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Lactobacillus Spp. strains isolation, identification, preservation and quantitative determinations from the intestinal content and faeces of weaned piglets

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ABSTRACT

The study aimed to isolate, identify, preserve and evaluate the quantitative level of the *Lactobacillus* strains from gut content and faeces of weaned piglets, 30-107 days old; to test the viability of these strains preserved at 4°C and room temperature. *Lactobacillus* strains were isolated, phenotypically identified and preserved from gut content and faeces of 20 weaned piglets. Identification was performed by morphological, cultural and biochemical character examination, using apiweb™ and ABIS online software. *Lactobacillus* spp. from intestinal content and faeces (10^6 – 10^9 CFU/g) and the viability of strains preserved at 4°C and at room temperature were also determined (from 38 days to 4 months). Twenty-six strains of *L. acidophilus*, *L. fermentum*, *L. plantarum*, *L. salivarius* and *L. delbrueckii* ssp. *delbrueckii*, from gut content and faeces of weaned piglets were isolated, phenotypic identified and preserved. Of these, *L. fermentum*, *L. delbrueckii* ssp. *delbrueckii* and *L. acidophilus* biotype 2 isolates were technologically and ecologically suitable for continuing the testing of probiotic traits.

Keywords: *Lactobacillus* spp.; weaned piglets; phenotypic identification; preservation.

INTRODUCTION

The mammal's intestinal microbiota has beneficial roles for the host, in carbohydrates digestion, vitamins production, immune system regulation, and protection from pathogens (Buffie et al., 2013; Kamada et al., 2013). As a complex ecosystem, the pig intestinal microbiota is characterized by a

dynamic composition (Isaacson and Kim, 2012). Gastrointestinal (GI) colonization is started at birth and is formed by saw's milk, which provide lactic acid bacteria (Frese et al., 2015). *Escherichia coli* and *Streptococcus* spp. create an anaerobic environment for *Lactobacillus*, *Bifidobacterium*, *Bacteroides,* and *Clostridium* (Petri et al., 2010). In the ilea of piglets at 26 days old, the most frequently identified genera included *Lactobacillus*, *Clostridium*, *Streptococcus*, *Helicobacter*, *Ruminococcus,* and *Veillonella* (Dowd et al., 2008). The most abundant genera at the age of 10-22 weeks included *Prevotella*, *Lactobacillus*, *Blautia*, *Streptococcus*, *Faecalibacterium* and *Roseburia* (Kim et al., 2011). There are bacterial genera that form an essential microbiota within the pig's GI tract regardless of country, diet, age or breed (Holman et al. 2017). Among all GI samples (including fecal samples), the genera *Clostridium*, *Prevotella*, *Alloprevotella*, *Ruminococcus*, *Blautia*, *Lactobacillus*, *Roseburia*, *Subdoligranulum* and RC9 gut group were found in more than 90% of all samples from 3-24 weeks old pigs. These taxa contain well-adapted bacteria to the pig's gut and, important, may serve as markers of a typical swine gut microbiota (Holman et al., 2017). The dietary transition and environmental changes at weaning are linked to modifying in piglet GI microbiota, which could be etiologically involved in enteric infections and post-weaning diarrhea (Lalles et al., 2007). Konstantinov et al. (2006), Su et al. (2008) and Wei et al. (2017) have informed a diminution in *Lactobacillus* and a loss of microbial diversity, whereas *Clostridium* spp., *E. coli*, *Prevotella* spp. was positively impacted through the weaning transition (Gresse et al., 2017). *Lactobacillus* spp. is recognised for its beneficial effects on the host's health (Duar et al., 2017). The sudden decrease of *Lactobacillus* spp., as major players in disease prevention, can amplify the increase of enteric infections (Konstantinov et al., 2006). Anyway, *Lactobacillus* is more abundant in the gastric mucosa, but is present in all types of GI samples, being able to attach to the epithelial and mucosal layers, where form biofilm-like communities (Pedersen and Tannock, 1989; van Winsen et al., 2001; Tannock, 2004; Walter 2008; Dumitru et al., 2020). Among these genus, *L. fermentum*, *L. acidophilus*, *L. salivarius*, *L. sobrius, L. reuteri*, *L. plantarum*, *L. delbrueckii* spp. *bulgaricus* and *L. crispatus* were most frequently detected (Pedersen and Tannock, 1989; De Angelis et al., 2006; Pieper et al., 2006; Janczyk et al., 2007). This diversity raises the issue of selecting the best strain for developing bacterial-based feed additives in pig nutrition. *L. johnosii*, *L. mucosae*, *L. acidophilus*, *L. casei*, *L. plantarum*, *L. sobrius* and *L. rhamnosus* were used as probiotic bacteria as different additives for pigs of 3-25 weeks old, with beneficial effects: decrease in *E. coli* (enterotoxigenic *E. coli* including), faecal coliforms and *Clostridia*, increase in *Lactobacillus* spp. and *Bifidobacterium* spp., decrease the incidence of diarheea, increase of villus height and higher production of short chain fatty acids (SCFAs) (Konstantinov et al., 2008; Zhang et al., 2010; Chiang et al., 2015; Barszcz et al., 2016; Dowarah et al., 2017; Shin et al., 2019; Wang et al.,

