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In vitro characterization of lactic acid bacteria and bifidobacteria from wild and domestic pigs: probiotic potential for post-weaning piglets

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Abstract

Background Gastrointestinal diseases in weaned piglets are a frequent cause of high morbidity and mortality in domestic pigs. The use of antibiotics is problematic due to increasing antibiotic resistance in bacterial populations, for which reason the use of suitable probiotics is highly recommended to maintain animal health and welfare.

Results In this study, 57 strains of biologically safe lactic acid bacteria (LAB) and bifidobacteria originating from the gastrointestinal tract (GIT) of pigs were identified and characterized in terms of their probiotic properties for potential use in weaned piglets. These strains were divided into two sets based on their origin – from the GIT of wild boars ($n=41$) and from the GIT of domestic pigs ($n=16$). Strains obtained from wild boars exhibited greater taxonomic diversity compared to isolates from domestic pigs. While searching for coding sequences (CDS) encoding bacteriocins and bile salt hydrolases (BSH), no significant difference was detected between the two tested groups. On the other hand, CDS encoding adhesinlike factors were more frequent in the dataset isolated from wild boars than in the dataset obtained from domestic pigs. Moreover, more CDS encoding carbohydrateactive enzymes (CAZymes) were carried in the genomes of strains obtained from wild boars. Utilization of important selected carbohydrate substrates, such as starch, D-raffinose, D-mannose, Dcellobiose and gentiobiose, was confirmed by API testing. Antimicrobial activity against at least one of the five tested pathogens was found in 51% of wild boar strains but in none of the isolates from domestic pigs.

Conclusion This suggests that the intestinal microbiota of wild boars could serve as a promising source of probiotics for domestic pigs.

Keywords Probiotics, Wild boars, Domestic pigs, Antimicrobial activity, CAZymes, Carbohydrate utilization

Introduction

The weaning and postweaning period represents a critical phase in the life of piglets characterized by significant changes in gut microbiota and resulting health risks. Early weaning is stressful due to separation from the sow, handling, transport, adjustment to a new environment and significant dietary change [1]. The transition from a milkbased diet to a plant-based diet leads to changes in

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the gut environment, nutrient availability, and host physiology, which, in turn, influence the establishment and development of the gut microbiota [2, 3].

A solid diet rich in complex carbohydrates fibre and plant-based components provides an ideal source of energy for the various species of gut microbiota including opportunistic pathogens such as *Escherichia coli* (*E. coli*) [1]. An increased abundance of these pathogens in the composition of the gut microbiota, together with a weakened microbial barrier function of the intestinal tract, can lead to diarrhoea associated with transient anorexia, watery stool, dehydration, weight loss, decreased growth rates and abdominal discomfort in weaned piglets [4]. Postweaning diarrhoea is also one of the most common causes of increased morbidity and mortality in pig farming and can lead to substantial economic losses associated with reduced productivity on farms, higher economic costs of treatment, and negative impacts on animal welfare [5].

Efforts to control diarrhoea in post-weaning piglets have historically relied on antibiotics. In recent decades, antibiotics have been widely used in pig husbandry, not only for preventing diarrheal diseases during the weaning period, but also as growth promoters to increase piglet growth and enhance production efficiency. However, the use of antibiotics is questionable due to concerns about their loss of effectiveness, the selection of resistant clones, and the spread of resistance genes among bacterial populations colonizing domestic animals and humans [6, 7]. For these reasons, restrictions have been imposed on the use of antibiotics as growth promoters in European Union member states [8]. With the reduction in antibiotic use and the ban on the use of zinc oxide at therapeutic doses in feed for food-producing animals to prevent diarrhoea, efforts are underway to find new alternatives to prevent gastrointestinal diseases in piglets [9]. This is why plant extracts, organic acids, prebiotics and probiotics are tested to improve the health and welfare of animals and to enhance breeding efficiency [10].

Probiotics are live microorganisms (bacteria or yeasts) that, when administered in sufficient amounts, can improve the health of the host [11]. The effectiveness of probiotic strains may depend on various factors, including environmental or animal source of origin [12–14] or host specificity [15, 16]. The selection of functional probiotics relies not only on individual strain properties, but also on the synergistic effects of appropriate combinations in multistrain probiotics. This approach potentiates the positive effects on host health [17–19].

Safety requirements belong among the crucial criteria for selecting probiotic strains, including the absence of horizontally-acquired resistance genes and virulence factors [20, 21]. An important requirement for strains surviving in the intestinal environment is the presence of bile salt hydrolase, an enzyme that degrades bile salts found in the intestine [22]. After passage through the gastrointestinal tract (GIT), the ability to adhere to intestinal mucus is a crucial aspect of probiotic effectiveness. Adhesive factors enable adherence to the epithelium and also stimulate the host's immune system. Probiotic strains also suppress pathogen multiplication by means of the production of various metabolites or the expression of bacteriocins [23].

One property of probiotic strains is their ability to utilize substrates from feed that are not degraded by host enzymes. The effectiveness of pig digestion is particularly influenced by the presence of endogenous digestive enzymes and microbial fermentation in the GIT, especially the hindgut. Utilization of these substrates can lead to higher feed intake and improve feed conversion in farm animals [24].

In the past five years, several papers have focused on the gut microbiota of wild boars. Most of these studies describe the composition of the gut microbiota of wild boars using a 16 S rRNA metagenomic approach [25–29]. Fewer studies have described the specific properties of isolated LAB strains [12, 30, 31].

In this study, we tested whether wild boars can be used as a source of lactic acid bacteria (LAB) to be used as probiotics in domestic pigs. To achieve this aim, a total of 76 strains obtained from both wild boars and domestic pigs were tested for antibiotic resistance, and strains carrying antimicrobial resistance (AMR) genes were eliminated from further examination. A final set of 57 isolates was subjected to wholegenome sequencing. Bioinformatic analysis was used to identify genes associated with health benefits, e.g. bacteriocins, carbohydrate-active enzymes (CAZymes), bile salt hydrolases (BSH), and adhesinlike factors. Furthermore, the versatility of potential probiotic strains was further evaluated by examination of their carbohydrate metabolism profiles and antimicrobial activity assays against commonly encountered pathogens.

