ORIGINAL PAPER
Polish Journal of Microbiology
2022, Vol. 71, No 3, 395–409
https://doi.org/10.33073/pjm-2022-035

New Potentially Probiotic Strains Isolated from Humans – Comparison of Properties with Strains from Probiotic Products and ATCC Collection

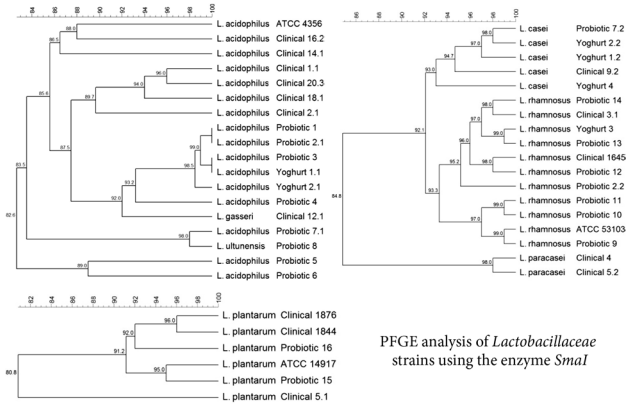
ANNA ZAWISTOWSKA-ROJEK[1, 2*], AGNIESZKA KOCISZEWSKA[1], TOMASZ ZARĘBA[1] and STEFAN TYSKI[1, 2]

[1] Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland
[2] Department of Pharmaceutical Microbiology, Medical University of Warsaw, Warsaw, Poland

Submitted 12 May 2022, accepted 14 July 2022, published online 19 September 2022

Abstract

Lactic acid bacteria are used in various types of probiotic products. Due to the constantly growing probiotics market, new strains with pro-health properties are sought. The present study compared 39 strains of *Lactobacillus, Lacticaseibacillus,* and *Lactiplantibacillus,* isolated from probiotic products and healthy people. The current research aimed to search for new, potentially probiotic strains. For this purpose the relationship between *Lactobacillaceae* strains was carried out; moreover, the basic properties of probiotic microorganisms, such as survival at low pH and bile salt environment, antibiotic susceptibility, aggregation and antagonism were estimated. The properties of these isolates were also compared with the properties of probiotic strains from the ATCC collection. In comparing the genetic relationship (PFGE method) between the tested isolates, it was observed that some of them show a high degree of similarity. All tested strains tolerated an environment with a pH value of 3.0, and the addition of 0.3% bile salt; showed auto-aggregation properties and displayed antagonism against pathogenic microorganisms. In the present study, the bacteria were susceptible to tetracycline, chloramphenicol and ampicillin; the resistance to vancomycin depended on the bacteria type. All the properties were strain-depended. Most of the tested strains had properties comparable to the reference strains. Three *L. acidophilus* strains isolated from cervical swabs seem to be promising candidates for probiotic strains.



PFGE analysis of *Lactobacillaceae* strains using the enzyme *SmaI*

K e y w o r d s: lactobacilli, PFGE, antimicrobial susceptibility, antagonism

Introduction

Strains belonging to the family *Lactobacillaceae* are classified in the lactic acid bacteria (LAB) group and have been used for years as food additives (Markowiak and Śliżewska 2017; Zawistowska-Rojek and Tyski 2022). Beside, *Bifidobacterium* strains are also frequently present in probiotic products (Zawistowska-Rojek and Tyski 2022). Live microorganisms that, when given in an appropriate number, have a beneficial effect on the health of the host, as defined by International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2014, are referred to as probiotics (Hill et al. 2014). In accordance with the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) guidelines (FAO/WHO 2002), probiotics should also have the following properties: resistance to low pH in the stomach, resistance to bile acids, adhesion to mucus and human epithelial cells and cell lines, antagonistic activity towards potentially

* Corresponding author: A. Zawistowska-Rojek, Department of Antibiotics and Microbiology, National Medicines Institute, Warsaw, Poland; Department of Pharmaceutical Microbiology, Medical University of Warsaw, Warsaw, Poland; **e-mail: a.zawistowska@nil.gov.pl**
© 2022 Anna Zawistowska-Rojek et al.
This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 License (https://creativecommons.org/licenses/by-nc-nd/4.0/).

pathogenic bacteria, the ability to limit the adhesion of pathogens to cell surfaces, and bile salt hydrolase activity. Moreover, they should be isolated from humans or animals.

Probiotics which are part of medicines, dietary supplements and fermented foods, are often used during antibiotic therapy to prevent diarrhoea associated with the consumption of antibiotics by normalising intestinal microbiota (Blaabjerg et al. 2017; Zawistowska-Rojek and Tyski 2022). Microorganisms used in food or drugs should have the Generally Recognized as Safe (GRAS) or Qualified Presumption of Safety (QPS) status (FAO/WHO 2002; Markowiak and Śliżewska 2017, Zawistowska-Rojek et al. 2022a). When analysing the safety of a microbial strain, particular attention should be paid to the lack of genes encoding the proteins responsible for resistance to antibiotics, which are located in mobile genetic elements, e.g., plasmids and transposons (Zawistowska-Rojek and Tyski 2018; Colautti et al. 2022).

Probiotic microorganisms produce several substances, e.g., short-chain fatty acids (SCFA) (acetic, propionic, butyric and lactic acid), hydrogen peroxide, bacteriocins, and deconjugated bile acids, which inhibit the growth of other microorganisms. The SCFAs maintain a low pH in the intestinal lumen; bacteriocins, on the other hand, increase cell membrane permeability, leading to cell death (Kerry et al. 2018; Zawistowska-Rojek and Tyski 2022). They also show bactericidal or bacteriostatic activity towards closely related species and other types of bacteria (Hernandez-Gonzalez et al. 2021). Probiotic bacteria exhibit antagonistic action towards numerous bacterial pathogens of the gastrointestinal tract, such as *Salmonella enterica*, *Shigella sonnei*, enteropathogenic strains of *Escherichia coli*, *Staphylococcus aureus*, *Campylobacter jejuni* and *Clostridioides difficile*. They hinder the adhesion of pathogens to the intestinal mucosa as a result of receptor binding competition and inhibit their multiplication through competition for nutrients (Markowiak and Śliżewska 2017; Kerry et al. 2018).

Some of the bacteriocins produced by lactic acid bacteria have inhibitory effects on the growth of closely related microorganisms: lactocycline Q produced by *Lactococcus* sp. OU12 may inhibit the growth of *Lactococcus lactis, Lactobacillus* spp., *Bacillus* and *Enterococcus*; plantaricin LpU4 produced by the strain *Lactiplantibacillus paraplantarum* LpU4 inhibits the growth of strains *Lactiplantibacillus plantarum*, *L. lactis, Enterococcus faecalis* and *Enterococcus faecium* (Trejo-Gonzalez et al. 2021); plantaricin JLA-9 produced by *L. plantarum* JLA-9 inhibits the growth of strains belonging to the *Bacillus* spp. genus, while fermenticin HV6b produced by *Limosilactobacillus fermentum* HV6b may inhibit the growth of *E. faecalis* (Darbandi et al. 2021).

Lactobacillaceae are also characterised by the ability to auto-aggregation. This characteristic is associated with, i.a., exopolysaccharides located on the surface of cells, which can play an essential role in the strength and speed of cell interaction. In addition, it is suggested that the shape of the bacterial cell is also vital for aggregation; longer cells, due to their larger surface area, are characterised by more significant aggregation. Thanks to this, lactic acid bacterial cells can remain in the host's gastrointestinal tract for a long time (Rajab et al. 2020; Zawistowska-Rojek et al. 2022b). According to some researchers, bacterial auto-aggregation is correlates with these bacteria's ability to adhere to and colonise the gastrointestinal tract (Hojjati et al. 2020).

The current research aimed to search for new potentially probiotic bacteria and to compare the basic probiotic properties (e.g., survival at low pH, survival in a bile salt environment, antibiotic susceptibility, and the aggregation or growth inhibition of both pathogenic and closely related microorganisms) of bacteria isolated from probiotic products, yoghurts and from clinical material with the properties of probiotic bacteria from the ATCC collection. In addition, the genetic relationship between *Lactobacillaceae* strains was determined.

Materials and Methods

Bacterial strains. Twenty-four *Lactobacillaceae* strains used in the study were isolated from various probiotic products available on the Polish market (dietary supplements, food for special medical purposes, medical devices, yoghurts) (Table I). Each probiotic product tested (Probiotic 1–16 and Yoghurt 1–4) was a unique sample; several packages of the same batch or several batches of the same product were not tested. Fifteen strains of *Lactobacillaceae* isolated from clinical material were analysed (cervix or anus swabs of healthy women). Strains were obtained from the collection of the Department of Pharmaceutical Microbiology of the Medical University of Warsaw (Table II). In addition, *Lactobacillus acidophilus* ATCC® 4356™, *Lacticaseibacillus rhamnosus* GG ATCC® 53103™, and *Lactiplantibacillus plantarum* ATCC® 14917™ were used as reference strains. All strains were identified using API 50 CHL biochemical tests (bioMérieux, France) or MALDI-TOF MS (Brucker System in ALAB Laboratories, Poland). Strains of lactic acid bacteria were cultured in De Man, Rogosa and Sharpe (MRS-Agar, Merck Millipore, Germany) agar in an atmosphere of 5% CO_2 at 37°C for 48–72 h.

In the study, 132 strains of potentially pathogenic bacteria from the collection of the Department of Pharmaceutical Microbiology of the Medical University of Warsaw and belonging to the following species: *E. coli*

Table I
Lactobacillaceae strains derived from probiotic products.

Source of isolation	*Lactobacillaceae* species declared by the manufacturer	*Lactobacillaceae* species identified by API/MALDI-TOF MS	Strain
Dietary supplement	*L. acidophilus*	*L. acidophilus*	Probiotic 1
Dietary supplement	*L. acidophilus* *L. rhamnosus* *L. casei*	*L. acidophilus* *L. rhamnosus*	Probiotic 2
Dietary supplement	*L. acidophilus*	*L. acidophilus**	Probiotic 3
Dietary supplement	*L. acidophilus*	*L. acidophilus*	Probiotic 4
Food for special medical purposes	*L. acidophilus* *L. delbrueckii* subsp. *bulgaricus*	*L. acidophilus*	Probiotic 5
Dietary supplement	*L. acidophilus* *L. paracasei*	*L. acidophilus*	Probiotic 6
Dietary supplement	*L. casei* *L. acidophilus*	*L. casei* *L. acidophilus*	Probiotic 7
Dietary supplement	*L. acidophilus*	*L. ultunensis**	Probiotic 8
Dietary supplement	*L. rhamnosus*	*L. rhamnosus**	Probiotic 9
Food for special medical purposes	*L. rhamnosus*	*L. rhamnosus**	Probiotic 10
Food for special medical purposes	*L. rhamnosus*	*L. rhamnosus**	Probiotic 11
Medical device	*L. rhamnosus*	*L. rhamnosus*	Probiotic 12
Dietary supplement	*L. rhamnosus*	*L. rhamnosus*	Probiotic 13
Dietary supplement	*L. rhamnosus* *L. plantarum*	*L. rhamnosus*	Probiotic 14
Dietary supplement	*L. fermentum* *L. gasseri* *L. plantarum* *L. rhamnosus*	*L. plantarum*	Probiotic 15
Dietary supplement	*L. plantarum*	*L. plantarum**	Probiotic 16
Yoghurt	*L. acidophilus* *L. casei*	*L. acidophilus* *L. casei*	Yoghurt 1
Yoghurt	*L. acidophilus* *L. casei*	*L. acidophilus* *L. casei*	Yoghurt 2
Yoghurt	*L. acidophilus* *L. paracasei*	*L. rhamnosus*	Yoghurt 3
Yoghurt	*L. casei*	*L. casei*	Yoghurt 4

* – strains identified by MALDI-TOF MS (ALAB Laboratory, Warsaw, Poland)

(n = 11), *Klebsiella pneumoniae* (n = 12), *Proteus mirabilis* (n = 10), *Serratia marcescens* (n = 10), *Enterobacter cloaceae* (n = 10), *Pseudomonas aeruginosa* (n = 11), *Acinetobacter baumannii* (n = 11), *Stenotrophomonas maltophilia* (n = 12), *E. faecalis* (n = 15), *S. aureus* (n = 17) and *Staphylococcus epidermidis* (n = 13), were used. The strains were identified using the VITEK (bioMérieux, France) system. Potentially pathogenic bacteria were cultured on Tryptic Soy Agar (TSA; Merck Millipore, Germany) at 37°C for 24 h.

All strains were stored in Microbank beads (Pro-Lab Diagnostics, Canada) in a deep freeze at < –70°C.

Pulsed field gel electrophoresis (PFGE). Agarose discs containing the DNA of *Lactobacillaceae* strains were prepared based on the methodology presented by Gosiewski et al. (2012) and Domingo-Lopes et al. (2017), with some modifications.

SmaI (5'-CCC^GGG-3') (Thermo Fischer Scientific, USA) and *ApaI* (5'-GGGCC^C-3') (Thermo Fischer Scientific, USA) endonuclease were used to differentiate the strains. Digestion was conducted at a temperature of 30°C (*SmaI*) or 37°C (*ApaI*) for 16–18 h. Then, electrophoretic separation was performed in the CHEFF III (Bio-Rad, USA) apparatus in 1% agarose (Pulsed Field Certified Agarose, Bio-Rad, USA) gel in 0.5 × TBE under conditions suitable for the strains (Table III). Lambda PFG Ladder (New England BioLabs, USA) was used as a molecular weight marker.

Table II
Lactobacillaceae strains isolated from clinical material.