2019). Safety of piglets from postweaning infections by probiotics could occur through modulation of the resident GI microbiota (composition and activity), immune stimulation (stimulation of lymphocytes, production of antibodies and cytokines, improvement of intestinal barrier integrity) and pathogen inhibition (competition for sites of adherence, secretion of antibacterial molecules, inhibition of virulence genes) (Greese et al., 2017; Shin et al., 2019; Wang et al., 2019). In this perspective, the isolation and testing of *Lactobacillus* spp. strains as probiotic tools is a promising way for better management of a weaning transition, with non-antibiotic strategies able to restore a balanced GI microbiota (Greese et al., 2017; Wang et al., 2019).

The current study purpose to isolate, identify, preserve and assess the quantitative level of the *Lactobacillus* strains from the intestinal content and faeces of twenty weaned piglets, 30-107 days old, in order to further testing their probiotic traits and to select the best strains as intestinal flora stabilizers in pig diet.

MATERIALS AND METHODS

Pigs were treated in accordance with Romanian legislation (Law 199/2018) for handling and protection of animals used for experimental purposes.

Bacterial strains isolation and determination of CFU/g intestinal content. The method of Mountzouris et al. (2007), adapted by Sorescu et al. (2019) was used in this study. Intestinal content (ileum and cecum, respectively) or faeces per capita, were collected from twenty weaned TOPIGS hybrid piglets, 30-107 days old.

Sample preparation: 1 g of sample was homogenized with 7 ml Oxoid BHI (Brain Heart Infusion) broth and 2 ml glycerol, and immediately frozen at –20˚C until testing (no more of two months). After defrost, decimal dilutions in Oxoid PBS (Phosphate Buffered Saline) were done: 0.1 ml from 10^{-4}, 10^{-5}, 10^{-6} dilutions, from every sample was inoculated on three Petri dishes with Oxoid MRS (Man, Rogosa, Sharpe) agar. The agar plates were cultural inspected. The procedure was presented in a previous paper (Sorescu et al., 2019) for the isolation and counting of *Lactobacillus* CFU from gut chickens. The colonies of each cultural type were calculated after 48 hours of anaerobic incubation (Oxoid jar with Anaerogen 2.5 L at 37°C). Gram-stained smears of each colony type were made, for investigative morphological characters and confirmation of L*actobacillus*.

Bacterial strains identification

Phenotypic identification of isolated bacterial strains was performed by morphological, cultural and biochemical characters examination, according to

apiwebTM API50CHL software BioMerieux (France), Bergey's Manual of Systematic Bacteriology (Hammes and Hertel, 2009) and ABIS on line software (Stoica and Sorescu, 2018), following the protocol previously described (Sorescu et al, 2019). The results obtained by Pelinescu (2009) were also considered.

Bacterial strains preservation

The medium-term preservation (weeks, months) was done by culture in MRS broth, the bacteria viability being assessed after 38 days-4 months. Long-time preservation (years) was done at -80°C, with addition of glycerol 20%, and bacteria viability is to be evaluated every 2 years.

RESULTS

The taxonomic classification of *Lactobacillus* spp. was made through morphologically (Gram-positive, non-spore forming rods), culturally (anaerobic growth) and biochemically characters (negative catalase test). The identification of *Lactobacillus* spp. was performed based on their biochemical characters. Thus, twenty-six strains of the genus *Lactobacillus* (*L. acidophilus* biotype 1 IBNA 88, *L. acidophilus* biotype 2 IBNA 91, *L. acidophilus* biotype 3 IBNA 70, 76, 79, 81, 89, 93, 94, 96-99, *L. fermentum* biotype 1 IBNA 71, 75, 78, 85, 90, 92, 95, *L. plantarum* biotype 1 IBNA 84, *L. salivarius* IBNA 86, 87, 100 and *L. delbrueckii* ssp. *delbrueckii* IBNA 72, 77) were isolated, identified and preserved.