Materials and methods

Sample origin and primary isolation of the strains

Samples of small intestine and colon content from 83 wild boars and 67 domestic pigs were collected primarily between 2018 and 2020 (Table 1), with eight of the tested strains collected during 2013–2015. Wild boars were shot during regular seasonal hunts in 16 different locations in the Czech Republic. Intestinal content samples from adult domestic pigs were collected during slaughter in five different slaughterhouses. No animals were deliberately killed for sampling for this study. Samples of intestinal content were preserved under anaerobic conditions (1% CO_2, 1% H_2, 8% N_2) and transported immediately to

Table 1 Origins of selected and tested strains

Strain	Identification	Animal	GIT content	Sampling year	Biosample
6 A	*Limosilactobacillus mucosae*	WB	C	2013	SAMN35847944
174 A	*Limosilactobacillus mucosae*	WB	C	2015	SAMN35847943
383 A	*Limosilactobacillus mucosae*	WB	C	2013	SAMN35847947
598 A	*Limosilactobacillus mucosae*	WB	SI	2013	SAMN35847945
609 A	*Limosilactobacillus mucosae*	WB	C	2013	SAMN35847946
M65A	*Limosilactobacillus mucosae*	WB	C	2019	SAMN35847952
M86A	*Limosilactobacillus mucosae*	WB	C	2019	SAMN35847953
M184A	*Limosilactobacillus mucosae*	WB	C	2019	SAMN35847948
M193A	*Limosilactobacillus mucosae*	WB	C	2019	SAMN35847949
M212A	*Limosilactobacillus mucosae*	WB	C	2019	SAMN35847950
M223A	*Limosilactobacillus mucosae*	WB	C	2019	SAMN35847951
M387A	*Limosilactobacillus mucosae*	WB	C	2018	SAMN35847939
M580A	*Limosilactobacillus mucosae*	WB	C	2019	SAMN35847940
M585A	*Limosilactobacillus mucosae*	WB	SI	2019	SAMN35847941
M592A	*Limosilactobacillus mucosae*	WB	C	2019	SAMN35847942
350 A	*Lactobacillus amylovorus*	WB	C	2013	SAMN31135161
M356A	*Lactobacillus amylovorus*	WB	C	2018	SAMN31135165
M374A	*Lactobacillus amylovorus*	WB	SI	2018	SAMN31135166
M388A	*Lactobacillus amylovorus*	WB	SI	2018	SAMN31135167
M477A	*Lactobacillus amylovorus*	WB	C	2019	SAMN31135168
M490A	*Lactobacillus amylovorus*	WB	SI	2019	SAMN31135169
M581A	*Lactobacillus amylovorus*	WB	C	2019	SAMN31135171
M583A	*Lactobacillus amylovorus*	WB	C	2019	SAMN31135172
M597AA	*Lactobacillus amylovorus*	WB	C	2019	SAMN31135173
M597B	*Lactobacillus amylovorus*	WB	C	2019	SAMN31135174
M624A	*Lactobacillus amylovorus*	WB	C	2019	SAMN31135175
M668A	*Lactobacillus amylovorus*	WB	C	2020	SAMN31135176
M696A	*Lactobacillus amylovorus*	WB	C	2019	SAMN31135177
M702A	*Lactobacillus amylovorus*	WB	C	2020	SAMN31135179
332 A	*Limosilactobacillus reuteri*	WB	C	2014	SAMN35847981
396 A	*Limosilactobacillus reuteri*	WB	C	2014	SAMN35847954
M495A	*Limosilactobacillus reuteri*	WB	SI	2019	SAMN35847955
M51A	*Pseudoscardovia radai*	WB	SI	2019	SAMN35847984
M569A	*Pseudoscardovia radai*	WB	SI	2019	SAMN35847983
M600A	*Lactobacillus porci*	WB	SI	2019	SAMN35847933
M616A	*Lactobacillus porci*	WB	SI	2019	SAMN35847932
M258A	*Lactobacillus equicursoris*	WB	C	2019	SAMN35847931
M494A	*Ligilactobacillus salivarius*	WB	C	2019	SAMN35847937
M587A	*Lacticaseibacillus paracasei* subsp. *paracasei*	WB	C	2019	SAMN35847929
M591A	*Lentilactobacillus parabuchneri*	WB	C	2019	SAMN35847934
M675A	*Lactobacillus delbrueckii* subsp. *bulgaricus*	WB	C	2020	SAMN35847930
M738A	*Lactobacillus amylovorus*	DP	SI	2019	SAMN31135182
M838B	*Lactobacillus amylovorus*	DP	C	2019	SAMN35847977
M1020A	*Lactobacillus amylovorus*	DP	SI	2019	SAMN31135188
M726A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847960
M733A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847963
M744A	*Limosilactobacillus reuteri*	DP	SI	2019	SAMN35847965
M746A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847966
M767A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847975

Table 1 (continued)

Strain	Identification	Animal	GIT content	Sampling year	Biosample
M770A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847976
M773A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847978
M778A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847980
M824A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847956
M832A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN35847964
M838A	*Limosilactobacillus reuteri*	DP	C	2019	SAMN31135185
M966A	*Limosilactobacillus reuteri*	DP	SI	2019	SAMN35847962
M978A	*Limosilactobacillus reuteri*	DP	SI	2019	SAMN35847967

WB wild boars, *DP* domestic pigs, *C* colon, *SI* small intestine

the laboratory of Microbiology of Veterinary Research Institute, Brno. Samples were cultured on De Man Rogosa Sharp (MRS) agar (Oxoid, Basingstoke, UK) within 24 h of sampling Primary cultivation occurred under microaerophilic conditions at 37 °C. Selected isolates were cross-streaked three times and stored at -70 °C. Seventysix isolates of LAB and *Bifidobacterium* spp. originating from the gastrointestinal tract of wild boars and domestic pigs were collected. From this set of isolates, 57 strains were selected based on the absence of antibiotic resistance genes in their genomes and absence of haemolytic activity (see appendix "Selection of the Strains") and their ability to grow in liquid MRS medium and produce a sufficient biomass under laboratory conditions.

Identification of the isolates

Taxonomic identification of the 57 selected strains was performed by 16 S rRNA gene sequencing following PCR amplification with primers 16S27f (AGAGTTTGATCMTGGCTCAG) and 16S1492r (TACGGYTACCTTGTTACGACTT). Following the PCR, the resulting products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, CA, USA) and sequenced in both the forward and reverse directions using a Mix2Seq Kit (Luxembourg City, Luxembourg). The obtained sequences were compared with reference sequences in the GenBank and EzBioCloud databases (https://www.ezbiocloud.net; accessed on 1 October 2020).

Whole-genome sequencing and De Novo assembly

Total DNA was purified using the Quick-DNA™ Fecal/Soil Microbe Microprep Kit following the manufacturer's instructions (Zymo Research, Irvine, CA, USA). Subsequently, a DNA sequencing library was constructed using the Nextera Library Preparation Kit (Illumina, Inc., San Diego, CA, USA). Pairend sequencing was performed on a NextSeq platform with a NextSeq 500/550 High-Output Kit v.2.5 (Illumina, Inc., San Diego, CA, USA). Trim Galore v.0.6.6 (https://www.bioinformatics.babraham.ac.uk; accessed on 1 December 2020) was used for trimming primer sequences followed by use of the Cutadapt v.0.6.6 tool to remove lowquality reads. The quality of the read sequences was assessed using MultiQC v.1.9 [32]. *De novo* genome assembly was performed using Unicycler v.0.4.9b [33] and SPAdes v.3.14.1 [34]. The number of contigs, contig size, L50, N50 value and GC content of assembled genomes are shown in Supplementary Tables 1 and Supplementary Table 2.