Source of isolation	*Lactobacillaceae* strains identified by API/MALDI-TOF MS	Strain
Cervical swabs	*L. acidophilus**	Clinical 1.1
	L. acidophilus	Clinical 2.1
	L. acidophilus	Clinical 14.1
	L. acidophilus	Clinical 16.2
	L. acidophilus	Clinical 18.1
	L. acidophilus	Clinical 20.3
	*L. gasseri**	Clinical 12.1
	*L. rhamnosus**	Clinical 3.1
Rectal swabs	*L. rhamnosus**	Clinical 1645
Cervical swabs	*L. casei*	Clinical 9.2
	L. paracasei	Clinical 4
	L. paracasei	Clinical 5.2
Rectal swabs	*L. plantarum**	Clinical 1876
	L. plantarum	Clinical 1844
Cervical swabs	*L. plantarum**	Clinical 5.1

* – strains identified by MALDI-TOF MS (ALAB Laboratory, Warsaw, Poland)

Table III
Pulsed field gel electrophoresis (PFGE) parameters.

Genus	Time	Temperature	Voltage	Pulse length
Lactobacillus	22 h	14°C	6 V	1–6 s
Lacticaseibacillus	22 h	14°C	5 V	1–15 s
Lactiplantibacillus	20.5 h	14°C	5.5 V	1–10 s

The gels were stained in an ethidium bromide 5 µg/ml solution (Sigma-Aldrich, USA). Dendrograms were prepared using the Unweighted Pair Group Method with Aritmetic Mean (UPGMA) method using the GelCompar II 6.6 (Applied Maths, Belgium) program.

Antagonistic activity. The antagonism test of *Lactobacillaceae* strains towards pathogenic bacteria and closely related bacteria was evaluated using the agar slab method (Ślizewska et al. 2021) on MRS agar.

One ml of lactic acid bacteria suspension (10^8 CFU/ml) was introduced to the MRS agar and incubated at a temperature of 37°C for 24 h in an atmosphere of 5% CO_2. Then, 9 mm diameter agar slabs were cut and put on TSA containing pathogenic bacterial strains (10^6 CFU/ml). Plates were incubated for 24 h under aerobic conditions, and then growth inhibition zones of pathogenic strains were measured. The antagonism test was performed against 87 Gram-negative bacterial strains from eight species and 45 Gram-positive strains from three species; all tests were performed in triplicate.

To determine clinical isolates auto-antagonism (antagonism to closely related species) (Table II) to probiotic isolates (Table I), 1 ml of *Lactobacillaceae* strains (10^8 CFU/ml), isolated from the clinical material, was introduced to the MRS agar medium and then incubated at 37°C for 24 h in an atmosphere of 5% CO_2. Subsequently, 9 mm diameter agar slabs were cut out and applied on MRS agar containing *Lactobacillaceae* isolated from probiotic products (10^6 CFU/ml). The plates were incubated for 24 h in a 5% CO_2 environment, and then the growth inhibition zones of probiotic strains were measured. All tests were performed in triplicate.

Survival of *Lactobacillaceae* strains at low pH. *Lactobacillaceae* strains were grown in MRS broth with a pH of 2.0 and 3.0, acidified with 1 M HCl solution. One ml suspension of *Lactobacillaceae* strains at a density of approximately 10^8 CFU/ml was added to MRS broth of a suitable acidic pH (2.0 or 3.0), and to MRS broth (pH 6.4) as a control and then incubated in an environment of 5% CO_2 at 37°C. After 1 h and 2 h of incubation, decimal dilutions were prepared and 1 ml of suspension was inoculated on MRS Agar. After incubation of the plates in an environment of 5% CO_2 at 37°C for 72 h, the number of *Lactobacillaceae* colonies was counted.

Survival of *Lactobacillaceae* strains in a presence of bile salt. One hundred µl of the 18–24 h bacterial culture of *Lactobacillaceae* strains in MRS broth was transferred to MRS broth containing 0.3% bile salt (Oxgall, Difco) (MRSO) and to MRS broth without bile (control), based on the methodology of Liu et al. (2022) with modifications. They were incubated at 37°C in a 5% CO_2 for 6 h. Absorbance at 620 nm was measured after the incubation of the MRS broth (A_{620} MRS Broth) and of the MRS broth with the addition of bile salt (A_{620} MRSO). The bacterial growth inhibition factor in the bile salt environment (Ch) was calculated using the formula:

$$Ch = \frac{A_{620}\,MRS\,Broth - A_{620}\,MRSO}{A_{620}\,MRS\,Broth} \qquad (2)$$

Auto-aggregation test. Auto-aggregation analysis was performed following Kos et al. (2003) and Zawistowska-Rojek et al. (2022b) with modifications. *Lactobacillaceae* strains were cultured for 20 h in MRS broth in an environment of 5% CO_2 at 37°C. The bacteria were centrifuged (5,000 × *g*, 20 min) and washed twice in Phosphate Buffered Saline (PBS; Gibco, Thermo Fisher Scientific, USA). The precipitate was suspended in PBS to standardise the density of the bacterial suspension to 0.25 ± 0.05 at 600 nm (A_{600}). The suspension (4 ml) was vortexed and then incubated for 24 h at 37°C, after which the absorbance was measured again at 600 nm (A_{24h}). Auto-aggregation was expressed by the equation:

$$Auto - aggregation\ (\%) = \left[1 - \left(\frac{A_{24h}}{A_{600}}\right) \times 100\right] \qquad (1)$$

Antibiotic susceptibility testing. Antibiotic susceptibility testing of *Lactobacillaceae* strains was performed

in accordance with the tests carried out by Kang et al. (2020) and the guidelines of the European Food Safety Authority (EFSA 2018) with modifications. Using an 18–24 h bacterial culture of the *Lactobacillaceae* strains, a bacterial suspension was prepared in 0.9% NaCl with a 5×10^5 CFU/ml density. The minimum inhibitory concentration (MIC) value for the selected antibiotics and chemotherapeutic agents was determined using MRS broth; the plates were incubated for 48 h at 37°C in an atmosphere of 5% CO_2. The following antibiotics were used in the study: erythromycin, clindamycin, vancomycin, chloramphenicol, ampicillin, tetracycline, cefotaxime, ceftazidime and ceftriaxone at concentrations from 0.06 mg/l to 256 (or 512) mg/l. The interpretation of the results for ampicillin, vancomycin, erythromycin, clindamycin, tetracycline, chloramphenicol, and imipenem was carried out in accordance with the EFSA Guidelines (EFSA 2018) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2021). For the other antibiotics – cefotaxime, ceftazidime and ceftriaxone, there are no antibiotic susceptibility guidelines for the *Lactobacillacae* family; therefore, only the minimum inhibitory concentration (MIC) is presented.

Statistical analysis. All presented results are expressed as three independent experiments' mean and standard deviation. A one-way analysis of variance (single-factor ANOVA) was performed, followed by Tukey's post-hoc test for multiple comparisons. For results that did not show a normal distribution, a non-parametric Kruskal-Wallis test was used, followed by a pairwise comparison. Values of $p < 0.05$ were considered significant. Statistical analysis was performed using SPSS software (version 28.0.1.0, IBM, USA).

Results

Pulsed Field Gel Electrophoresis (PFGE). The PFGE analysis of 42 *Lactobacillaceae* strains resulted in 40 different pulsotypes (16 for bacteria of the *Lactobacillus*, 18 for *Lacticaseibacillus,* and 6 for *Lactiplantibacillus*) after application of the *SmaI* enzyme, and 41 profiles after application of the *ApaI* enzyme (Fig. 1–3). *L. acidophilus* isolated from Probiotic 1 and Probiotic 2 has a 100% (*SmaI*) compliant genetic profile, as do bacteria of the same species derived from Probiotic 3 and Yoghurt 1. Using the *ApaI* enzyme, 100% compliance was observed for *L. acidophilus* strains isolated from Yoghurt 1 and Yoghurt 2. A very high percentage of similarity can be observed between some analysed strains belonging to the same species, e.g., strains of *Lactobacillus* sp. derived from Probiotic 1, 2, 3 as well as from Yoghurt 1 and 2, which show similarity at the level of 98.5% (*SmaI* enzyme digestion), and 96.3% (*ApaI* digestion); while strains from Probiotic 7 and 8 demonstrate a similarity of 98% (*SmaI*) and 93% (*ApaI*). None of the analysed probiotic and clinical strains showed a similarity above 90% to the reference strain *L. acidophilus* ATCC® 4356™ (Fig. 1). A high level of similarity can also be observed for strains of *Lacticaseibacillus* (Fig. 2). The strain isolated from Probiotic 9 shows a 99% (*SmaI*)/95% (*ApaI*) similarity to the reference strain *L. rhamnosus* ATCC® 53103™; a relative level of similarity can be observed between strains from Probiotic 10 and Probiotic 11. All four described strains show a similarity of 97% (*SmaI*).

For the bacteria of *Lactiplantibacillus* (Fig. 3), a substantial percentage of similarity was observed between the strain from Probiotic 15 and the reference strain *L. plantarum* ATCC® 14917™ – 99.2% (*ApaI*) and 95% (*SmaI*). Clinical strains 1876 and 1844 were also closely related – 99.6% (*ApaI*) and 96% (*SmaI*). The most minor degree of similarity and the most remarkable differences between the enzymes used were observed for the clinical strain 5.1; the similarity to the other strains is less than 81% (*SmaI*), and only 43% for the *ApaI* enzyme. It can also be observed that strains of the *Lactobacillus* sp. – Clinical 1.1, 18.1, and 20.3 were not closely related to any of the analysed strains derived from probiotic products (similarity, 83.5%), just like the strains *Lacticaseibacillus* – Clinical 4 and 5.2 (84.8%).

Antagonistic activity. The tested *Lactobacillaceae* strains were characterised by a different level of antagonism against pathogenic bacteria (Table IV), depending on the type of lactic acid bacteria and the species of pathogenic bacteria. The weakest antagonistic properties for all tested pathogenic species were shown by bacteria of the *Lactobacillus* sp. (statistically significant differences compared to the genera of *Lacticaseibacillus* and *Lactiplantibacillus*, $p < 0.05$). Most of the tested strains were characterised by a low level of growth inhibition (mean zone of growth inhibition 10–15 mm) or no growth inhibition (< 10 mm). Only three strains of *L. acidophilus* (two from probiotic products and one clinical isolate) showed a strong growth inhibition (16–22 mm) concerning *E. faecalis* strains. However, no statistically significant difference was observed compared to *L. acidophilus* ATCC® 4356™ reference strain. Some of the tested strains derived from probiotic products (Probiotic 5 and 6) and isolated from clinical material (Clinical 2.1, 14.1, 20.3, 12.3), compared to the reference strain *L. acidophilus* ATCC® 4356™, inhibited the growth of *E. cloaceae* strains to a lower degree ($p < 0.05$). In turn, Probiotic 7.1 and Clinical 1.1 strains were characterised by a more substantial antagonistic effect towards *S. epidermidis* than the reference strain. There were no statistically significant differences in the inhibition of pathogenic bacteria growth by *Lacticaseibacillus* and *Lactiplantibacillus* strains ($p > 0.05$). Most

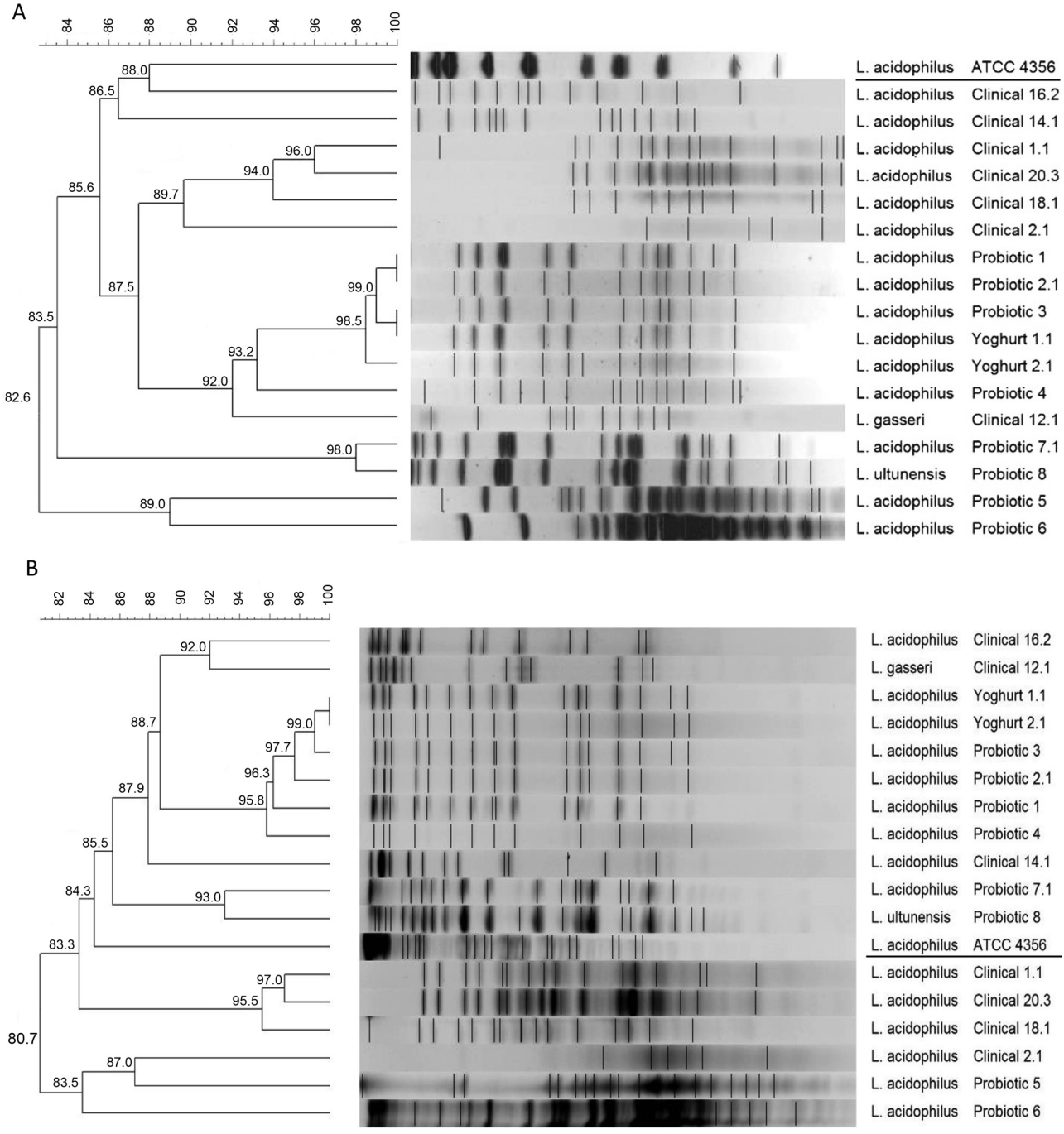


Fig.1. PFGE analysis of bacteria of *Lactobacillus* sp. The dendrogram shows the percentage similarity of PFGE profiles after DNA digestion with two enzymes: A – *SmaI*, B – *ApaI*.