The morphological, cultural and biochemical traits of the identified strains are presented in Table 1.

Table 1. Morphological, cultural and biochemical characteristics of the *Lactobacillus* strains isolated from intestinal content and faeces of weaned piglets.

Tests	1	2	3	4	5	6	7
Morphological characters	a	a	a, c	a, b, c	b	b	a
Cultural characters	x	x	x, y, z	x, y, z	y	y	x, z
Catalase test	0(1)*	0(1)	0(11)	0(7)	0(1)	0(3)	0(2)
Fermentation (API50CHL)							
L-arabinose	0(1)	0(1)	0(11)	6(7)	0(1)	0(3)	0(2)
D-ribose	0(1)	?(1)	0(11)	7(7)	1(1)	2(3)	0(2)
D-xylose	0(1)	1(1)	1(11)	6(7)	0(1)	0(3)	0(2)
D-adonitol	0(1)	0(1)	0(11)	0(7)	0(1)	1(3)	0(2)
Methyl-βD-xylopyr.	0(1)	0(1)	0(11)	1(7)	0(1)	0(3)	0(2)
D-galactose	0(1)	1(1)	9?(11)	7(7)	1(1)	3(3)	0(2)
D-fructose	1(1)	1(1)	11(11)	0(7)	1(1)	3(3)	2(2)
D-mannose	1(1)	?(1)	2(11)	0(7)	1(1)	3(3)	?(2)
L-rhamnose	0(1)	0(1)	0(11)	0(7)	0(1)	2(3)	0(2)
D-mannitol	0(1)	0(1)	0(11)	0(7)	1(1)	3(3)	0(2)
D-sorbitol	0(1)	0(1)	0(11)	0(7)	1(1)	3(3)	0(2)
N-acetyl glucosamine	1(1)	1(1)	6?(11)	0(7)	1(1)	3(3)	2(2)
amygdalin	?(1)	0(1)	0(11)	0(7)	1(1)	?(3)	0(2)
arbutin	1(1)	0(1)	0(11)	0(7)	1(1)	1(3)	0(2)
esculin	1(1)	1(1)	8(11)	0(7)	1(1)	1(3)	?(2)
salicin	1(1)	0(1)	0(11)	0(7)	1(1)	1(3)	0(2)
D-cellobiose	1(1)	1(1)	8(11)	0(7)	1(1)	?(3)	?(2)
D-lactose	1(1)	1(1)	9(11)	7(7)	1(1)	3(3)	0(2)
D-melibiose	0(1)	1(1)	3(11)	7(7)	1(1)	3(3)	0(2)

D-trehalose	1(1)	0(1)	1(11)	0(7)	1(1)	3(3)	0(2)
D-raffinose	1(1)	1(1)	11(11)	7(7)	1(1)	3(3)	0(2)
starch	?(1)	1(1)	11?(11)	0(7)	0(1)	0(3)	0(2)
gentibiose	1(1)	?(1)	7?(11)	0(7)	?(1)	0(3)	0(2)
D-arabitol	0(1)	0(1)	0(11)	0(7)	0(1)	1(3)	0(2)
potassium gluconate	0(1)	0(1)	0(11)	3?(7)	?(1)	0(3)	0(2)
potassium 5-ketogluconate	0(1)	0(1)	0(11)	2?(7)	0(1)	0(3)	0(2)

1=*L. acidophilus* biotype 1 IBNA 88; 2= *L. acidophilus* biotype 2 IBNA 91; 3= *L. acidophilus* biotype 3 IBNA 70, 76, 79, 81, 89, 93, 94, 96-99; 4= *L. fermentum* biotype 1 IBNA 71, 75, 78, 85, 90, 92, 95; 5= *L. plantarum* biotype 1 IBNA 84; 6= *L. salivarius* IBNA 86, 87, 100; 7= *L. delbrueckii* ssp. *delbrueckii* IBNA 72, 77.

a= Gram positive non-spore forming rods, grouped in pairs, chains, filaments, irregular clumps or, rarely, in palisade; b= Gram positive short rods, with rounded end, non-spore forming, arranged in pairs, short chains or irregular clumps; c= Gram positive short and thick rods or coccoid cells, non-spore forming, arranged in short chains and irregular clumps.

x= small colonies, 0.5-1.5 mm in diameter, rarely larger, smooth type, round, opaque, semi-transparent or transparent, and whitish, grey or colourless on MRS agar; y= large colonies, 2.0-4.0 mm in diameter, rarely smaller, smooth type, round, opaque, white or whitish; z= small colonies, 1.0-1.5 mm in diameter, rarely larger, rough type, transparent or semi-transparent, flattened, round, colourless.