Average nucleotide identity

Taxonomic identification based on 16S rRNA sequencing of the 57 isolates proved inadequate, as it yielded multiple taxonomic species with similar identity percentages. To address this, ANI (Average Nucleotide Identity) calculations were conducted using FastANI v1.32 [35] for further clarification (see Table 2). The genome sequences of *Limosilactobacillus reuteri* (*L. reuteri*) DSM 20,016, *Lactobacillus amylovorus* (*L. amylovorus*) DSM 20,531, *Lacticaseibacillus paracasei* subsp. *paracasei* (*L. paracasei*) ATCC 25,302, *Lentilactobacillus parabuchneri* (*L. parabuchneri*) DSM 5707, *Lactobacillus porci* (*L. porci*) KCTC 21,090 and *Lactobacillus delbrueckii* subsp. *bulgaricus* (*L. delbrueckii* subsp. *bulgaricus*) ATCC 11,842 were included as references for ANI analysis (https://lpsn.dsmz.de/).

The genome annotation of the strains

Gene prediction and annotation were carried out using Prokka v.1.14.6, with searches performed against a number of databases including ISfinder, the NCBI Bacterial Antimicrobial Resistance Reference Gene database, and UniprotKB (SwissProt) [36].

Protein sequences annotated by Prokka were then utilized for functional annotation through predicted orthology assignments using the EggNOG mapper tool emapper v.2.1.6 25 g1502c0F [37]. Further comparisons of protein sequences were made using DIAMOND v.2.0.11 protein aligner against EggNoggDB v.5.0.2 [38].

Table 2 Strain identification by 16S rRNA sequencing or ANI calculation (wild boars origin)

Strain	16 S rRNA Sequencing		Average Nucleotide Identity		Final Identification
	Identification	16 S (%)	Reference Strain	ANI (%)	
6 A	*L. mucosae*	99.70	-		*L. mucosae*
174 A	*L. mucosae*	99.86	-		*L. mucosae*
383 A	*L. mucosae*	100.00	-		*L. mucosae*
598 A	*L. mucosae*	99.59	-		*L. mucosae*
609 A	*L. mucosae*	99.38	-		*L. mucosae*
M65A	*L. mucosae*	99.32	-		*L. mucosae*
M86A	*L. mucosae*	99.45	-		*L. mucosae*
M184A	*L. mucosae*	99.93	-		*L. mucosae*
M193A	*L. mucosae*	100.00	-		*L. mucosae*
M212A	*L. mucosae*	99.52	-		*L. mucosae*
M223A	*L. mucosae*	99.59	-		*L. mucosae*
M387A	*L. mucosae*	100.00	-		*L. mucosae*
M580A	*L. mucosae*	99.51	-		*L. mucosae*
M585A	*L. mucosae*	99.59	-		*L. mucosae*
M592A	*L. mucosae*	99.52	-		*L. mucosae*
350 A	*L. amylovorus/kitasotonis/crispatus*	99.93/99.58/98.81	DSM 20,531	96.94	*L. amylovorus*
M356A	*L. amylovorus/kitasatonis/crispatus*	99.79/99.44/98.74	DSM 20,531	97.12	*L. amylovorus*
M374A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.58/98.81	DSM 20,531	97.25	*L. amylovorus*
M388A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.58/98.81	DSM 20,531	97.05	*L. amylovorus*
M477A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.58/98.81	DSM 20,531	97.03	*L. amylovorus*
M490A	*L. amylovorus/kitasatonis/crispatus*	99.86/99.51/98.81	DSM 20,531	96.85	*L. amylovorus*
M581A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.60/98.78	DSM 20,531	96.90	*L. amylovorus*
M583A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.60/98.78	DSM 20,531	96.84	*L. amylovorus*
M597AA	*L. amylovorus/kitasatonis/crispatus*	99.93/99.60/98.78	DSM 20,531	98.79	*L. amylovorus*
M597B	*L. amylovorus/kitasatonis/crispatus*	99.87/99.54/98.73	DSM 20,531	98.65	*L. amylovorus*
M624A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.60/98.78	DSM 20,531	98.60	*L. amylovorus*
M668A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.60/98.78	DSM 20,531	96.95	*L. amylovorus*
M696A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.60/98.78	DSM 20,531	98.76	*L. amylovorus*
M702A	*L. amylovorus/kitasatonis/crispatus*	99.87/99.54/98.84	DSM 20,531	96.96	*L. amylovorus*
332 A	*L. reuteri*	99.60	-		*L. reuteri*
396 A	*L. reuteri*	99.38	-		*L. reuteri*
M495A	*L. reuteri*	99.79	-		*L. reuteri*
M51A	*P. radai*	99.64	-		*P. radai*
M569A	*P. radai*	99.78	-		*P. radai*
M600A	*L. delbrueckii*	96.00	KCTC 21,090	96.53	*L. porci*
M616A	*L. delbrueckii* subsp. *indicus*	96.00	KCTC 21,090	96.06	*L. porci*
M258A	*L. equicursoris*	99.00	-		*L. equicursoris*
M494A	*L. salivarius*	99.86	-		*L. salivarius*
M587A	*L. paracasei/zeae/casei/rhamnosus*	100.00/99.21/98.55	ATCC 25,302	98.11	*L. paracasei*
M591A	*L. parabouchneri/sinkii/otakiensis/kefiri/bauchneri*	99.87/99.30/99.30/98.93/98.90	DSM 5707	98.06	*L. parabauchneri*
M675A	*L.d.* subsp. *bulg/indicus/lactis/sunkii*	99.15/99.23/99.06/99.06	ATCC 11,842	95.12	*L. delbrueckii* subsp. *bulgaricus*
M738A	*L. amylovorus/kitasatonis/crispatus*	99.89/99.51/98.74	DSM 20531	97.14	*L. amylovorus*
M838B	*L. amylovorus/kitasatonis/crispatus*	99.93/99.58/98.81	DSM 20531	97.07	*L. amylovorus*
M1020A	*L. amylovorus/kitasatonis/crispatus*	99.93/99.58/98.81	DSM 20531	97.11	*L. amylovorus*
M726A	*L. reuteri*	99.73	-		*L. reuteri*
M733A	*L. reuteri*	99.66	-		*L. reuteri*
M744A	*L. reuteri*	99.73	-		*L. reuteri*

Table 2 (continued)

Strain	16 S rRNA Sequencing		Average Nucleotide Identity		Final Identification
	Identification	16 S (%)	Reference Strain	ANI (%)	
M746A	*L. reuteri*	99.72	-		*L. reuteri*
M767A	*L. reuteri*	99.90	-		*L. reuteri*
M770A	*L. reuteri*	99.59	-		*L. reuteri*
M773A	*L. reuteri*	99.81	-		*L. reuteri*
M778A	*L. reuteri*	99.79	-		*L. reuteri*
M824A	*L. reuteri*	99.73	-		*L. reuteri*
M832A	*L. reuteri*	99.79	-		*L. reuteri*
M838A	*L. reuteri*	99.72	-		*L. reuteri*
M966A	*L. reuteri*	99.90	-		*L. reuteri*
M978A	*L. reuteri*	99.72	-		*L. reuteri*

For the annotation of CAZyme gene families, the run_dbcan4 tool (v3.0.4) [39] was employed utilizing three substrate prediction approaches: HMMER3, DIAMOND, and MMSeq2 [40]. Only genes identified by at least two prediction approaches were considered CAZyme genes.