of the tested strains of the given genus weakly inhibited the growth of the pathogenic strains (10–15 mm). *Lacticaseibacillus* bacteria most strongly inhibited the growth of *E. faecalis* (strong and very strong antagonism). Strong inhibition of *S. maltophilia* growth was also observed. The *L. rhamnosus* strain isolated from clinical material (Clinical 3.1) showed a weaker antagonistic effect towards the majority of pathogenic microorganisms tested compared to the reference strain.

L. rhamnosus ATCC® 53103™ ($p < 0.05$). *Lactiplantibacillus* isolates strongly inhibited the growth of *P. aeruginosa, S. maltophilia,* and *E. faecalis.* The clinical strain *L. plantarum* 1844 showed practically no antagonism towards pathogenic microorganisms (growth inhibition zones from 9 to 10.7 mm); its effect was also weaker than the reference strain *L. plantarum* ATCC® 14917™. The weakest inhibitory effect of *Lactobacillaceae* strains was towards *P. mirabilis* (weak or no inhibition).

It was also examined whether clinical *Lactobacillaceae* isolates inhibit the growth of closely related bacteria derived from probiotic products (Fig. 4). The clinical strains of *L. acidophilus* (n = 6) inhibited all

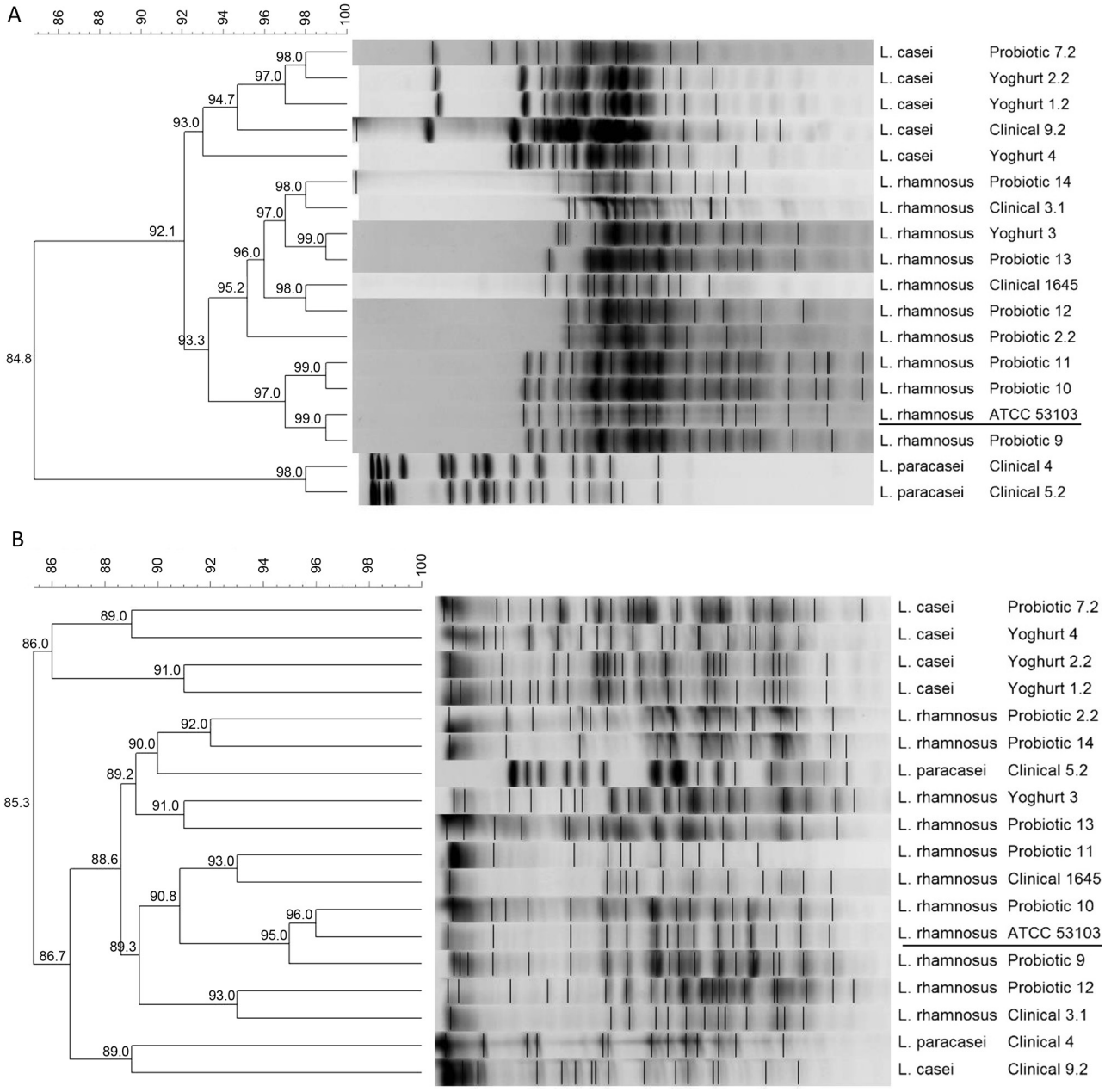


Fig. 2. PFGE analysis of bacteria of *Lacticaseibacillus* sp. The dendrogram shows the percentage similarity of PFGE profiles after DNA digestion with two enzymes: A – *SmaI*, B – *ApaI*.

potentially probiotic strains to a weak or strong degree. The clinical strains of *L. acidophilus* and *L. rhamnosus* (n = 2) inhibited probiotic bacteria of the *Lactobacillus* sp. the most, while the weakest – strains of *Lactiplantibacillus* sp. ($p < 0.05$). The clinical isolates of *L. plantarum* (n = 3) inhibited *Lactobacillus* sp. strains more strongly compared to *Lactiplantibacillus* sp. ($p < 0.05$). In turn, no statistically significant differences between the tested types of potentially probiotic bacteria were observed in the antagonistic effect toward the clinical strains of *Lacticaseibacillus paracasei* (n = 2).

Survival of *Lactobacillaceae* strains at low pH. In most of the tested strains, both those derived from probiotic products and clinical isolates, a significant reduction in growth was observed in pH 2.0 after 1 and 2 hours of incubation (Table V). The reduction of 5–7 log in the number of bacteria after 2 h incubation was observed. After incubation of LAB bacteria at pH 3.0, 30% to almost 100% of the bacteria were alive except for the clinical strain *L. paracasei* 4, whose only 1% of the population survived. Most *Lactobacillus* sp. strains survived in higher numbers in pH 3.0 after 2 h incubation than the reference strain *L. acidophilus* ATCC® 4356™. In turn, the tested *L. rhamnosus* and *L. plantarum* strains survived at a similar level to the reference strains of the same species. The exception was the

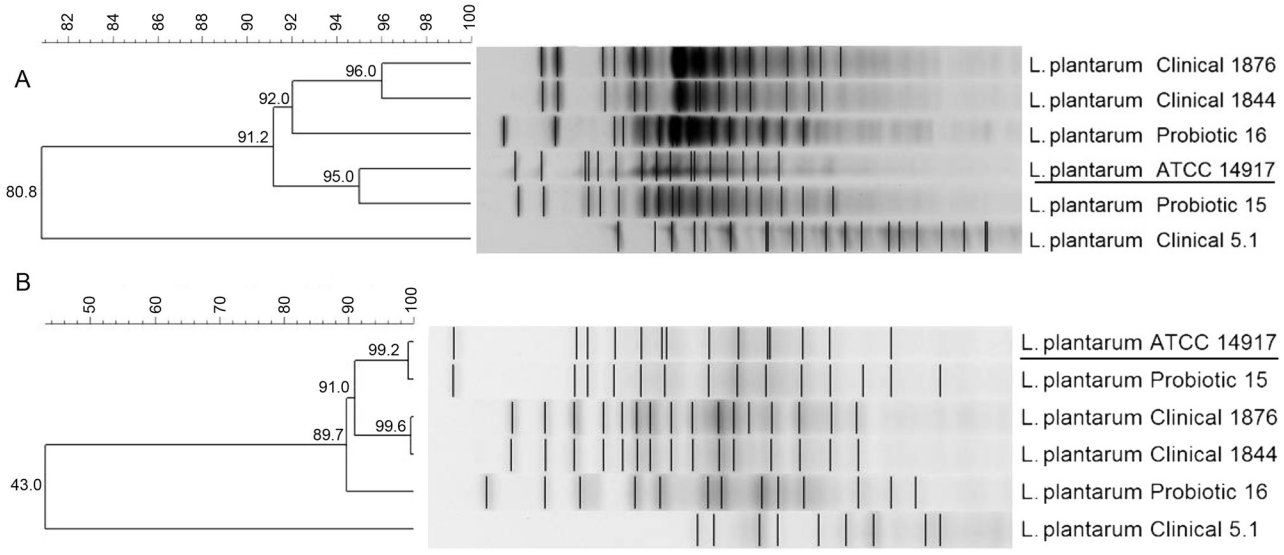


Fig. 3. PFGE analysis of bacteria of *Lactiplantibacillus* sp. The dendrogram shows the percentage similarity of PFGE profiles after DNA digestion with two enzymes: A – *SmaI*, B –*ApaI*.

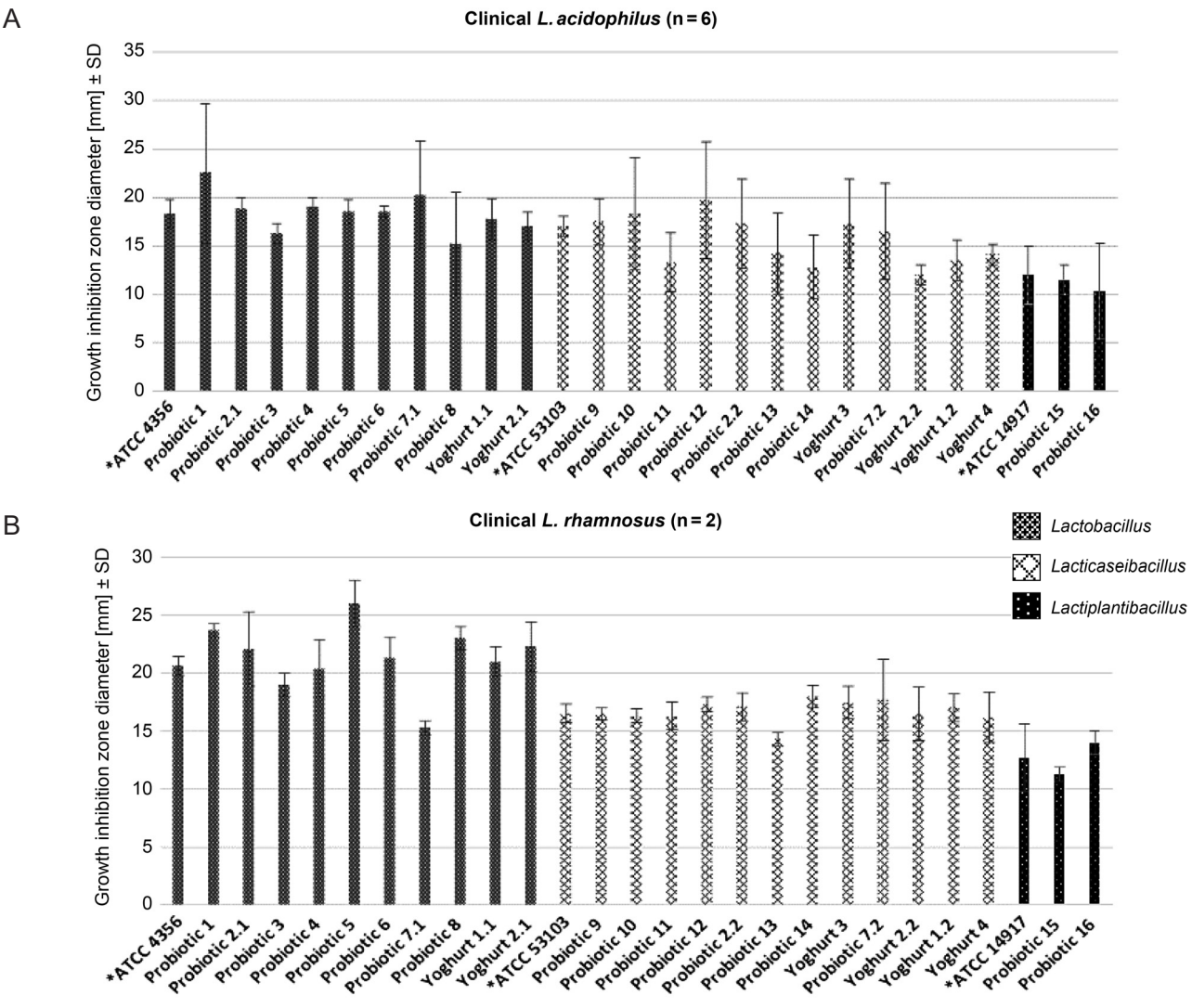


Fig. 4 – A, B. Auto-antagonism of clinical *Lactobacillaceae* isolates towards *Lactobacillaceae* isolated from probiotic products. The mean growth inhibition zone (mm) ± standard deviation. The test was performed in triplicate.

