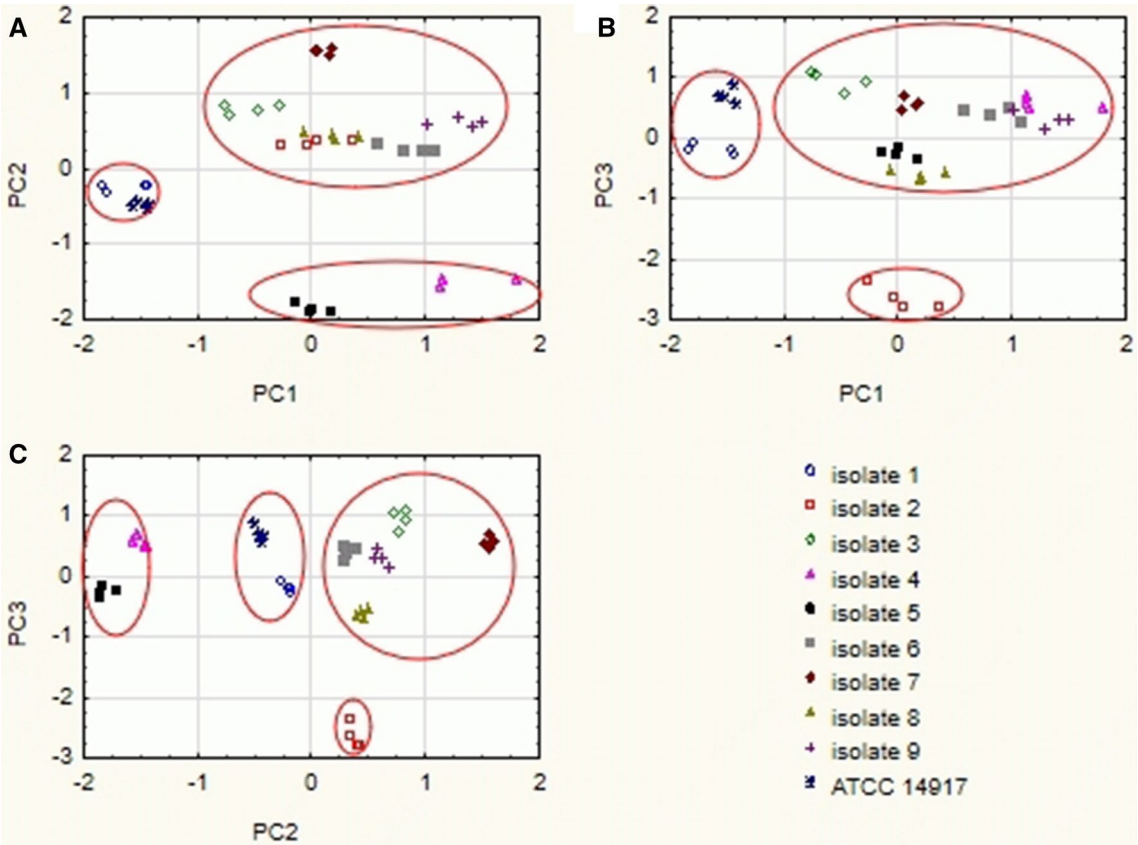

Fig. 4 Factor scores in principal component coordinate systems. Projection of the *Lb. plantarum* isolates in the space of PC1 and PC2 (**a**), PC1 and PC3 (**b**), PC2 and PC3 (**c**)

high tolerance to low pH of isolates belonging to Group 1. The highest differences according to PC1 were observed in Group 4. The highest value of PC2 was characteristic of isolates belonging to Group 3 and 4, whereas a lower value of PC2 was calculated for the isolates from Group 1 and the lowest one from Group (2) The variation in the acid tolerance of the lactic acid bacteria has been linked to the difference in induction of H+-ATPase activity resulting in removal of protons (H+), alkalization of the external environment, and changes in the composition of the cell envelope [34]. Graph PC1–PC3 (Fig. 4b) presents even higher differences between the isolates within the groups. The low value of PC3 can be seen only for isolate 2, which is probably different from the others in terms of the cell surface charge and the presence of external receptor proteins. We can assume that isolate 2 has a low capability of auto- and coaggregation. In graph PC2–PC3 (Fig. 4 C), four separate groups clearly correlating with the results of UPGMA analysis can be distinguished. Low differences between the isolates were observed within the groups. High values of PC3 were found in groups 1, 2, and 4. Isolates belonging to these groups are characterized by a high value of auto- and coaggregation. It should be pointed out that isolates belonging to group 4 and isolate 1 are very similar as regards the functional traits to reference strain *Lb. plantarum* ATCC 14917. Consistently, low values of PC3 were noted in group 3, which comprised only isolate 2. The differences observed in the functional traits connected with the cell surface charge and receptor proteins (PC3) of isolate 2 can be explained by variation in the level of expression of cell surface proteins [35]. To sum up the PCA results, it can be claimed that factors such as the composition of the external cell envelope (PC2) as well as the cell surface charge and the presence of external receptor proteins (PC3) are important contributors while screening the functional traits of the examined isolates. These factors have a strong impact on such functional traits as auto- and coaggregation as well as resistance to lysozyme and simulated intestinal fluid. It is worth pointing out that the same variables (functional traits) were taken into consideration in both the UPGMA and PCA analyses, and it is clearly seen that the results of these analyses indicated the same groups of isolates with similar values of functional traits (Tables 2, 3).