The presence of antibiotic resistance genes was assessed using Abricate v.1.0.1 software (https://github.com/tseemann/abricate; accessed on 7 February 2022), which compared the genomes against several databases including the Comprehensive Antibiotic Resistance Database (CARD) [41], ResFinder [42], Argannot [43], Megares [44], and NCBI AMRFinderPlus [45]. Abricate software was configured with a minimum DNA identity threshold of 80% and a minimum seqence coverage of 80%.

The BAGEL4 web tool was employed to search for bacteriocinencoding genes (http://bagel4.molgenrug.nl/). Coding sequences (CDS) encoding adhesinlike factors and bile salt hydrolases were identified using annotation generated by Prokka and Eggnog-mapper.

Blood haemolysis assay

Single colonies of the isolates grown on MRS agar plates were inoculated onto Columbia agar plates supplemented with 5% sterile sheep blood (Oxoid, Basingstoke, UK). The plates were incubated at 37 °C for 72 h in an anaerobic atmosphere and haemolytic activity was evaluated visually.

Antimicrobial activity assay

All 57 isolates were precultivated on MRS agar for 48 h at 37 °C. The tested pathogenic bacteria including enteropathogenic *E. coli* 971, *E. coli* 973, *E. coli* 974, *Salmonella* Typhimurium 970 and *Yersinia enterocolitica* M108/15 were cultivated on Tryptone Soya agar (TSA; Himedia, Brno, Czech Republic) at 37 °C for 24 h. Bacterial suspensions with a density of 1.3 McFarland were prepared from the tested strains in physiological saline solution. Suspensions of all tested pathogens with a density of 0.5 McFarland were also prepared. Subsequently, 5 µl of inoculum from each tested strain was applied as spots on MRS agar. After the spots had dried, Petri dishes containing MRS agar inoculated with bacterial spots were covered with TSA agar supplemented with phosphate buffer adjusted to pH 7. Once the TSA agar solidified, a suspension of individual pathogens was evenly applied on top of the agar in each Petri dish using cotton swabs. The inoculated Petri dishes were incubated at 37 °C for 48 h. After incubation, any zones indicating inhibition of pathogen growth were measured. All samples were tested in duplicates. The growth of pathogens on TSA agar without LAB inoculation was also performed as a negative control.

Carbohydrate metabolic profile

The Analytical Profile Index – API® 50 CH (Biomerieux, Marcy-l'Étoile, France) was utilized to classify the tested strains according to their ability to ferment or utilize a total of 49 different substrates. The testing procedure was conducted in accordance with the manufacturer's instructions.

Results

Selection of the strains

Transferable antimicrobial resistance-encoding genes were found in 19 out of 76 strains, with *erm*B, *tet*W and *tet*L being the most prevalent. A detailed characterization of the resistant strains has been published in a previous study [46]. The 57 strains were selected for further characterization due to their absence of AMR determinants and absence of haemolysin activity.

Species classification

Among the 57 strains, 14 were identified as *Lactobacillus amylovorus*, 15 as *Limosilactobacillus mucosae* (*L. mucosae*), three as *Lactobacillus reuteri*, two as *Lactobacillus porci* and two as *Pseudoscardovia radai* (*P. radai*), a genus belonging to *Bifidobacteriaceae*. The remaining five isolates were identified as *Ligilactobacillus salivarius* (*L. salivarius*), *Lentilactobacillus parabuchneri*, *Lacticaseibacillus paracasei* subsp. *paracasei*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Lactobacillus equicursoris* (*L. equicursoris*). All these strains were isolated from wild boars. Another 13 strains of *L. reuteri* and three strains of *L. amylovorus* were isolated from domestic pigs. The results of ANI calculations for *Lactobacillus amylovorus* and *Lactobacillus mucosae* strains have been published in previous studies [46, 47], though these are included here for the sake of completeness (Table 2).

Genotypic characterization

The genomes of all 57 selected strains were evaluated for the presence of CDS associated with potential probiotic properties, such as the production of adhesinlike factors, bacteriocins, bile salt hydrolases and carbohydrate-active enzymes (Fig. 1).

Adhesin-like factors

CDS encoding fibronectin-binding proteins were found in 55 out of 57 isolates. CDS encoding peptidase M60like family proteins were present in 15 strains (with two copies in *L. mucosae* strain M585A), while CDS encoding Slayer associated proteins (SLAP) domain were identified in 16 strains. Prepilinrelated signal peptidase CDS was found in 19 strains. Additionally, CDS encoding prokaryotic Nterminal methylation motifs were found in the genomes of two isolates, specifically in *Pseudoscardovia radai* strains M51A and M569A, with both strains encoding five copies each.

Bacteriocins

CDS encoding various bacteriocins were found in the genomes of 43 out of the total 57 strains. The most common bacteriocins were enterolysin A found in 41 strains and helveticin J found in 21 strains. Both enterolysin A and helveticin J were confirmed in all *L. amylovorus* strains. Carnocin was identified in *L. paracasei* M587A and the CDS for coagulin detected in *L. delbrueckii* subsp. *bulgaricus* M675A. Tenuecyclamid A was confirmed in *L. equicursoris* M258A, acidocin in *L. reuteri* M495A, and salivaricin in *L. salivarius* M494A.

Bile salt hydrolases

The *cbh* gene (EC 3.5.1.24/ko: K01442) encoding cholylglycine hydrolase was found in all 57 tested strains. Two copies of the *cbh* gene were determined in all *L. mucosae* strains, except for the strain 6 A which carried three copies of this gene in the genome. Two copies of the *cbh* gene were also found in *L. amylovorus* isolates M356A, M624A, M702A and M838B and the *P. radai* M51A and M569A strains. Three copies of CDS encoding BSH were found in strains M597AA, M597B, M696A, M738A and M1020A (all *L. amylovorus)*, as well as *L. salivarius* M494A and *L. parabuchneri* M591A.

Carbohydrateactive enzymes

CAZymes are primarily represented by GHs (glucoside hydrolases), GTs (glucoside transferases) and CBMs (carbohydratebinding modules). The CDS responsible for different GHs identified in the genome sequences of the tested isolates were grouped together based on their enzymatic activity (Fig. 2).

The GH1 family was one of the most common groups of glucoside hydrolases, since all isolates of *L. amylovorus*, *L. porci*, *L. equicursoris* and *L. paracasei* possessed more than four copies of CDS for the GH1 family in their genomes (Fig. 2). CDS encoding 6phosphoβglucosidase (EC 3.2.1.86) from the GH1 family were found in all *L. amylovorus* isolates (17/17) and in six out of 16 *L. mucosae* isolates (Fig. 3).