* – reference strains from ATCC collection: *L. acidophilus* ATCC® 4356™, *L. rhamnosus* GG ATCC® 53103™, *L. plantarum* ATCC® 14917™

clinical strain *L. plantarum* 1844, whose survived in pH 3.0 in lower numbers than the reference strain ($p < 0.05$).

Survival of *Lactobacillaceae* strains in the presence of bile salt. All 42 tested strains were characterised by a growth inhibition coefficient below 0.5 (Table V). *Lactobacillus* strains from probiotic products were less tolerant to bile salts present in the medium (the value of the growth inhibition coefficient ranged from 0.07 to 0.41) compared to clinical isolates of this genus (growth inhibition coefficient in the range of 0.06–0.18). The clinical strains 1.1, 2.1, 18.1 and strains 1.1 and 2.1 isolated from yoghurts had a growth inhibition coefficient lower than the reference strain *L. acidophilus* ATCC® 4356™, at a statistically significant level ($p < 0.05$). For *Lacticaseibacillus* strains, significant differences in the growth inhibition coefficient were also observed, depending on the strain tested. The lowest coefficient was shown by the *Lacticaseibacillus casei* Clinical 9.2 strain (0.05), while the lowest tolerance to the prevailing conditions was demonstrated by the *L. rhamnosus* Probiotic 2.2 strain (0.48). The Probiotic 2.2 strain isolated from the dietary supplement and the clinical strain Clinical 4 were characterised by a significantly higher value (statistically significant, $p < 0.05$) of the growth inhibition coefficient compared to the reference strain *L. rhamnosus* ATCC® 53103™. Most *Lactiplantibacillus* strains had a growth inhibition coefficient in the range of 0.13–0.24 (statistically insignificant differences, $p > 0.05$). The exception was the *L. plantarum* Clinical 1844 strain, whose coefficient was 0.42 and significantly higher ($p < 0.05$) than the coefficient of the reference strain of *L. plantarum* ATCC® 14917™.

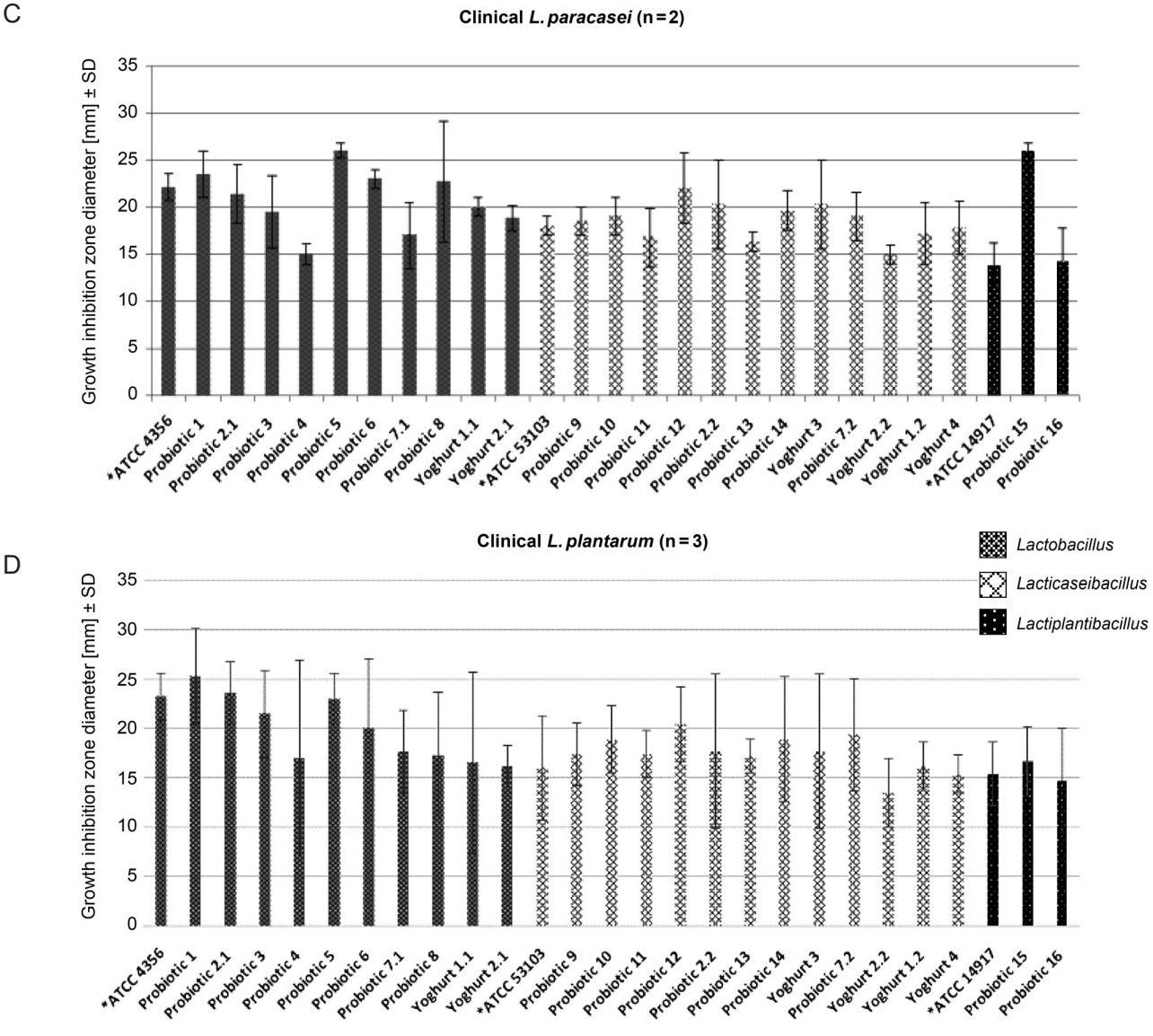


Fig. 4 – C, D. Auto-antagonism of clinical *Lactobacillaceae* isolates towards *Lactobacillaceae* isolated from probiotic products. The mean growth inhibition zone (mm) ± standard deviation. The test was performed in triplicate.

* – reference strains from ATCC collection: *L. acidophilus* ATCC® 4356™, *L. rhamnosus* GG ATCC® 53103™, *L. plantarum* ATCC® 14917™

Auto-aggregation. *Lactobacillaceae* strains auto-aggregated, which varied depending on the strain tested (Table V). Auto-aggregation values after 24 h incubation ranged from 37% for the strain of *L. plantarum* isolated from the probiotic product to 89% for *L. rhamnosus* strain derived from yoghurt. The auto-aggregation values ranged from 50–85%, and 52–81% for *Lactobacillus* sp. strains from probiotic products and clinical isolates. The three tested strains of *L. acidophilus* (Probiotic 3, Clinical 14.1, and Yoghurt 1.1) had an auto-aggregation value significantly lower ($p < 0.05$) than the reference strain *L. acidophilus* ATCC® 4356™.The strains of Probiotic 6 and Yoghurt 2.2 achieved a significantly higher value of this parameter ($p < 0.05$). Probiotic *L. rhamnosus* strains reached an auto-aggregation value of 48–89%, while clinical isolates from this species achieved 57–65%. Probiotic 2.2, 11, 12, 13, isolated from probiotic products, and clinical isolates Clinical 3.1 and 1645 had auto-aggregation values significantly lower ($p < 0.05$) than that reference strain *L. rhamnosus* ATCC® 53103™. *L. casei* strains from probiotic products presented significantly lower values of auto-aggregation (40–47%) as was also observed for *Lactiplantibacillus* strains (40–60%). Probiotic 15, Clinical 1844, and Clinical 5.1 had lower values of auto-aggregation compared to the reference strain *L. plantarum* ATCC® 14917™ ($p < 0.05$).

Antibiotic susceptibility. The drug susceptibility to antibiotics and chemotherapeutic agents was determined (Table VI). *Lactobacillaceae* strains possessed different sensitivity to antibiotics depending on the type of bacteria and the strain. *L. rhamnosus* strain isolated from the medical device (Probiotic 12) was resistant to clindamycin and erythromycin. In addition, both strains of *L. paracasei* and *L. plantarum* Clinical 1876 were resistant to erythromycin. Resistance to imipenem was also observed for all *Lacticaseibacillus* strains, four out of six (67%) strains of *Lactiplantibacillus* genus and four out of 18 (22%) strains of *Lactobacillus*. In addition, most strains of *Lacticaseibacillus* (94%) and *Lactiplantibacillus* (83%) were resistant to vancomycin.

Discussion

Many probiotic products available on the market, dietary supplements, foods for special medical purposes, and fermented foods contain lactic acid bacteria. Manufacturers, however, very often give only the name of the species, without detailed description of the strain. Probiotics, in addition to the species name, should have a specific description at the strain level, which can be marked with the catalogue number of the recognised culture collection, or with the commercial affiliation of the strain (Binda et al. 2020). The presented study examined the relationship between all analysed strains using the PFGE method. A very high level of similarity between the strains from various probiotic products was observed (e.g., Probiotic 1, Probiotic 2.1, Probiotic 3, Yoghurt 1.1 and Yoghurt 1.2). It may indicate the use of the same probiotic strain by different manufacturers, but in none of the cases was the description of the strain. A similar situation occurs for strains from Probiotic 9, Probiotic 10 and Probiotic 11, which are very similar to the reference strain *L. rhamnosus* GG (ATCC® 53103™). Thus, it can be assumed that this strain is present in these products. A high level of similarity between some clinical isolates and strains isolated from probiotic products (e.g., Clinical 3.1 and Probiotic 14, or Clinical 1645 and Probiotic 12) could also be observed. It may be the result of consumption of the given probiotic product by person from whom the strain was isolated. Domingos-Lopes et al. (2017) analysed 14 different strains of *L. paracasei* subsp. *paracasei* and five strains of *L. plantarum* using the PFGE method, resulting in 11 and five different pulsotypes, respectively. In turn, Yang and Yu (2019) analysing 43 different *Lactobacillaceae* strains, obtained 24 different profiles; this proves the existence of a large variety of *Lactobacillus* strains.

One of the properties that probiotic bacteria should present is antagonism towards pathogenic microorganisms. The study showed that bacteria from the *Lactobacillus, Lacticaseibacillus,* and *Lactiplantibacillus* inhibited the growth of various pathogenic bacteria – *P. aeruginosa, S. marcescens, E. coli, K. pneumoniae*, *P. mirabilis, S. maltophilia, A. baumannii, E. cloaceae, E. faecalis, S. aureus,* and *S. epidermidis*. The growth inhibition was comparable to the effects of reference strains from the given genera. The observed level of growth inhibition varied depending on the LAB strain used and the pathogenic strain. It was confirmed by other researchers, as it is known that antagonistic properties are strain-dependent (Asadi et al. 2022). In the current study, the most substantial growth inhibition of *E. faecalis* and *S. matophilia* clinical isolates was observed for *Lacticaseibacillus* strains. Asadi et al. (2022) demonstrated the antagonistic effect of *L. plantarum*, *L. acidophilus* and *L. casei* toward *E. coli, Salmonella typhi, Shigella dysenteriae, Neisseria gonorrhoea,* and *Streptococcus agalactiae*. In turn, Lashani et al. (2020) showed the inhibitory effect of *L. rhamnosus*, *L. paracasei*, and *L. plantarum* toward *B. cereus*, *S. enteritidis*, *E. coli, S. flexneri*, *S. aureus*, and *L. monocytogenes* from food samples. One of the strains tested in the above study – *L. plantarum* Clinical 1844 did not show antagonistic properties to most strains; it also inhibited their growth to a minimal extent, having a weaker effect than the tested reference strain *L. plantarum* ATCC® 14917™. In addition, the clinical strain *L. rhamnosus* Clinical 3.1

Table IV
Antagonism of *Lactobacillaceae* strains towards strains of pathogenic species (n = 132).