Table 2 Resistance to low pH after 24 h of incubation and survival of *Lb. plantarum* isolates at lysozyme and simulated intestinal fluid (SIF) after 30 min of incubation

Isolate	pH 4 (OD_{600})	Lysozyme (CFU/ml)	SIF (CFU/ml)
1	1.079	5.71	34.23
	1.108	5.54	34.85
	1.175	5.83	34.52
	1.143	6.39	35.08
2	0.703	6.89	22.06
	0.623	7.3	22.15
	0.735	6.72	22.72
	0.623	7.49	21.51
3	0.861	10.28	58.5
	0.752	10.55	57.94
	0.813	10.87	58.23
	0.755	10.98	58.17
4	0.669	67.96	22.86
	0.652	68.34	22.99
	0.635	67.5	23.3
	0.633	68.92	23.65
5	0.727	77.19	14.51
	0.652	77.83	14.94
	0.617	76.64	14.12
	0.71	78.34	15.87
6	0.378	24.68	20.66
	0.394	24.35	21.05
	0.352	24.11	20.82
	0.4	25.82	20.55
7	0.741	1.2	72.03
	0.701	1.56	71.87
	0.671	1.34	71.92
	0.688	1.38	72.18
8	0.607	19.13	38.85
	0.583	19.58	38.94
	0.564	18.99	39.25
	0.597	19.22	39.64
9	0.673	35.62	61.53
	0.625	35.14	61.92
	0.753	36.01	62.14
	0.636	36.75	61.77
10	0.91	27.03	6.05
	0.97	28.15	5.78
	0.936	26.98	5.89
	0.991	26.4	5.92

Conclusion

To conclude, it should be noted that the use of PCA and UPGMA together to analyze data from the same microbiological experiments provided information about the similarity of the examined isolates in terms of functional traits. Additionally, the level of the analyzed functional traits within the particular groups of isolates was shown. The presented approach is the basis for choosing isolates that are most closely related to the reference strain isolated from pickled cabbage. In terms of their use as starter cultures, the isolates from Group 4 are the most promising. Based on the UPGMA and PCA analysis, it can be stated that the composition of the external cell envelope

Table 3 Percent of aggregation of *Lb. plantarum* isolates after 5 h of incubation and ß-glucosidase activity

Isolate	Autoaggregation (%)	Coaggregation (%)		ß-Glucosidase (U/ml)
		B. cereus	*L. monocytogenes*	
1	65.012	8.542	8.142	4.263
	64.823	9.015	7.928	4.342
	65.267	8.876	7.645	3.971
	65.478	8.407	8.537	4.024
2	72.359	13.394	2.568	4.011
	72.016	13.649	2.212	4.235
	71.989	12.692	2.751	3.884
	72.564	13.571	2.321	3.670
3	48.562	6.120	6.648	4.001
	48.716	5.875	6.116	4.245
	48.494	5.632	6.056	3.684
	50.160	6.457	6.472	3.870
4	41.231	6.516	11.468	6.113
	40.882	6.945	11.716	5.929
	41.112	7.053	11.125	5.844
	40.695	7.022	10.855	6.991
5	55.458	7.118	10.663	4.571
	55.720	7.577	11.026	4.432
	55.854	7.264	11.454	4.019
	55.684	7.393	10.061	4.095
6	44.356	4.341	4.216	4.126
	44.521	4.859	4.648	4.543
	44.763	4.122	4.935	4.821
	44.072	5.218	4.357	4.990
7	52.013	6.341	3.721	4.623
	51.994	6.723	4.052	4.581
	52.278	6.929	3.947	4.120
	52.347	6.367	3.532	4.594
8	65.751	6.813	4.230	4.278
	65.698	7.154	4.762	4.591
	65.812	7.228	4.613	4.430
	66.527	6.737	4.471	5.141
9	44.563	8.156	5.323	6.023
	44.912	7.832	5.185	5.985
	45.029	7.914	4.991	5.741
	44.920	8.414	4.585	5.491
10	56.739	4.238	5.146	3.862
	56.968	4.015	4.865	3.916
	57.117	3.871	4.923	3.880
	56.628	3.496	5.270	4.422

together with the cell surface charge and external receptor proteins has a significant impact on bacterial functional traits such as tolerance to lysozyme and SIF as well as auto- and coaggregation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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