The GH13 family of glucoside hydrolases which encodes starchdegrading enzymes was found in multiple copies in all *L. amylovorus* genomes, as well as in *P. radai*, *L. porci*, *L. equicursoris*, *L. delbrueckii* subsp. *bulgaricus* and *L. paracasei*. Specifically, the gene encoding pullulanase (EC 3.2.1.41)/αglucosidase (EC 3.2.1.20) belonging to the GH13 family was found in the genomes of all *L. amylovorus* and *L. mucosae* strains (Fig. 3). αglucosidase/oligo-1,6glucosidase (EC 3.2.1.10) was also found in all genomes of *L. amylovorus*. CDS encoding oligo-1,6glucosidase (EC 3.2.1.10) was detected in *L. reuteri* genomes. CDS for cyclomaltodextrinase (EC 3.2.1.54), maltogenic amylase (EC 3.2.1.133) and neopullulanase (EC 3.2.1.135) were present in all genomes of the *L. amylovorus* group and *L. mucosae* and in two copies in *L. parabuchneri* M587A and *L. salivarius* M494A isolates. CDS encoding 1,4−α−glucanbranching enzyme (EC 2.4.1.18) was detected in all isolates originating from wild boar GIT, with the exception of three strains of *L. amylovorus* and two strains of *L. reuteri*. Similarly, CDS for α,αphosphotrehalase (EC 3.2.1.93) was found only in genomes of lactobacilli obtained from wild boars.

The presence of genetic elements responsible for the hydrolysis of galactans was confirmed by CDS encoding αgalactosidase (EC 3.2.1.22) belonging to the GH36 family and βgalactosidase (EC 3.2.1.23) belonging to the GH2 and GH42 families. CDS encoding αgalactosidase and β-galactosidase were found in 49 and 53 strains,

Fig. 1 The distribution of genetic determinants associated with probiotic characteristics in the genomes of tested strains. The numbers given in the table represent the counts of specific CDS associated with adesins, bacteriocins, bile salt hydrolases and carbohydrate-active enzyme production. The heatmap is divided into strains isolated from wild boars (upper part) and domestic pigs (bottom part)

Fig. 2 The distribution of CDS encoding glucoside hydrolase families in the genomes of tested isolates. Strains isolated from wild boars are grouped in the upper part of the heatmap and strains originating from domestic pigs are shown in the bottom part

respectively. Four CDS responsible for galactan hydrolases were identified in *L. amylovorus* strains M477A and M490A. *L. reuteri* 332 A, 396 A, M495A, M773A, M778A and M824A each also had four CDS encoding galactan hydrolases. *Lactobacillus parabuchneri* M591A also possessed four CDS for galactandegrading enzymes.

CDS encoding arabinoxylan hydrolysis were found in the genomes of *L. amylovorus* M477A and M490A, both harbouring six specific CDS, and in one isolate of *L. mucosae* (M212A) possessing five CDS encoding arabinoxylanhydrolysing enzymes. Genes for βxylosidase (EC 3.2.1.37) belonging to the GH43 family were found in the genomes of nine *L. mucosae* strains and in 14 genomes of *L. amylovorus*. Similarly, genetic elements encoding αLarabinofuranosidase (EC 3.2.1.55) belonging to GH51 were found in the genomic sequences of six isolates of the total number of 15 *L. mucosae* strains. Both CDS together were presented in *L. mucosae* 174 A, M212A, M580A and M65A. The presence of both enzymes was also detected in two strains of the *L. amylovorus* group – M477A and M490A – both isolated from the GIT of wild boars.

Other enzymes involved in carbohydrate metabolism

Of the enzymes involved in the metabolism of saccharides but not classified as CAZymes, CDS encoding 1phosphofructokinase (EC 2.7.1.56) was identified in all genomes of *L. amylovorus* (Fig. 3). CDS encoding Lribulose-5phosphate-4epimerase (EC 5.1.3.4), Lribulokinase (EC 2.7.1.16) and Larabinose isomerase (EC 5.3.1.4) were found mainly in the genomes of *L. mucosae* and *L. reuteri*. All three CDS were present in 9 out of 15 *L. mucosae* genomes and 12 out of 16 *L. reuteri* genomes.

Phenotypic characterization

Antimicrobial activity and API tests were performed to confirm inhibition activity against pathogenic bacteria and the metabolic profiles of the strains.

Antimicrobial activity assay

Antimicrobial activity was detected only in isolates originating from the GIT of wild boars. None of the strains derived from the GIT of domestic pigs revealed an inhibition zone against any of the five tested pathogenic bacteria. The greatest antimicrobial activity was observed

Fig. 3 Individual CDS encoding specific enzymes involved in the metabolism of saccharides. The CAZymes in the heatmap are grouped according to their GH family affiliation. The enzymes 1-phospho-fructokinase (EC 2.7.1.56), Lribulose-5phosphate-4epimerase (EC 5.1.3.4), Lribulokinase (EC 2.7.1.16) and Larabinose isomerase (EC 5.3.1.4) are not classified as CAZymes, though these are involved in the metabolism of Dmannose (EC 2.7.1.56) and Larabinose (EC 5.1.3.4, EC 2.7.1.16, EC 5.3.1.4)

in *L. amylovorus* strains isolated from the GIT of wild boars, in which seven out of 14 tested isolates inhibited the growth of one or more tested pathogenic bacteria (Fig. 4). Of these, *L. amylovorus* strains M668A and M624A revealed large inhibition zones against five and three pathogens, respectively. High antimicrobial activity against tested pathogens was also detected in *L. salivarius* M494A, *L. paracasei* M587A and *P. radai* M51A. *L. mucosae* strains also showed antimicrobial activity in ten out of a total of 15 isolates, but for no more than two tested pathogens.

Substrate utilization

The majority of strains belonging to *L. mucosae* (12/15) and all strains of *L. reuteri* (16/16) were capable of degrading Larabinose (Fig. 5). Conversely, Darabinose could be utilized only by a single strain from the entire set of all isolates (*L. mucosae* M193A). Dribose and Dxylose were degraded by all *L. mucosae* isolates and by the majority of *L. reuteri* strains, irrespective of their source of isolation. Most strains from the *L. amylovorus* group fermented Dmannose (15/17), Nacetylglucosamine (16/17), Dcellobiose (16/17), gentiobiose (15/17) and starch (14/17). Other strains belonging to different taxa showed the degradation of these saccharides only in individual cases (Fig. 5). The degradation of Dtrehalose was common across the strains in this study, including three isolates of *L. mucoase* (6 A, M212A, M592A), five isolates of *L. amylovorus* (M597AA, M597B, M624A, M668A, M696A), *L. salivarius* M494A, *L. paracasei* M587A, *L. bulgaricus* M675A and *L. reuteri* M746A.