Genus	Species	Strain	*P. aeruginosa* (n = 11)	*S. marcescens* (n = 10)	*E. coli* (n = 11)	*K. pneumoniae* (n = 12)	*P. mirabilis* (n = 10)	*S. maltophilia* (n = 10)	*A. baumannii* (n = 11)	*E. cloaceae* (n = 10)	*E. faecalis* (n = 15)	*S. aureus* (n = 17)	*S. epidermidis* (n = 13)
Lactobacillus	***L. acidophilus***	**ATCC® 4356™**	11.0 ± 1.1	11.0 ± 1.0	11.1 ± 1.0	10.6 ± 1.7	10.7 ± 0.8	12.8 ± 2.1	11.2 ± 1.1	11.0 ± 1.0	15.1 ± 1.9	10.3 ± 1.1	11.0 ± 1.5
	L. acidophilus	Probiotic 1	12.4 ± 1.2	10.0 ± 2.0	11.0 ± 1.6	10.2 ± 1.6	10.7 ± 1.1	14.1 ± 1.5	11.8 ± 1.9	10.0 ± 0.4	14.5 ± 1.7	10.1 ± 0.8	11.1 ± 1.8
	L. acidophilus	Probiotic 2.1	12.3 ± 1.1	10.4 ± 1.6	11.2 ± 1.4	10.3 ± 1.4	10.6 ± 0.8	13.8 ± 1.2	11.6 ± 1.3	10.2 ± 0.5	14.7 ± 1.1	10.2 ± 0.5	11.4 ± 1.2
	L. acidophilus	Probiotic 3	12.5 ± 1.3	10.6 ± 1.4	11.0 ± 1.0	10.5 ± 1.2	10.8 ± 1.2	14.3 ± 1.4	11.9 ± 1.2	10.3 ± 0.4	14.5 ± 1.2	10.1 ± 0.6	11.0 ± 1.1
	L. acidophilus	Probiotic 4	12.5 ± 2.5	12.0 ± 1.9	11.3 ± 2.0	10.1 ± 1.4	10.6 ± 0.8	14.4 ± 2.3	12.5 ± 2.9	10.0 ± 0.6	13.7 ± 1.6	10.5 ± 1.4	11.1 ± 1.8
	L. acidophilus	Probiotic 5	11.4 ± 0.7	11.0 ± 0.6	11.7 ± 2.0	10.5 ± 0.3	9.3 ± 0.5	12.2 ± 1.1	10.6 ± 0.5	9.1 ± 0.3*	16.4 ± .2.2	9.3 ± 0.6	11.5 ± 3.3
	L. acidophilus	Probiotic 6	12.0 ± 0.7	9.2 ± 0.5	9.3 ± 0.5	9.2 ± 0.4	9.0 ± 0	11.6 ± 0.5	9.0 ± 0*	9.0 ± 0*	12.4 ± 2.0	9.2 ± 0.4	9.8 ± 1.5
	L. acidophilus	Probiotic 7.1	11.6 ± 0.5	10.4 ± 0.4	11.7 ± 2.9	10.4 ± 0.9	9.0 ± 0	11.8 ± 0.8	10.6 ± 0.9	10.4 ± 0.9	14.6 ± 2.3	11.6 ± 1.3	15.5 ± 3.1*
	L. ultunensis	Probiotic 8	11.9 ± 0.9	11.0 ± 0.9	11.0 ± 1.7	9.8 ± 0.4	9.3 ± 0.5	12.6 ± 0.5	10.1 ± 0.2	9.9 ± 0.9	17.6 ± 3.1	9.6 ± 0.8	11.5 ± 0.5
	L. acidophilus	Clinical 1.1	11.6 ± 0.5	10.2 ± 0.5	11.8 ± 2.8	10.5 ± 0.8	9.2 ± 0.4	11.8 ± 0.8	10.7 ± 0.9	10.4 ± 0.9	14.8 ± 2.3	11.6 ± 1.3	15.5 ± 3.1*
	L. acidophilus	Clinical 2.1	12.0 ± 0.7	9.6 ± 0.4	9.3 ± 0.4	9.3 ± 0.4	9.0 ± 0	11.6 ± 0.5	10.1 ± 0.2	9.2 ± 0.2*	12.4 ± 2.0	9.3 ± 0.3	9.9 ± 1.5
	L. acidophilus	Clinical 14.1	11.3 ± 0.4	11.3 ± 0.7	11.7 ± 2.1	10.5 ± 0.4	9.3 ± 0.6	12.3 ± 1.1	10.6 ± 0.5	9.1 ± 0.2*	15.8 ± 2.4	9.3 ± 0.5	11.5 ± 3.3
	L. acidophilus	Clinical 16.2	11.5 ± 0.5	10.4 ± 0.5	11.6 ± 2.5	10.5 ± 0.5	9.3 ± 0.3	11.8 ± 0.8	10.8 ± 0.8	10.4 ± 0.5	14.6 ± 2.2	11.6 ± 1.3	9.9 ± 1.2
	L. acidophilus	Clinical 18.1	11.8 ± 0.8	11.2 ± 0.9	11.0 ± 1.6	9.9 ± 0.4	9.3 ± 0.5	12.6 ± 0.5	10.3 ± 0.4	9.9 ± 0.7	17.6 ± 3.1	9.6 ± 0.6	11.5 ± 0.6
	L. acidophilus	Clinical 20.3	12.0 ± 0.6	9.5 ± 0.4	9.6 ± 0.4	9.3 ± 0.4	9.0 ± 0	11.6 ± 0.5	9.6 ± 0.3	9.3 ± 0.2*	12.8 ± 2.0	9.2 ± 0.3	10.2 ± 1.3
	L. gasseri	Clinical 12.1	12.3 ± 0.4	10.0 ± 0.6	9.8 ± 0.5	9.5 ± 0.5	9.0 ± 0	11.6 ± 0.6	9.3 ± 0.2*	9.1 ± 0.1*	12.2 ± 1.6	9.4 ± 0.2	10.4 ± 1.1
	L. acidophilus	Yoghurt 1.1	12.4 ± 1.0	10.4 ± 1.2	11.2 ± 1.2	10.1 ± 1.1	10.7 ± 1.0	14.0 ± 1.2	11.6 ± 1.7	10.2 ± 0.3	14.6 ± 1.4	10.1 ± 0.8	11.2 ± 1.2
	L. acidophilus	Yoghurt 2.1	12.6 ± 0.8	10.6 ± 0.8	11.4 ± 0.9	10.3 ± 0.9	10.5 ± 0.7	14.2 ± 1.1	11.7 ± 0.9	10.3 ± 0.4	14.8 ± 1.8	10.2 ± 0.6	11.4 ± 1.3
Lacticaseibacillus	***L. rhamnosus***	**ATCC® 53103™**	15.0 ± 2.1	14.0 ± 2.3	13.0 ± 3.5	14 ± 2.3	11.3 ± 1.2	17.1 ± 1.6	14.4 ± 2.7	12.4 ± 2.3	21.0 ± 1.7	11.6 ± 1.8	13.7 ± 2.2
	L. rhamnosus	Probiotic 9	15.0 ± 2.9	14.0 ± 1.9	13.4 ± 4.0	13 ± 0.9	11.6 ± 2.2	16.9 ± 1.8	12.2 ± 2.3	11.7 ± 1.6	22.0 ± 3.1	12.1 ± 1.2	13.7 ± 3.8
	L. rhamnosus	Probiotic 10	14.9 ± 3.0	13.0 ± 2.1	13.0 ± 3.5	13.3 ± 0.6	11.2 ± 1.6	16.4 ± 1.7	12.4 ± 2.6	11.6 ± 1.2	23.3 ± 4.3	11.9 ± 1.2	13.2 ± 2.3
	L. rhamnosus	Probiotic 11	15.0 ± 2.7	13.0 ± 2.0	13.1 ± 2.8	13.0 ± 0.8	11.1 ± 1.2	15.0 ± 2.1	11.7 ± 2.4	11.1 ± 1.7	24.0 ± 4.3	11.3 ± 0.9	12.7 ± 2.0
	L. rhamnosus	Probiotic 12	15.6 ± 2.2	13.0 ± 2.1	12.7 ± 2.6	10.1 ± 1.2**	10.8 ± 0.6	16.5 ± 2.7	14.5 ± 2.1	10.7 ± 1.3	16.2 ± 1.7**	11.8 ± 2.4	13.3 ± 2.2
	L. rhamnosus	Probiotic 2.2	15.8 ± 2.9	13.0 ± 1.1	12.5 ± 2.0	12.8 ± 1.3	10.7 ± 0.9	16.8 ± 2.6	14.9 ± 2.5	11.3 ± 1.8	17.7 ± 2.2	12.0 ± 1.2	13.7 ± 2.9
	L. rhamnosus	Probiotic 13	14.0 ± 1.9	14.0 ± 2.0	12.8 ± 3.3	12.0 ± 1.2	11.3 ± 1.8	16.2 ± 2.9	11.7 ± 2.2	10.8 ± 1.6	21.0 ± 3.8	11.2 ± 1.1	15.7 ± 3.4
	L. rhamnosus	Probiotic 14	15.0 ± 2.9	13.0 ± 1.7	12.7 ± 3.2	13.0 ± 1.1	11.0 ± 1.2	15.4 ± 2.6	12.0 ± 2.4	12.5 ± 0.9	24.0 ± 4.2	11.6 ± 1.2	13.8 ± 3.3
	L. rhamnosus	Clinical 3.1	10.0 ± 1.5**	10.0 ± 0.5**	11.2 ± 1.8	9.7 ± 0.8**	10.7 ± 0.8	12.1 ± 2.1**	11.0 ± 1.9**	9.6 ± 1.1**	12.0 ± 2.5**	10.1 ± 1.2	10.7 ± 1.4**
	L. rhamnosus	Clinical 1645	14.6 ± 0.5	13.0 ± 0.5	12.3 ± 3.2	11.0 ± 0.7	9.0 ± 0**	14.8 ± 0.5	11.2 ± 0.4	12.3 ± 0.7	19.8 ± 4.9	10.0 ± 0.7	16.0 ± 2.2
	L. rhamnosus	Yoghurt 3	15.0 ± 2.8	13.2 ± 1.5	12.7 ± 3.0	13.0 ± 1.1	11.0 ± 1.2	15.5 ± 2.3	12.1 ± 2.2	12.5 ± 0.9	24.1 ± 3.8	11.5 ± 1.1	13.7 ± 3.2
	L. casei	Probiotic 7.2	14.5 ± 0.7	14.4 ± 1.6	12.6 ± 3.0	12 ± 1.2	11.2 ± 1.1	16.2 ± 2.7	11.6 ± 2.1	10.7 ± 1.4	21.4 ± 2.5	11.1 ± 1.0	15.5 ± 3.1
	L. casei	Yoghurt 2.2	15.0 ± 0.4	12.0 ± 0.7	12.0 ± 2.0	10.6 ± 0.9	9.3 ± 0.5	14.8 ± 0.4	11.3 ± 0.3	12.3 ± 1.3	18.9 ± 4.2	10.1 ± 0.8	11.8 ± 0.8
	L. casei	Yoghurt 1.2	15.3 ± 0.7	12.6 ± 0.5	12.2 ± 1.4	10.5 ± 0.7	9.5 ± 0.3	14.5 ± 0.2	11.5 ± 0.5	12.4 ± 1.2	19.4 ± 2.5	10.3 ± 0.9	12.2 ± 09
	L. casei	Yoghurt 4	14.8 ± 0.9	13.1 ± 0.4	12.6 ± 1.6	10.7 ± 0.8	9.4 ± 0.4	15.0 ± 0.6	11.6 ± 0.4	12.1 ± 1.1	18.5 ± 2.2	10.4 ± 0.6	11.9 ± 1.1
	L. casei	Clinical 9.2	12.8 ± 0.4	12.0 ± 0.5	12.0 ± 3.4	11.2 ± 1.3	9.3 ± 0.6	11.8 ± 0.5**	10.5 ± 0.3**	11.4 ± 1.7	15.6 ± 3.1**	9.5 ± 0.7	14.3 ± 1.9
	L. paracasei	Clinical 4	15.4 ± 0.9	12.1 ± 0.7	12.0 ± 1.6	10.6 ± 0.9	9.3 ± 0.3	14.7 ± 0.5	11.4 ± 0.5	12.4 ± 1.2	19.0 ± 4.1	10.1 ± 0.9	11.8 ± 0.9
	L. paracasei	Clinical 5.2	15.1 ± 1.0	11.8 ± 0.6	11.9 ± 1.7	10.8 ± 0.7	9.5 ± 0.4	14.5 ± 0.8	11.6 ± 0.6	11.5 ± 1.6	19.4 ± 2.2	10.3 ± 0.7	12.2 ± 1.2
Lactiplantibacillus	***L. plantarum***	**ATCC® 14917™**	16.0 ± 4.3	14.0 ± 1.9	13.7 ± 3.5	14.0 ± 2.9	11.1 ± 1.1	18.6 ± 2.1	15.1 ± 3.1	13.6 ± 2.0	19.0 ± 2.2	13.2 ± 1.6	15.8 ± 1.5
	L. plantarum	Probiotic 15	15.4 ± 2.1	12.0 ± 1.3	12.8 ± 3.1	13.1 ± 2.1	12.2 ± 1.2	19.0 ± 5.4	14.5 ± 2.5	12.5 ± 1.6	18.0 ± 2.1	12.5 ± 2.1	14.0 ± 1.6
	L. plantarum	Probiotic 16	17.0 ± 3.3	14.0 ± 1.5	14.3 ± 4.3	13.0 ± 2.4	11.4 ± 2.1	18.9 ± 2.7	14.7 ± 2.6	13.9 ± 2.5	16.0 ± 3.1	14.2 ± 3.6	15.8 ± 2.3
	L. plantarum	Clinical 1876	18.0 ± 1.2	13.0 ± 0.5	13.3 ± 4.1	12.8 ± 1.6	10.0 ± 0	16.4 ± 0.6	13.0 ± 1.2	14.3 ± 0.7	21.4 ± 3.5	11.4 ± 0.9	17.8 ± 2.4
	L. plantarum	Clinical 1844	9.6 ± 0,5***	9.0 ± 0***	10.7 ± 2.0	9.0 ± 0***	9.0 ± 0***	9.4 ± 0.5***	9.0 ± 0***	9.4 ± 0.7***	10.8 ± 0.4***	9.0 ± 0***	10.0 ± 0.8***
	L. plantarum	Clinical 5.1	15.8 ± 1.1	13.1 ± 1.1	13.3 ± 3.0	13.6 ± 2.2	11.5 ± 1.1	18.8 ± 2.2	14.8 ± 2.7	12.9 ± 1.5	18.4 ± 2.5	12.7 ± 1.7	14.6 ± 1.2

The mean growth inhibition zone (mm) ± standard deviation for isolates of the same species. The test was performed in triplicate.
* – statistically significant differences ($p < 0.05$) compared to the reference strain *L. acidophilus* ATCC® 4356™
** – statistically significant differences ($p < 0.05$) compared to the reference strain *L. rhamnosus* ATCC® 53103™
*** – statistically significant differences ($p < 0.05$) compared to the reference strain *L. plantarum* ATCC® 14917™

– no growth inhibition (< 10 mm)
– weak growth inhibition (10–15 mm)
– strong growth inhibition (16–22 mm)
– very strong growth inhibition (> 23 mm)

Table V
Survival in a low pH, in presence of bile salt, and auto-aggregation *Lactobacillaceae* strains.