*= number of positive strains from number of tested strains;?= dubious, weekly positive.

All strains were negative for the fermentation of glycerol, erythritol, D-arabinose, L-xylose, L-sorbose, dulcitol, inositol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, inulin, D-melezitose, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, L-arabitol and potassium 2-ketoglucunonate. All strains were positive for the fermentation of D-glucose, D-maltose, D-saccharose.

Figures 1-2 illustrate smears from *L. acidophilus* IBNA 88 and *L. delbrueckii* ssp. *delbrueckii* IBNA 72 cultures in/on MRS broth/agar (Gram staining, x 1000).

Figure 1. *L. acidophilus* IBNA 88 in MRS broth medium

Figure 2. *L. delbrueckii* ssp. *delbrueckii* IBNA 72 on MRS agar medium

In Table 2 are presented the origin (faeces and intestinal content) and the level of the isolate's existence in the natural niche.

In Table 3 are presented the results of strains identification by apiweb™ soft, API50CHL V.5.1, BioMerieux (France), and ABIS online software.

Details on the meaning and mode of calculation of % SIM for ABIS and API % ID were presented in a previous article (Sorescu et al., 2019).

In Table 4 are presented the results of the viability test for *Lactobacillus* strains which are preserved at 4°C and at room temperature.

Table 2. The origin and the number of *Lactobacillus* spp. presence in the ecological niche (gut content and faeces of weaned piglets).

Strains	Origin; sample number	CFU/g intestinal content (log10)
L. acidophilus biotype 1 IBNA 88	faeces, 30 days old piglet; 109 (small colonies)*.	8.51
L. acidophilus biotype 2 IBNA 91	faeces, 30 days old piglet; 112.	8.60
L. acidophilus biotype 3 IBNA 70,	cecum content, 81 days old piglet; 99 (opaque, large and smooth colonies).	9.69
76,	ileum content, 81 days old piglet; 101 (small and smooth colonies).	8
79,	ileum content, 107 days old piglet; 103 (opaque and smooth colonies).	7.90
81,	cecum content, 107 days old piglet; 103 (semi-transparent and rough colonies).	7.60
89,	faeces, 30 days old piglet; 110.	8
93,	faeces, 30 days old piglet; 115 (transparent and rough colonies).	8.39
94,	faeces, 30 days old piglet; 116.	8.60
96,	faeces, 30 days old piglet; 120.	8.90
97,	faeces, 30 days old piglet; 121.	9.14
98,	faeces, 30 days old piglet; 122.	9.90
99.	faeces, 30 days old piglet; 126 (small, transparent and rough colonies).	8.39
L. plantarum biotype 1 IBNA 84	ileum content, 107 days old piglet; 106.	5.84
L. delbrueckii ssp. *delbrueckii* IBNA	ileum content, 81 days old piglet; 99 (opaque, large/middle and smooth colonies).	8.30
72,	cecum content, 81 days old piglet; 101	9.60
77.	(large/small and rough colonies).	
L. fermentum biotype 1 IBNA 71,	cecum content, 81 days old piglet; 99 (semi-transparent, large and rough colonies).	8.77
75,	ileum content, 81 days old piglet; 100.	6.60
78,	cecum content, 107 days old piglet; 102.	7.30
85,	ileum content, 107 days old piglet; 107.	6.60
90,	faeces, 30 days old piglets; 112.	8.00
92,	faeces, 30 days old piglets; 115 (opaque and	8.39
95.	smooth colonies).	
	faeces, 30 days old piglets; 119.	8.30
L. salivarius IBNA	faeces, 30 days old piglets; 108.	
86,	faeces, 30 days old piglets; 109 (large colonies).	7.54
87,	faeces, 30 days old piglets; 126 (large, opaque and smooth colonies).	7.87
100.		9.39

*= differential cultural characters only for the different strains isolated from same sample.

Table 3. The identification of strains by apiweb™ soft, API50CHL V.5.1, BioMerieux, and ABIS online software.