The most metabolically active strains in this study were *L. mucosae* M387A from the GIT of domestic pigs

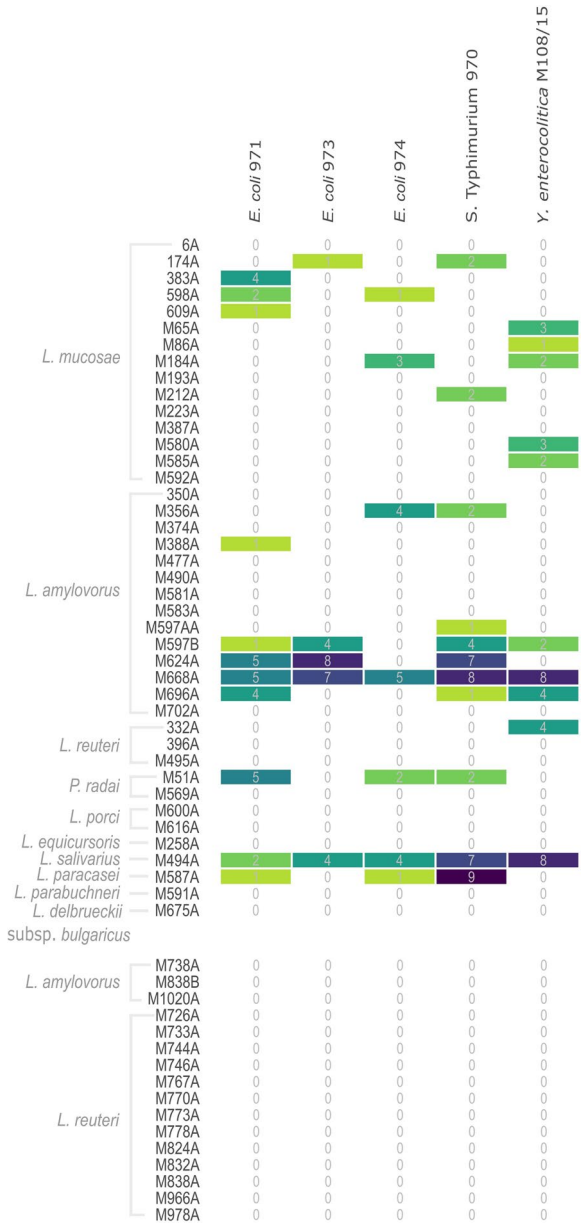

Fig. 4 Heatmap of the antimicrobial activity of tested strains against five selected intestinal pathogenic bacteria. The numbers in cells represent the size of the inhibition zones against tested pathogens in millimetres.The upper part of the heatmap represents isolates originating from the GIT of wild boars and the lower part of the heatmap represents isolates from the GIT of domestic pigs. *E. coli* – *Escherichia coli*, *S.* Typhimurium – *Salmonella enterica serovar Typhimurium*, *Y. enterocolitica* – *Yersinia enterocolitica*

utilizing 24 substrates and *L. reuteri* M746A (23 substrates), *L. paracasei* M587A (22 substrates) and *L. mucosae* 6 A, M193A, M212A (20 substrates), all four isolated from the GIT of wild boars.

Discussion

A total of 57 LAB and *Bifidobacteria* isolates obtained from the GIT of wild boars and domestic pigs were characterized according to the requirements related to the development of potential probiotics for piglets. The 57 strains tested also confirmed the absence of AMR genes and haemolytic activity.

Strains of LAB and bifidobacteria isolated from the GIT of wild boars were diverse and exhibited broader taxonomical variability as compared to the strains from the GIT of domestic pigs. The majority of the strains originating from domestic pigs were identified as *L. reuteri*. *L. reuteri* is described as an abundant gut bacterium colonizing the GIT of various vertebrates such as pigs, rodents, chickens and humans [48]. Consistent with the existing literature, *L. mucosae* and *L. amylovorus* were the dominant species in the group of isolates from the GIT of wild boars. *L. mucosae* has been repeatedly reported as an abundant strain in the intestines of wild boars [12, 31, 49]. *L. amylovorus* strains are frequently used as food additives and are also predominant species in the GIT of pigs [50–52]. The taxonomical diversity of the isolates originating from the GIT of wild boars was increased by the presence of other species such as *L. salivarus*, *L. paracasei*, *L. equicursoris*, *L. porci* and *P. radai*. *L. salivarius* and *L. paracasei* are commonly isolated from the digestive tract of domestic pigs [30]. *L. porci* was described as a new species in 2018 and is closely related to *L. delbrueckii* subsp. *bulgaricus* and *L. equicursoris* [53]; both these species were also detected in the intestines of wild boars in this study. *Pseudoscardovia radai*, belonging to *Bifidobacteriaceae*, was originally isolated from the GIT of wild boars [54]. Holman et al., 2023 found that the abundance of *P. radai* in the digestive tract of pasture-raised pigs is higher than that in conventionally raised pigs. Pastureraised pigs are kept in conditions more similar to those of wild boar as compared to conventionally raised pigs [55]. For this reason, their microbiota may be more diverse and similar to the gut microbiota of wild boars [56].

One of the key properties when characterizing the probiotic features of bacterial strains is their ability to adhere to the host gut epithelium. This adherence aids in the transient colonization of the intestine, stimulates immunomodulatory functions, supports the gut barrier and facilitates a number of metabolic processes. Various types of adhesins have been identified in different strains of *Lactobacillus* species, such as MapA, MUB and CmbA in *L. reuteri* [57]. Similarly, the majority of LAB and bifidobacteria isolates in our study harboured multiple copies of genes encoding adhesins in their genomes, particularly the isolates originating from the GIT of wild boars.