Genus	Species	Strain	Survivability in an acid environment							Growth inhibition coefficient	Auto-aggregation
			Control	pH 2.0				pH 3.0		0.3% bile salts	24 h
				1 h		2 h		2 h			
			log CFU/ml	log CFU/ml	%	log CFU/ml	%	log CFU/ml	%		
Lactobacillus	***L. acidophilus***	**ATCC® 4356™**	7.05 ± 0.1	0.59 ± 0.11	< 0.01	no growth	nd	6.57 ± 1.00	33.6^{ab}	0.28 ± 0.09^{abcd}	67.85% ± 4.70^{cdefg}
	L. acidophilus	Probiotic 1	6.40 ± 0.52	4.44 ± 1.75	6.4	2.95 ± 2.62	0.6	6.24 ± 0.54	69.5^{cd}	0.20 ± 0.04^{bcdef}	59.21% ± 1.83^{abc}
	L. acidophilus	Probiotic 2.1	6.18 ± 0.40	5.20 ± 0.78	13.9	1.97 ± 2.31	0.4	6.09 ± 0.36	81.9^{cde}	0.22 ± 0.03^{bcdef}	79.05% ± 6.48^{h}
	L. acidophilus	Probiotic 3	8.03 ± 0.35	3.70 ± 0.33	< 0.01	0.79 ± 1.36	< 0.01	7.96 ± 0.31	86.4^{cde}	0.32 ± 0.07^{abc}	49.73% ± 3.38^{a}
	L. acidophilus	Probiotic 4	6.39 ± 1.19	2.24 ± 1.27	0.3	0.67 ± 0.65	< 0.01	6.33 ± 1.19	87.4^{de}	0.41 ± 0.09^{a}	60.95% ± 3.36^{bcd}
	L. acidophilus	Probiotic 5	6.15 ± 1.12	4.36 ± 1.11	2.6	2.56 ± 0.90	0.1	6.01 ± 1.09	72.7^{cd}	0.16 ± 0.07^{cdef}	65.58% ± 0.89^{cdef}
	L. acidophilus	Probiotic 6	7.05 ± 1.56	3.77 ± 1.55	13.3	1.04 ± 0.92	< 0.01	7.03 ± 1.53	95.9^{e}	0.28 ± 0.05^{abcd}	82.78% ± 0.72^{j}
	L. acidophilus	Probiotic 7.1	6.16 ± 0.71	1.61 ± 0.26	< 0.01	1.18 ± 0.14	< 0.01	6.04 ± 0.72	75.9^{cd}	0.37 ± 0.01^{ab}	72.73% ± 3.92^{efgh}
	L. ultunensis	Probiotic 8	7.04 ± 0.15	4.38 ± 0.78	0.6	0.39 ± 0.26	< 0.01	6.94 ± 0.16	80.4^{cde}	0.25 ± 0.01^{abcde}	75.04% ± 1.29^{fgh}
	L. acidophilus	Clinical 1.1	8.11 ± 0.16	1.31 ± 1.87	< 0.01	no growth	nd	7.99 ± 0.20	77.0^{cde}	0.06 ± 0.03^{f}	63.56% ± 5.25^{cde}
	L. acidophilus	Clinical 2.1	7.83 ± 0.05	0.06 ± 0.10	< 0.01	0.77 ± 0.008	< 0.01	7.75 ± 0.04	82.7^{cde}	0.06 ± 0.02^{f}	71.04% ± 1.96^{defgh}
	L. acidophilus	Clinical 14.1	7.41 ± 0.03	2.91 ± 0.60	< 0.01	no growth	nd	7.29 ± 0.01	75.3^{cd}	0.12 ± 0.05^{def}	52.34% ± 1.61^{ab}
	L. acidophilus	Clinical 16.2	6.01 ± 0.97	3.34 ± 1.03	0.6	1.00 ± 1.73	0.1	5.90 ± 0.99	76.9^{cde}	0.14 ± 0.08^{cdef}	67.73% ± 3.39^{cdefg}
	L. acidophilus	Clinical 18.1	6.94 ± 0.49	0.53 ± 0.50	< 0.01	0.76 ± 0.85	< 0.01	6.62 ± 0.55	47.6^{b}	0.08 ± 0.07^{ef}	80.77% ± 1.24^{i}
	L. acidophilus	Clinical 20.3	7.21 ± 0.82	3.89 ± 1.80	1.6	no growth	nd	6.61 ± 0.65	26.3^{a}	0.18 ± 0.09^{cdef}	69.11% ± 3.12^{cdefgh}
	L. gasseri	Clinical 12.1	7.02 ± 0.20	5.53 ± 0.50	3.8	5.09 ± 0.15	1.2	7.00 ± 0.16	96.4^{e}	0.12 ± 0.10^{def}	77.04% ± 6.28^{gh}
	L. acidophilus	Yoghurt 1.1	7.37 ± 0.97	4.52 ± 1.42	13.4	1.77 ± 2.04	0.1	7.26 ± 1.01	78.7^{cde}	0.07 ± 0.01^{ef}	51.78% ± 2.40^{ab}
	L. acidophilus	Yoghurt 2.1	6.24 ± 0.54	3.33 ± 2.49	3.8	1.46 ± 1.40	< 0.01	6.07 ± 0.51	67.4^{c}	0.07 ± 0.02^{ef}	85.17% ± 0.15^{k}
Lacticaseibacillus	***L. rhamnosus***	**ATCC® 53103™**	5.65 ± 0.34	No growth	nd	No growth	nd	5.56 ± 0.30	81.3^{defgh}	0.15 ± 0.05^{abcd}	81.54% ± 6.25^{a}
	L. rhamnosus	Probiotic 9	7.23 ± 0.33	1.91 ± 0.76	< 0.01	0.36 ± 0.62	< 0.01	7.20 ± 0.35	94.8^{h}	0.17 ± 0.07^{abcd}	86.93% ± 3.89^{a}
	L. rhamnosus	Probiotic 10	7.76 ± 0.14	No growth	nd	No growth	nd	7.64 ± 0.12	76.8^{defgh}	0.33 ± 0.10^{def}	87.31% ± 5.28^{a}
	L. rhamnosus	Probiotic 11	7.21 ± 0.36	0.61 ± 0.81	< 0.01	No growth	< 0.01	7.12 ± 0.37	81.7^{fgh}	0.18 ± 0.07^{abcd}	66.08% ± 1.36^{b}
	L. rhamnosus	Probiotic 12	7.11 ± 0.58	0.77 ± 0.68	< 0.01	0.10 ± 0.17	< 0.01	7.06 ± 0.61	89.0^{fgh}	0.22 ± 0.02^{abcd}	47.92% ± 2.73^{def}
	L. rhamnosus	Probiotic 2.2	7.84 ± 0.05	2.14 ± 0.59	< 0.01	0.10 ± 0.17	< 0.01	7.69 ± 0.03	70.9^{cdef}	0.48 ± 0.07^{f}	63.49% ± 2.05^{bc}
	L. rhamnosus	Probiotic 13	7.94 ± 0.09	0.75 ± 0.65	< 0.01	No growth	nd	7.81 ± 0.11	73.7^{defg}	0.21 ± 0.01^{abcd}	59.84% ± 2.50^{bc}
	L. rhamnosus	Probiotic 14	7.70 ± 0.11	1.71 ± 0.60	< 0.01	0.42 ± 0.49	< 0.01	7.62 ± 0.10	83.1^{defgh}	0.32 ± 0.09^{cdef}	86.56% ± 1.58^{a}
	L. rhamnosus	Clinical 3.1	7.48 ± 0.22	0.33 ± 0.58	< 0.01	No growth	nd	7.44 ± 0.24	90.9^{defgh}	0.21 ± 0.05^{abcd}	64.70% ± 4.68^{b}
	L. rhamnosus	Clinical 1645	7.44 ± 0.14	1.66 ± 0.03	< 0.01	0.85 ± 0.93	< 0.01	7.40 ± 0.16	89.9^{gh}	0.24 ± 0.09^{bcde}	57.14% ± 1.99^{bcd}
	L. rhamnosus	Yoghurt 3	7.32 ± 0.06	2.77 ± 0.15	< 0.01	No growth	nd	7.24 ± 0.10	83.4^{fgh}	0.14 ± 0.01^{abc}	89.36% ± 3.53^{g}
	L. casei	Probiotic 7.2	7.73 ± 0.04	1.61 ± 1.46	< 0.01	0.18 ± 0.31	< 0.01	7.68 ± 0.05	88.0^{b}	0.12 ± 0.08^{ab}	47.29% ± 2.78^{def}
	L. casei	Yoghurt 2.2	7.95 ± 0.20	1.05 ± 1.82	< 0.01	No growth	nd	7.67 ± 0.17	52.7^{defgh}	0.17 ± 0.04^{abcd}	47.21% ± 1.25^{def}
	L. casei	Yoghurt 1.2	7.97 ± 0.26	1.98 ± 1.85	< 0.01	0.57 ± 0.68	< 0.01	7.78 ± 0.25	64.8^{efgh}	0.32 ± 0.08^{cdef}	42.15% ± 4.76^{ef}
	L. casei	Yoghurt 4	7.52 ± 0.61	1.09 ± 0.67	< 0.01	No growth	< 0.01	7.45 ± 0.63	86.4^{bc}	0.14 ± 0.03^{abcd}	39.88% ± 3.88^{f}
	L. casei	Clinical 9.2	7.27 ± 0.44	5.09 ± 1.37	2.1	1.77 ± 1.56	< 0.01	6.97 ± 0.38	50.4^{a}	0.05 ± 0.06^{a}	77.29% ± 3.43^{a}
	L. paracasei	Clinical 4	7.42 ± 0.39	No growth	nd	No growth	nd	5.08 ± 0.52	0.7^{bcde}	0.41 ± 0.05^{ef}	65.16% ± 2.57^{b}
	L. paracasei	Clinical 5.2	8.12 ± 0.75	No growth	nd	No growth	nd	7.95 ± 0.76	67.7^{bcd}	0.28 ± 0.00^{bcde}	52.85% ± 3.85^{cde}
Lactiplanti-bacillus	***L. plantarum***	**ATCC® 14917™**	6.96 ± 1.22	1.00 ± 1.73	< 0.01	No growth	nd	6.91 ± 1.18	91.0^{a}	0.14 ± 0.04^{a}	59.80% ± 1.63^{a}
	L. plantarum	Probiotic 15	6.79 ± 0.18	1.65 ± 0.13	< 0.01	0.26 ± 0.24	< 0.01	6.72 ± 0.14	85.7^{a}	0.14 ± 0.02^{a}	37.23% ± 1.75^{c}
	L. plantarum	Probiotic 16	7.87 ± 0.30	0.38 ± 0.66	< 0.01	0.20 ± 0.35	< 0.01	7.85 ± 0.29	93.9^{a}	0.24 ± 0.05^{a}	52.77% ± 3.41^{ab}
	L. plantarum	Clinical 1876	7.89 ± 0.31	2.17 ± 0.28	< 0.01	0.85 ± 1.47	< 0.01	7.84 ± 0.32	89.5^{a}	0.13 ± 0.02^{a}	57.17% ± 2.46^{ab}
	L. plantarum	Clinical 1844	7.64 ± 0.36	2.67 ± 0.16	< 0.01	No growth	nd	7.44 ± 0.40	62.1^{b}	0.42 ± 0.05^{b}	39.86% ± 5.04^{c}
	L. plantarum	Clinical 5.1	7.62 ± 0.14	0.10 ± 0.17	< 0.01	0.10 ± 0.17	< 0.01	7.60 ± 0.15	95.2^{a}	0.15 ± 0.08^{a}	50.70% ± 2.16^{b}

Mean value ± standard deviation.
Letter symbols in the same column for a given microbial family indicate statistically significant differences ($p < 0.05$).
nd – no data

Table VI
Antibiotic susceptibility of *Lactobacillaceae* strains.