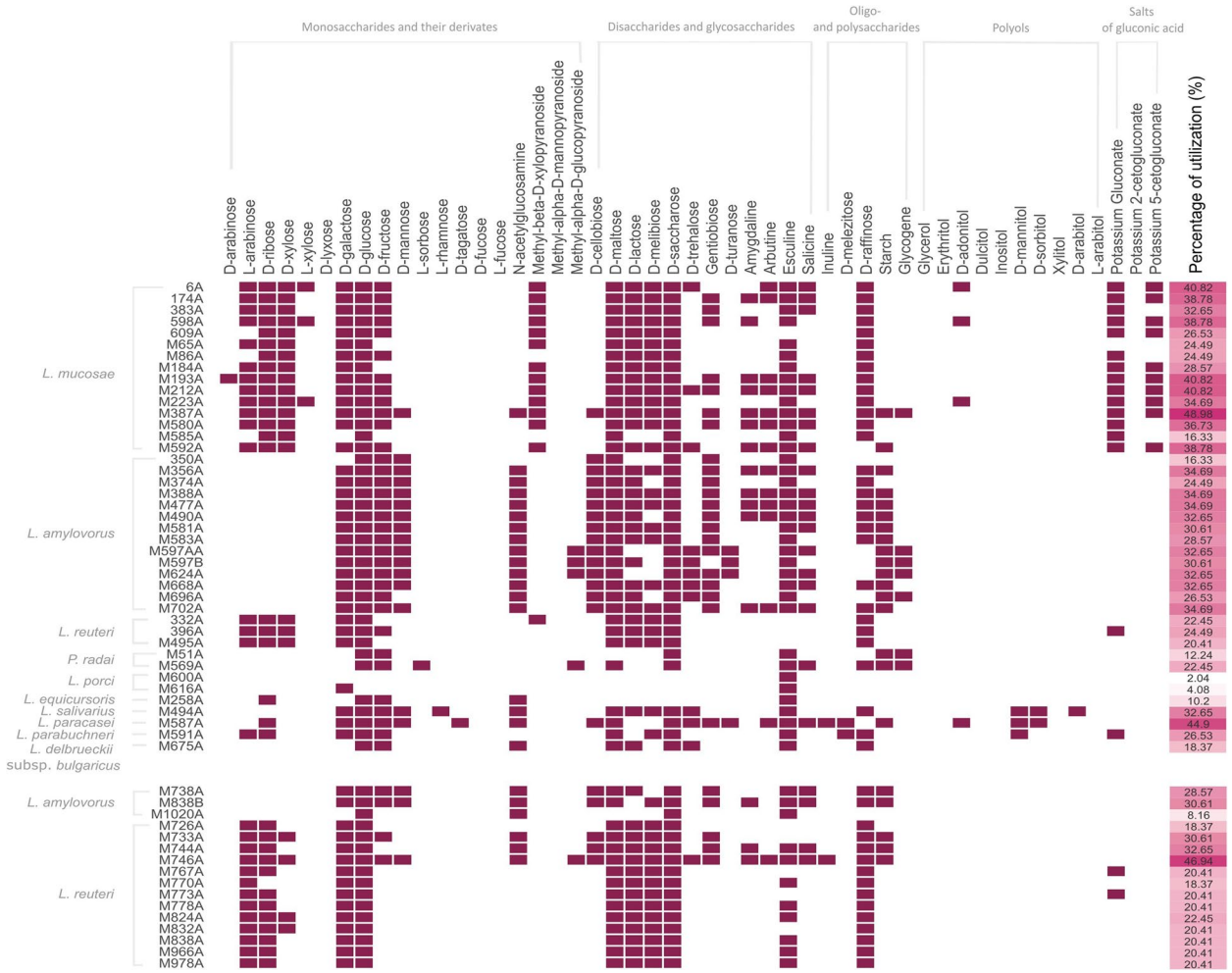

Fig. 5 Substrate utilization ability. Each isolate's capacity to utilize the tested spectrum of substrates is presented as a percentage in the last column of the heatmap. The upper part of the heatmap displays isolates from wild boars, while the lower part represents strains originating from domestic pigs

The ability to survive in the different conditions found in the gastrointestinal tract of the host represents other important feature of potentially probiotic bacteria. During the transition through the host's digestive system, the bacteria must contend with the low pH of the stomach and elevated bile salt concentrations in the small intestine. One mechanism for surviving increased concentrations of bile salts is the production of bile salt hydrolases, enzymes that enable bacteria to deconjugate primary bile acids [58]. The ability of LAB to produce BSH [59, 60], as well as the presence of the *bsh* gene in their genomes [61], has been extensively described. These findings correlate with the data obtained in our study, in which all of the 57 studied strains of LAB and bifidobacteria possessed at least one copy of the *bsh* gene.

The presence of helveticin J and enterolysin A encoding genes was confirmed in the genomes of all *L. amylovorus* isolates, including those from wild boars and domestic pigs. The presence of the enterolysin A encoding gene was also confirmed in the genomes of *L. mucosae* strains, as described in our previous study [47]. Helveticin J and enterolysin A are described as heatlabile bacteriocins belonging to class III bacteriocins [62, 63] and their presence in probiotic bacteria is relatively common. The presence of helveticin J has been confirmed in *Lactobacillus acidophilus* [62] as well as in *L. amylovorus* strains [64, 65]. Similarly, the ability to produce enterolysin A has been commonly detected in enterococci [66] and in *L. mucosae* [67]. The presence of both helveticin J and enterolysin A has also been observed in *L. kefiranofaciens* [62].

Genes encoding the bacteriocins acidocin and salivaricin were found in the genomes of *L. reuteri* M495A and *L. salivarius* M494A, respectively. Strain M494A also exhibited antimicrobial activity against all tested pathogens. Acidocin belongs to subclass IIA of bacteriocins and is known for its antilisterial effect, while salivaricin belongs to subclass IB of bacteriocins which are able to inhibit essential enzymes in target bacteria [63].

Bacteriocins are mostly found in gram-positive bacteria, particularly lactic acid bacteria. Bacteriocins produced by LAB have a narrow spectrum of effectiveness compared to antibiotics, meaning they do not inhibit other commensal microbiota in the GIT. Moreover, bacteriocins are not harmful to the mammalian host because the host's proteolytic enzymes are capable of bacteriocin inhibition [63]. In addition to bacteriocins, LAB are also known for producing other metabolic compounds such as organic acids (lactate, acetate, propionic and butyric acid) and hydrogen peroxide. All of these compounds can have an antimicrobial effect [68]. For instance, although the genome of the strain *P. radai* M51A did not show the presence of any CDS encoding bacteriocins, its in vitro ability to inhibit the growth of three out of five tested pathogens was observed.

Maldonado et al., 2018 reported antimicrobial activity against *E. coli* in *L. acidophilus*, *L. acidilactici*, *L. mucosae*, *L. amylovorus* and *L. plantarum* [69]. Moreover, Horvathova et al., 2023 tested selected representatives of pig GIT microbiota – *L. amylovorus*, *L. reuteri*, *L. paracasei* subsp. *tolerans* – and confirmed antimicrobial activity against *S.* Typhimurium [50]. Similarly, Angmo et al., 2016 demonstrated the antimicrobial activity of *Lactobacillus* spp. and *L. brevis* isolated from fermented food against *Yersinia enterocolitica* [70]. These findings correlate with the results of our study, in which particular isolates of *L. mucosae*, *L. amylovorus*, *L. reuteri*, *P radai*, *L. salivarius* and *L. paracasei* were able to inhibit in vitro growth of at least one pathogen. Interestingly, all these isolates were obtained from the GIT of wild boars, suggesting that these isolates may have greater probiotic potential for use in farmed pigs.

Dietary fibre can improve gut health by modulating gut microbiota, improving growth performance and reducing postweaning diarrhoea in pigs [71]. Dietary fibre is not well digested in the upper GIT of monogastric animals, but can be degraded in their hindgut by selected members of the gut microbiota, such as *Bacteroides*, *Lactobacilli* and *Bifidobacteria*. This results in the production of shortchain fatty acids (SCFAs) such as acetic, propionic and butyric acids, as well as lactate, as the final by products of their metabolism [72] that are beneficial to colonic health, provide energy to colonic cells and play a role in regulating the immune system.

Starch is one of the most abundant polysaccharides produced by agricultural crops [73] and represents one of the main sources of energy in the domestic pig diet [74]. Bacterial starchdegrading hydrolases of the GH13 family of CAZymes degrade α−1,4 and α−1,6 glycosidic linkages of starch [75]. Cyclomaltodextrinase, neopullulanase and maltogenicαamylase are enzymes sharing 40–60% ofsequence identity and capable of hydrolysing some or all of the following three types of oligo or polysaccharide substrates: cyclomaltodextrins, pullulan and starch [75]. Neopullulanase, along with other pullulanhydrolysing enzymes, is also capable of degrading starch, amylopectin and pullulan into smaller fermentable saccharides [73].

α−glucan branching enzyme (GBE, glycogen-branching enzyme, EC 2.4.1.18) was also detected in *L. amylovorus*. GBE modifies the biosynthesis of branched polysaccharides such as glycogen and amylopectin, increases the solubility of the glycogen molecule, and plays a role in starch and sucrose metabolism, i.e. it has the potential to increase the digestibility of starch [76, 77].