Genus	Species	Strain	Antibiotic MIC mg/l									
			VA	E	TET	CHL	AMP	CC	CTX	CAZ	CRO	IMP
Lactobacillus	***L. acidophilus***	**ATCC® 4356™**	0.5	0.5	4	4	<0.25	1	<0.5	<0.5	4	4
	L. acidophilus	Probiotic 1	1	0.5	4	4	0.5	4	<0.5	4	1	**8**
	L. acidophilus	Probiotic 2.1	0.5	0.5	2	4	<0.25	4	<0.5	1	1	1
	L. acidophilus	Probiotic 3	0.5	<0.06	0.25	4	0.5	0.125	<0.5	2	1	0.125
	L. acidophilus	Probiotic 4	0.5	0.5	0.5	4	<0.25	2	<0.5	1	8	2
	L. acidophilus	Probiotic 5	<0.25	0.25	0.25	2	<0.25	1	<0.5	2	<0.5	<0.06
	L. acidophilus	Probiotic 6	1	0.5	1	4	0.5	4	<0.5	1	2	4
	L. acidophilus	Probiotic 7.1	0.5	0.5	1	2	<0.25	4	2	8	4	0.125
	L. ultunensis	Probiotic 8	0.5	0.5	2	4	<0.25	4	4	4	8	2
	L. acidophilus	Clinical 1.1	1	0.25	2	2	<0.25	4	<0.5	1	<0.5	0.125
	L. acidophilus	Clinical 2.1	1	0.5	0.5	2	1	<0.06	1	128	2	1
	L. acidophilus	Clinical 14.1	1	0.5	2	4	<0.25	1	<0.5	2	<0.5	0.5
	L. acidophilus	Clinical 16.2	1	1	4	4	1	1	1	8	2	**8**
	L. acidophilus	Clinical 18.1	1	1	2	4	1	2	<0.5	8	1	**8**
	L. acidophilus	Clinical 20.3	0.5	1	4	4	0.5	0.5	0.5	8	2	1
	L. gasseri	Clinical 12.1	0.5	1	4	4	0.5	1	1	2	2	0.5
	L. acidophilus	Yoghurt 1.1	1	1	4	4	0.5	4	<0.5	4	1	**8**
	L. acidophilus	Yoghurt 2.1	0.5	1	2	4	<0.25	4	<0.5	1	1	1
Lacticaseibacillus	***L. rhamnosus***	**ATCC® 53103™**	**>256**	0.5	4	4	1	2	2	16	16	**>64**
	L. rhamnosus	Probiotic 9	**>256**	0.5	2	4	0.5	2	4	2	16	**>64**
	L. rhamnosus	Probiotic 10	**>256**	0.5	1	4	1	0.25	2	8	8	**32**
	L. rhamnosus	Probiotic 11	**>256**	0.5	2	4	1	1	2	4	16	**>64**
	L. rhamnosus	Probiotic 12	**>256**	**>256**	1	4	0.5	**>64**	8	32	32	**16**
	L. rhamnosus	Probiotic 2.2	**>256**	1	4	4	0.5	2	4	8	32	**>64**
	L. rhamnosus	Probiotic 13	**>256**	1	1	4	0.5	1	2	8	16	**64**
	L. rhamnosus	Probiotic 14	**>256**	1	4	4	0.5	1	4	4	16	**>64**
	L. rhamnosus	Clinical 3.1	**>256**	1	2	4	1	1	4	8	32	**>64**
	L. rhamnosus	Clinical 1645	**>256**	1	4	4	0.5	2	2	16	16	**>64**
	L. rhamnosus	Yoghurt 3	**>256**	1	1	4	0.5	1	4	8	32	**32**
	L. casei	Probiotic 7.2	**>256**	0.5	0.5	4	0.5	<0.06	1	8	16	**8**
	L. casei	Yoghurt 2.2	**>256**	0.5	1	4	0.5	0.125	1	8	16	**8**
	L. casei	Yoghurt 1.2	**>256**	0.5	0.5	4	0.5	0.125	1	8	16	**8**
	L. casei	Yoghurt 4	**>256**	0.5	1	4	0.5	0.125	1	8	16	**16**
	L. casei	Clinical 9.2	1	0.5	4	4	1	4	2	16	2	**>64**
	L. paracasei	Clinical 4	**4**	**16**	4	2	0.5	4	<0.5	2	1	**>64**
	L. paracasei	Clinical 5.2	**4**	**>256**	4	4	0.5	4	<0.5	2	<0.5	**>64**
Lactiplantibacillus	***L. plantarum***	**ATCC® 14917™**	**>256**	1	32	8	<0.25	0.5	<0.5	1	1	**>64**
	L. plantarum	Probiotic 15	**>256**	1	16	4	<0.25	0.125	<0.5	<0.5	<0.5	**16**
	L. plantarum	Probiotic 16	**>256**	1	32	4	<0.25	4	<0.5	2	<0.5	**>64**
	L. plantarum	Clinical 1876	**>256**	**2**	32	8	<0.25	4	128	256	128	**>64**
	L. plantarum	Clinical 1844	1	1	2	8	1	0.125	1	8	2	2
	L. plantarum	Clinical 5.1	**>256**	1	16	8	<0.25	<0.06	2	2	2	2

Strains resistant to a given compound were **darkened** (in accordance with EFSA (EFSA 2018) and EUCAST (EUCAST 2021) guidelines).
VA – vancomycin, GEN – gentamycin, E – erythromycin, K – kanamycin, S – streptomycin, TET – tetracycline, CHL – chloramphenicol, IMP – imipenem, AMP – ampicilin, CC – clindamycin, CTX – cefotaxime, CAZ – ceftazidime, CRO – ceftriaxone

also showed a weak antagonistic effect compared to the reference strain *L. rhamnosus* ATCC® 53103™. According to Marchwińska and Gwiazdowska (2021), not all lactic acid bacteria inhibit the growth of pathogenic bacteria; 87 out of 376 isolates did not show such properties. Due to the lack of inhibition of the growth of pathogenic microorganisms, these strains do not have all the properties normally associated with probiotic strains.

Lactic acid bacteria have antagonistic effects not only on pathogenic bacteria but also on closely related bacteria. This effect is probably exerted by the bacteriocin often produced by LAB. The current research found that *Lactobacillaceae* isolated from humans inhibited potentially probiotic bacteria from dietary supplements or food products. The phenomenon of self-antagonism was also described by Pellegrino et al. (2019); *Enterococcus mundtii* CRL 1656 strain inhibited the growth of *Enterococcus hirae* 7–3 and *Weissella cibaria* CRL 1833. Therefore, it is worth considering whether probiotic products containing many different species of lactobacilli do not mutually inhibit the growth of these strains after ingestion.

To consider microorganisms as probiotic, it is also necessary to examine their potential for survival in the gastrointestinal environment; this is why the survivability of strains is determined at low pH, as well as in 0.3% bile salt since this concentration is considered to be present in the human gastrointestinal tract (Liu et al. 2021). Most *Lactobacillaceae* strains tolerated pH 3.0; the survival rate ranged from 30% to almost 100%. These results are consistent with the data presented by other authors. In most reports, *Lactobacillaceae* tolerate an acidic environment well (pH 3.0), and significant growth inhibition in a pH 2.0 was observed after 1 h of incubation (Cizeikiene and Jagelaviciute 2021; Liu et al. 2021). It is consistent with the results obtained in this study. However, Śliżewska et al. (2021) showed that *L. paracasei* ŁOCK 1091, *L. pentosus* ŁOCK 1094, *L. plantarum* ŁOCK 0860, *L. reuteri* ŁOCK 1092, and *L. rhamnosus* ŁOCK 1087 well survived in pH 2.0 (about 90%), even after 4 hours of incubation.

All 42 tested strains had a growth inhibition coefficient below 0.5 (Table V), indicating good tolerance to bile salts (Liu et al. 2021). Marchwińska and Gwiazdowska (2021) stated that the content of bile salt caused a decrease in bacterial survival; however, for many of the strains studied by these authors, no statistically significant differences in growth were observed in the presence of 0.5 and 1% bile salt. In turn, in the studies carried out by Kowalska et al. (2020) and Śliżewska et al. (2021), most of the tested strains survived in an environment with a concentration of up to 2% bile salt.

The ability to auto-aggregate lactobacilli is an important feature that creates the barrier and hinders pathogenic strains' adhesion (Klopper et al. 2018). The value of auto-aggregation depends mainly on the incubation time (Piwat et al. 2015;) and on the strain tested (Zawistowska-Rojek et al. 2022b). In the current study, the auto-aggregation values ranged from 37% to 89%, depending on the strain used. The highest values were obtained for *Lactobacillus* strains, 50–85%. Cizeikiene and Jagelaviciute (2021) demonstrated that the auto-aggregation of *L. acidophilus* strains at 24 h was 87.5%, while for *L. gasseri* – 74.2%. A similar range of values in the current study was obtained *Lacticaseibacillus* strains – 40–89%. The differences in the value of auto-aggregation of individual strains are pretty significant, but similar phenomena could also be found in other works. For *Lacticaseibacillus* strains, some authors observed auto-aggregation values of 55–57% (Śliżewska et al. 2021), 57–68% (Cizeikiene and Jagelaviciute 2021), or even 93–98% (Kowalska et al. 2020). It confirms that this property is strain-dependent. In turn, *Lactiplantibacillus* strains in the current study had the lowest value of auto-aggregation, ranging from 40–60%. In other studies, the authors also obtained quite divergent results; for strains *L. plantarum,* Liu et al. (2022) observed auto-aggregation in the range of 45–90%, Cizeikiene and Jagelaviciute (2021) – 61.0–71.1%, and Śliżewska et al. (2021) – 95.5%.

Lactobacillaceae strains are part of probiotic preparations that are recommended for consumption, especially during antibiotic therapy and immediately after its completion, to prevent the development of antibiotic associated diarrhoea (Markowiak and Śliżewska 2017; Zawistowska-Rojek and Tyski 2022). The susceptibility *Lactobacillaceae* strains to antibiotics and chemotherapeutic agents is a crucial criterion when introducing microorganisms into the food chain. Numerous data indicate a variable susceptibility of this group of bacteria to antimicrobial agents (Stefańska et al. 2021). Lactic acid bacteria can be a potential reservoir of antibiotic resistance genes that can be transferred to other bacteria by mobile genetic elements (e.g., plasmids and transposons). Therefore, it is essential to determine the drug resistance profile of potentially probiotic strains that may be used in food products (Zawistowska-Rojek and Tyski 2018; Stefańska et al. 2021). All bacterial strains tested in the present study were susceptible to tetracycline, chloramphenicol, and ampicillin, which is consistent with studies conducted by others (Rozman et al. 2020; Nunziata et al. 2022). There are also reports on the strains resistant to these antibiotics, e.g., isolates from animals or fermented milk drinks, which were resistant to ampicillin (Dec et al. 2018; Yang and Yu et al. 2019). Moreover, some lactic acid bacteria often derived from fermented products could be resistant to chloramphenicol (Yang and Yu et al. 2019). Resistance to this antibiotic, which has been evidenced in many

Lactobacillaceae species, is usually associated with the presence of the *cat* gene (Stefańska et al. 2021). This gene is often located on plasmids, potentially allowing the transfer of the resistance gene from lactic acid bacteria to pathogenic bacteria (Nunziata et al. 2022). In turn, resistance to tetracycline, usually located in the *tetK, tetW*, or *tetM* genes, could also be passed on to other strains, since these genes are located on mobile genetic elements (Nunziata et al. 2022). Most LABs are also sensitive to clindamycin (Nunziata et al. 2022). In the current study, only one strain of *L. rhamnosus* isolated from the probiotic product (Probiotic 12) was resistant to this antibiotic, and resistance to erythromycin was also observed. Erythromycin resistance was also found in two clinical strains of *L. paracasei* and two strains of *L. plantarum*. Other researchers have also individual cases of resistance to the above antibiotics (Stefańska et al. 2021).

Lactic acid bacteria present a tremendous variation in their sensitivity to vancomycin. *Lacticaseibacillus* and *Lactiplantibacillus* strains had naturally occurring resistance to this antibiotic (Zheng et al. 2017); there are no requirements for these groups in the guidelines presented by EFSA (2018). However, for four tested strains (one *L. casei,* two *L. paracasei* and one *L. plantarum*), significantly lower MIC values were obtained compared to the remaining strains (1–4 mg/l); this may suggest the sensitivity of these clinical isolates to the antibiotic mentioned above. In turn, *Lactobacillus* strains are susceptible to this compound (Goldstein et al. 2015), which was also confirmed in the above analysis. The MIC values of three antibiotics belonging to the third generation of cephalosporins – cefotaxime, ceftazidime, and ceftriaxone were also determined. For most of the tested strains, low MIC values were observed for cephalosporins, except for one clinical isolate of *L. plantarum* 1876, for which the MIC values of the above antibiotics were 128–256 mg/l; it may suggest resistance of the above strain to this group of antibiotics. It was also noted that second-generation cephalosporins showed higher activity against *Lactobacillaceae* than third-generation cephalosporins (Salminen et al. 2006). On the other hand, some *Lactobacillaceae* can be susceptible to cephalosporins (Álvarez-Cisneros and Ponce-Alquicira 2018). It may be assumed that the susceptibility to this group of antibiotics is closely related to the strain. Similar conclusions can be drawn for imipenem. Both in the current study and in the available literature data is a considerable discrepancy between the MIC of imipenem values among lactobacilli (Goldstein et al. 2015). In addition, resistance to streptomycin, kanamycin, gentamycin, and ciprofloxacin (Yang i Yu 2019) is also commonly observed among lactobacilli, but usually, it is not transferred to other microorganisms (Liu et al. 2022). This resistance type has therapeutic and prophylactic benefits when consuming probiotics during antibiotic therapy (Liu et al. 2022).

Conclusions

The presented study compared some of the properties of strains isolated from probiotic products mainly dietary supplements, fermented foods, and medical devices, as well as bacteria belonging to probiotic species of human origin.

Thanks to the strain comparison performed by the PFGE method, it can be noted that some manufacturers use the same strains in their products. Moreover, a very high genetic similarity can be observed between the strains derived from probiotic products and those isolated from humans. The tested strains well tolerated low pH and the bile salt environment. They showed auto-aggregation and antagonized pathogenic microorganisms to varying degrees depending on the tested strain. Most strains displayed probiotic properties comparable to those of the reference strains (*L. acidophilus* ATCC® 4356™, *L. rhamnosus* ATCC® 53103™, and *L. plantarum* ATCC® 14917™), except for the strain *L. plantarum* Clinical 1844, whose potential probiotic properties were much weaker. An important aspect of the use of probiotics is also their safety profile. Probiotic bacteria should not have antibiotic resistance genes on mobile genetic elements, e.g., plasmids or transposons. Among lactic acid bacteria, genes resistant to tetracycline, erythromycin, or chloramphenicol are most often located on mobile genetic elements. Among the tested isolates, there were no strains resistant to tetracycline or chloramphenicol, while several tested strains were resistant to erythromycin.

When searching for new strains with probiotic properties, it is worth taking a closer look at the clinical isolates of *L. acidophilus* marked with numbers 1.1, 18.1, and 20.3 in the subsequent studies. These strains are not closely related to known strains derived from probiotic products; they survive in the gastrointestinal tract, are antagonistic to pathogenic species, and are susceptible to antibiotics such as erythromycin, tetracycline or chloramphenicol.

ORCID
Anna Zawistowska-Rojek https://orcid.org/0000-0002-6797-3240
Agnieszka Kociszewska https://orcid.org/0000-0002-2055-2588
Tomasz Zaręba https://orcid.org/0000-0003-2000-8248
Stefan Tyski https://orcid.org/0000-0003-3352-038X

Conflict of interest
The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Álvarez-Cisneros YM, Ponce-Alquicira E. Antibiotic resistance in lactic acid bacteria. In: Kumar Y, editor. Antimicrobial Resistance – A Global Threat. London (UK): IntechOpen; 2018. https://doi.org/10.5772/intechopen.80624

Asadi A, Lohrasbi V, Abdi M, Mirkalantari S, Esghaei M, Kashanian M, Oshaghi M, Talebi M. The probiotic properties and potential of vaginal *Lactobacillus* spp. isolated from healthy women against some vaginal pathogens. Lett Appl Microbiol. 2022 May;74(5):752–764. https://doi.org/10.1111/lam.13660

Binda S, Hill C, Johansen E, Obis D, Pot B, Sanders ME, Tremblay A, Ouwehand AC. Criteria to qualify microorganisms as "probiotic" in foods and dietary supplements. Front Microbiol. 2020 Jul 24; 11:1662. https://doi.org/10.3389/fmicb.2020.01662

Blaabjerg S, Artzi DM, Aabenhus R. Probiotics for the prevention of antibiotic-associated diarrhea in outpatients – A systematic review and meta-analysis. Antibiotics (Basel). 2017 Oct 12;6(4):21. https://doi.org/10.3390/antibiotics6040021

Cizeikiene D, Jagelaviciute J. Investigation of antibacterial activity and probiotic properties of strains belonging to *Lactobacillus* and *Bifidobacterium* genera for their potential application in functional food and feed products. Probiotics Antimicrob Proteins. 2021 Oct;13(5):1387–1403. https://doi.org/10.1007/s12602-021-09777-5

Colautti A, Arnoldi M, Comi G, Iacumin L. Antibiotic resistance and virulence factors in lactobacilli: something to carefully consider. Food Microbiol. 2022 May;103:103934. https://doi.org/10.1016/j.fm.2021.103934

Darbandi A, Asadi A, Mahdizade Ari M, Ohadi E, Talebi M, Halaj Zadeh M, Darb Emamie A, Ghanavati R, Kakanj M. Bacteriocins: Properties and potential use as antimicrobials. J Clin Lab Anal. 2022 Jan;36(1):e24093. https://doi.org/10.1002/jcla.24093

Dec M, Nowaczek A, Stępień-Pyśniak D, Wawrzykowski J, Urban-Chmiel R. Identification and antibiotic susceptibility of lactobacilli isolated from turkeys. BMC Microbiol. 2018 Oct 29;18(1):168. https://doi.org/10.1186/s12866-018-1269-6

Domingos-Lopes MFP, Stanton C, Ross PR, Dapkevicius MLE, Silva CCG. Genetic diversity, safety and technological characterization of lactic acid bacteria isolated from artisanal Pico cheese. Food Microbiol. 2017 May;63:178–190. https://doi.org/10.1016/j.fm.2016.11.014

EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0. Basel (Switzerland): The European Committee on Antimicrobial Susceptibility Testing; 2021 [cited 13 March 2022]. Available from https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf

EFSA (European Food Safety Authority). Guidance on the characterisation of microorganisms used as feed additives or as production organisms. EFSA J. 2018;16(3):5206. https://doi.org/10.2903/j.efsa.2018.5206

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). Guidelines for the evaluation of probiotics in food. Rome (Italy): Food and Agriculture Organization of the United Nations; 2002 [cited 2022 Mar 13]. p. 1–11. Available from https://4cau4jsaler1zglkq3wnmje1-wpengine.netdna-ssl.com/wp-content/uploads/2019/04/probiotic_guidelines.pdf

Goldstein EJ, Tyrrell KL, Citron DM. *Lactobacillus* species: Taxonomic complexity and controversial susceptibilities. Clin Infect Dis. 2015 May 15;60(Suppl_2):S98–S107. https://doi.org/10.1093/cid/civ072

Gosiewski T, Chmielarczyk A, Strus M, Brzychczy-Włoch M, Heczko PB. The application of genetics methods to differentiation of three *Lactobacillus* species of human origin. Ann Microbiol. 2012 Dec; 62:1437–1445. https://doi.org/10.1007/s13213-011-0395-2

Hernández-González JC, Martínez-Tapia A, Lazcano-Hernández G, García-Pérez BE, Castrejón-Jiménez NS. Bacteriocins from lactic acid bacteria. A powerful alternative as antimicrobials, probiotics, and immunomodulators in veterinary medicine. Animals (Basel). 2021 Apr 1;11(4):979. https://doi.org/10.3390/ani11040979

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014 Aug; 11:506–514. https://doi.org/10.1038/nrgastro.2014.66

Hojjati M, Behabahani BA, Falah F. Aggregation, adherence, anti-adhesion and antagonistic activity properties relating to surface charge of probiotic *Lactobacillus brevis* gp104 against *Staphylococcus aureus*. Microb Pathog. 2020 Oct;147:104420. https://doi.org/10.1016/j.micpath.2020.104420

Kang W, Pan L, Peng C, Dong L, Cao S, Cheng H, Wang Y, Zhang C, Gu R, Wang J, et al. Isolation and characterization of lactic acid bacteria from human milk. J Dairy Sci. 2020 Nov;103(11):9980–9991. https://doi.org/10.3168/jds.2020-18704

Kerry GR, Patra JK, Gouda S, Park Y, Shin HS, Das G. Benefaction of probiotics for human health: A review. J Food Drug Anal. 2018 Jul;26(3):927–939. https://doi.org/10.1016/j.jfda.2018.01.002

Klopper KB, Deane SM, Dicks LMT. Aciduric strains of *Lactobacillus reuteri* and *Lactobacillus rhamnosus*, isolated from human feces, have strong adhesion and aggregation properties. Probiotics Antimicrob Proteins. 2018 Mar;10(1):89–97. https://doi.org/10.1007/s12602-017-9307-5

Kos B, Susković J, Vuković S, Simpraga M, Frece J, Matosić S. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. J Appl Microbiol. 2003;94(6):981–987. https://doi.org/10.1046/j.1365-2672.2003.01915.x

Kowalska JD, Nowak A, Śliżewska K, Stańczyk M, Łukasiak M, Dastych J. Anti-salmonella potential of new *Lactobacillus* strains with the application in the poultry industry. Pol J Microbiol. 2020 Sep; 69(1):5–18. https://doi.org/10.33073/pjm-2020-001

Lashani E, Davoodabadi A, Soltan Dallal MM. Some probiotic properties of *Lactobacillus* species isolated from honey and their antimicrobial activity against foodborne pathogens. Vet Res Forum. 2020;11(2):121–126. https://doi.org/10.30466/vrf.2018.90418.2188

Liu C, Han F, Cong L, Sun T, Menghe B, Liu W. Evaluation of tolerance to artificial gastroenteric juice and fermentation characteristics of *Lactobacillus* strains isolated from human. Food Sci Nutr. 2021 Dec 10;10(1):227–238. https://doi.org/10.1002/fsn3.2662

Liu C, Xue WJ, Ding H, An C, Ma SJ, Liu Y. Probiotic potential of *Lactobacillus* strains isolated from fermented vegetables in Shaanxi, China. Front Microbiol. 2022 Feb 1;12:774903. https://doi.org/10.3389/fmicb.2021.774903

Marchwińska K, Gwiazdowska D. Isolation and probiotic potential of lactic acid bacteria from swine feces for feed additive composition. Arch Microbiol. 2021 Dec 23;204:61. https://doi.org/10.1007/s00203-021-02700-0

Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 2017 Sep 15;9(9):1021. https://doi.org/10.3390/nu9091021

Nunziata L, Brasca M, Morandi S, Silvetti T. Antibiotic resistance in wild and commercial non-enterococcal lactic acid bacteria and Bifidobacteria strains of dairy origin: An update. Food Microbiol. 2022 Jun;104:103999. https://doi.org/10.1016/j.fm.2022.103999

Pellegrino MS, Frola ID, Natanael B, Gobelli D, Nader-Macias MEF, Bogni CI. *In vitro* characterization of lactic acid bacteria isolated from bovine milk as potential probiotic strains to prevent bovine mastitis. Probiotics Antimicrob Proteins. 2019 Mar;11(1):74–84. https://doi.org/10.1007/s12602-017-9383-6

Piwat S, Sophatha B, Teanpaisan R. An assessment of adhesion, aggregation and surface charges of *Lactobacillus* strains derived from the human oral cavity. Lett Appl Microbiol. 2015 Jul;61:98–105. https://doi.org/10.1111/lam.12434

Rajab S, Tabandeh F, Shahraky MK, Alahyaribeik S. The effect of *Lactobacillus* cell size on its probiotic characteristics. Anaerobe. 2020 Apr;62:102103. https://doi.org/10.1016/j.anaerobe.2019.102103

Rozman V, Mohar Lorbeg P, Accetto T, Bogovič Matijašić B. Characterization of antimicrobial resistance in lactobacilli and bifidobacteria used as probiotics or starter cultures based on integration of phenotypic and *in silico* data. Int J Food Microbiol. 2020 Feb 2;314:108388. https://doi.org/10.1016/j.ijfoodmicro.2019.108388

Salminen MK, Rautelin H, Tynkkynen S, Poussa T, Saxelin M, Valtonen V, Järvinen A. *Lactobacillus* bacteremia, species identification, and antimicrobial susceptibility of 85 blood isolates. Clin Infect Dis. 2006 Mar 1;42(5):e35–e44. https://doi.org/10.1086/500214

Śliżewska K, Chlebicz-Wójcik A, Nowak A. Probiotic properties of new *Lactobacillus* strains intended to be used as feed additives for monogastric animals. Probiotics Antimicrob Proteins. 2021 Feb; 13:146–162. https://doi.org/10.1007/s12602-020-09674-3

Stefańska I, Kwiecień E, Jóźwiak-Piasecka K, Garbowska M, Binek M, Rzewuska M. Antimicrobial susceptibility of lactic acid bacteria strains of potential use as feed additives – The basic safety and usefulness criterion. Front Vet Sci. 2021 Jul 1;8:687071. https://doi.org/10.3389/fvets.2021.687071

Trejo-González L, Gutiérrez-Carrillo AE, Rodríguez-Hernández AI, Del Rocío López-Cuellar M, Chavarría-Hernández N. Bacteriocins produced by LAB isolated from cheeses within the period 2009–2021: A review. Probiotics Antimicrob Proteins. 2022 Apr; 14:238–251. https://doi.org/10.1007/s12602-021-09825-0

Yang C, Yu T. Characterization and transfer of antimicrobial resistance in lactic acid bacteria from fermented dairy products in China. J Infect Dev Ctries. 2019 Feb 28;13(2):137–148. https://doi.org/10.3855/jidc.10765

Zawistowska-Rojek A, Kośmider A, Stępień K, Tyski S. Adhesion and aggregation properties of *Lactobacillaceae* strains as protection ways against enteropathogenic bacteria. Arch Microbiol. 2022b Apr 27;204:285. https://doi.org/10.1007/s00203-022-02889-8

Zawistowska-Rojek A, Tyski S. Are probiotic really safe for humans? Pol J Microbiol. 2018;67(3):251–258. https://doi.org/10.21307/pjm-2018-044

Zawistowska-Rojek A, Tyski S. How to improve health with biological agents – Narrative review. Nutrients. 2022 Apr 20;14(9):1700. https://doi.org/10.3390/nu14091700

Zawistowska-Rojek A, Zaręba T, Tyski S. Microbiological testing of probiotic preparations. Int. Int J Environ Res Public Health. 2022a May 7;19(9):5701. https://doi.org/10.3390/ijerph19095701

Zheng M, Zhang R, Tian X, Zhou X, Pan X, Wong A. Assessing the risk of probiotic dietary supplements in the context of antibiotic resistance. Front Microbiol. 2017 May 19;8:908. https://doi.org/10.3389/fmicb.2017.00908