Cereal grains also contain non-starch polysaccharides such as arabinoxylans, β−glucans and cellulose. Wheat, corn, sorghum and barley are particularly rich in arabinoxylan content (Jha and Berrocoso, 2015). The presence of CDS encoding enzymes capable of degrading arabinoxylans, βxylosidase (EC 3.2.1.37) and αLarabinofuranosidase (EC 3.2.1.55) was proven in eight *L. amylovorus* strains and in six of the *L. mucosae* strains in our study.

α−galactooligosaccharides (α−GOS) or raffinose family oligosaccharides (RFOs) represent another type of fibre compound that is difficult to digest that can be metabolized by lactobacilli and bifidobacteria into SCFAs [78]. One RFO, Draffinose, is a trisaccharide composed of galactose, glucose and fructose that can be degraded by the enzyme αgalactosidase [79]. Draffinose was utilized by the majority of the strains from both wild and domestic pigs in this study.

Cellobiose and gentiobiose are disaccharides composed of two glucose units joined by β−1,4- or β−1,6glycosidic bonds. Cellobiose is a product of cellulose or plant glucan degradation. Cellobiose is not digested by the host, but can be degraded by microbial fermentation in the colon where it serves as a prebiotic and a source of energy for the gut microbiota. These disaccharides are degraded by 6phosphoβglucosidase [80, 81]. API testing of the isolates in our study confirmed the capability of utilizing Dcellobiose and gentiobiose in 14 strains of *L. amylovorus* and in *L. mucosae* M387A and *L. paracasei* M587A. All these isolates also carried CDS encoding 6phosphoβglucosidase (EC 3.2.1.86, GH1).

The utilization of another type of disaccharide, D-trehalose, was observed in 11 strains from wild boars and in

one strain from a domestic pig. α,αphosphotrehalase (EC 3.2.1.93, GH 13) was found in the genomes of Dtrehaloseutilizing strains, except for *L. amylovorus* M668A and *L. reuteri* M746A. Dtrehalose is present in many different natural sources, such as insects, invertebrates, fungi and various plants [82]. The presence of α,αphosphotrehalase primarily in the genomes of lactobacilli from wild boar suggests a possible connection with the more diverse diet of wild boars, which may include fungi or insects.

The majority of *L. amylovorus* strains, as well as *L. mucosae* M387A, *L. salivarius* M494A and *L. paracasei* M587A, fermented D-mannose. Buron-Moles et al., 2019 described a correlation between 1phosphofructokinase and the phenotypic utilization of Dmannose in lactic acid bacteria, highlighting the fact that 1phosphofructokinase is an important enzyme in Dmannose degradation [77]. In agreement with this, all the strains fermenting Dmannose in this study also encoded 1−phosphofructokinase.

The monosaccharide L−arabinose was mainly utilized by *L. mucosae* and *L. reuteri* strains of both domestic and wild pig origin. L−ribulokinase (EC 2.7.1.16), L−arabinose isomerase (EC 5.3.1.4) and Lribulose-5phosphate-4epimerase (EC 5.1.3.4) were found in the genomes of these strains, except for *L. mucosae* 598 A and *L. reuteri* M495A isolates. These enzymes degrade Larabinose into xylulose-5phosphate, a significant compound in the fermentation process in heterofermentative bacteria [79].

Lactobacillus amylovorus and *Lactobacillus porci* generally carried the largest number of CDS encoding CAZYmes in their genomes. No significant differences were observed concerning the distribution of genetic elements for CAZymes between *L. amylovorus* strains from the GIT of wild boars and domestic pigs. However, when considering the sum of CDS encoding CAZYmes, the isolates originating from domestic pigs exhibited lower enzymatic activity than those isolated from wild boars. This was primarily due to the predominance of *Lactobacillus reuteri* strains in the domestic pig isolates which had the lowest number of glycoside hydrolase families in their genomes. Three *L. reuteri* strains (332 A, 396 A, M495A) were also represented among the strains from wild boars, with similar numbers of CDS for CAZymes as *L. reuteri* strains from domestic pigs.

We propose that the higher taxonomical variability of the isolates from the GIT of wild boars played a role in the greater ability of these strains to inhibit in vitro the growth of selected pathogenic bacteria and to utilize a broader spectrum of saccharide substrates than the domestic pig isolates. Moreover, the strains originating from the GIT of wild boars showed lower resistance to selected antibiotics than those from domestic pigs, indicating that the digestive tract of wild boars represents a promising source of new probiotic strains for use in piglets on farms.

Conclusion

Only the isolates without AMR genes and haemolytic activity were selected from the original set of strains of lactic acid bacteria and bifidobacteria isolated from domestic pigs and wild boars. The group of isolates from the GIT of domestic pigs included only two taxa – *L. amylovorus* and *L. reuteri*, while the dataset from wild boars was more diverse. Although strains belonging to the same genus, but of different origin, possessed similar profiles of observed CDS in their genomes, the isolates from wild boars exhibited greater taxonomic variability which enhances the number of beneficial genes encoding potential probiotic properties, such as production of CAZymes, bacteriocins, adhesins or BSH. Many strains originating from the GIT of wild pigs also showed antimicrobial activity against tested pathogens, compared to the zero antimicrobial activity of domestic pig isolates. According to the phenotypic ability to utilize different types of substrates, the strains degrading the largest number of different carbohydrates were mostly identified as those originating from the GIT of wild boars.

While the lactic acid bacteria and bifidobacteria strains isolated from the GIT of domestic pigs were considered safe and exhibited some beneficial properties, the dataset from wild boars showed higher complexity. The intestinal microbiota of wild boars may present a unique and promising source of beneficial probiotic strains for domestic pigs, though this has to be tested since the different dietary composition of wild boars and domestic pigs may influence its ability to colonise and thereby express probiotic characteristics.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-024-03711-9.

Supplementary Material 1.

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Authors' contributions
Writing – original draft - K.K. Concetualization - K.K., M.C. Data curation - K.K., I.K. Formal analysis - K.K. Investigation - K.K., I.K., T.K., M.M., M.C. Methodology - K.K., I.K., M.C. Vizualization - K.K. Writing – review & editing - I.K., M.C. Funding acquisition - M.M., M.C.

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Data availability
The sequenced genomes have been deposited in the NCBI database under Bioproject accession number PRJNA886611 (available from April 2024). Supplementary data are deposited in the Figshare repository: https://figshare.com/articles/dataset/Supplementary_table_-_b_i_In_vitro_i_b_b_Selection_of_Lactic_Acid_Bacteria_and_Bifidobacteria_from_the_Digestive_Tract_of_Wild_and_Domestic_Pigs_Potential_Probiotic_Candidates_for_Post-weaning_Piglets_b_/26808520?file=48726811.

Declarations

Ethics approval and consent to participate
No animal were killed in order to obtain samples for this study. Material used as samples for this study were collected during regular hunts or from cadaver at slaughterhouse.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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