Strains	**API, % ID**	**ABIS, % SIM**
L. acidophilus biotype 1 IBNA 88	*L. acidophilus* 1, 61.5 *L. crispatus*, 36.1	*L. manihotivorans,* 92 *L. acidophilus,* 82
L. acidophilus biotype 2 IBNA 91	*L. acidophilus* 2, 66.2	*L. acidophilus,* 91
L. acidophilus biotype 3 IBNA 70,	*L. acidophillus* 3, 67.1	*L. intestinalis*, 94 *L. kefiranofaciens* ssp. *kefirgranum,* 93 *L. acidophilus,*85
76,	*L. acidophillus* 3, 90.1	*L.taiwanensis,* 82 *L. lindneri,* 82 *L. acidophilus,*75
79,	*L. acidophillus* 3, 90.7	*L. kunkeei*, 91 *L. pontis*, 89 *L. acidophilus,* 74
81,	*L. acidophillus* 3, 74.7	*L. acidophilus,* 88
89,	*L. acidophillus* 3, 85.8	*L. ultunensis*, 91 *L. aviarus* ssp. *aviarus*,90 *L. acidophilus,* 84
93,	*L. acidophillus* 3, 87.8	*L. acidophilus,* 91
94,	*L. acidophillus* 3, 93.9	*L. acidophilus,* 91
96,	*L. acidophillus* 3, 93.8	*L. acidophilus,* 88
97,	*L. acidophillus* 3, 72.7	*L. acidophilus,* 88
98,	*L. acidophillus* 3, 87.9	*L. acidophilus,* 88
99.	*L. acidophillus* 3, 95.3	*L. acidophilus,* 88
L. plantarum biotype 1 IBNA 84	*L. plantarum* 1, 15.4	*L. nantensis*, 92 *L. plantarum*, 90

L. delbrueckii ssp. *delbrueckii* IBNA 72, 77.	*L. delbrueckii* ssp. *delbruecki,* 98.5 *L. delbrueckii* ssp. *delbruecki,* 78	*L. delbrueckii* ssp. *delbruecki,* 95 *L. delbrueckii* ssp. *delbruecki,* 92
L. fermentum biotype 1 IBNA 71,	*L. fermentum* 1, 99.8	*L. fermentum*, 92
75,	*L. fermentum* 1, 97.4	*L. similis*, 88 *L. fermentum*, 85
78,	*L. fermentum* 1, 97.4	*L. similis*, 89 *L. fermentum*, 85
85,	*L. fermentum* 1, 96.9	*L. similis*, 89 *L. fermentum*, 86
90,	*L. fermentum* 1, 97.3	*L. similis*, 92 *L. fermentum*, 88
92,	*L. fermentum* 1, 99.1	*L. similis*, 85 *L. fermentum*, 85
95.	*L. fermentum* 1, 95.9	*L. similis*, 95 *L. fermentum*, 92
L. salivarius IBNA 86,	*L. salivarius,* 96.5	*L. nantensis,* 99 *L. agilis*, 95 *L. plantarum*, 95 *L. salivarius*, 87
87,	*L. salivarius,* 99.9	*L. salivarius,* 94
100.	*L. salivarius,* 99.9	*L. salivarius,* 97

For apiweb identification is presented the % ID (percentage of identification), and % SIM for ABIS (percentage of similarity with respectively specie).

Table 4. The viability of *Lactobacillus* spp. strains preserved at 4°C and room temperature

Strains	Viability at 4°C	Viability at room temperature
L. acidophilus biotype 1 IBNA 88	≥ 45 days	< 45 days
L. acidophilus biotype 2 IBNA 91	≥ 45 days	≥ 45 days
L. acidophilus biotype 3 IBNA 70,	≥ 38 days	55 days
76,	≥ 40 days	< 70 days
79,	≥ 40 days	< 60 days
81,	53 days	< 45 days
89,	45 days	45 days
93,	< 55 days	≥ 56 days
94,	< 55 days	< 55 days
96,	< 45 days	≥ 45 days
97,	< 45 days	< 45 days
98,	< 45 days	< 45 days
99.	nd*	nd*
L. plantarum biotype1 IBNA 84	≥ 45 days	< 45 days
L. delbrueckii ssp. *delbrueckii* IBNA	55 days	38 days
72,	≥ 40 days	< 70 days
77.		
L. fermentum biotype 1 IBNA 71,	55 days	45 days
75,	3.5 months	2.5 months
78,	≥ 4 months	2 months
85,	≥ 4 months	45 days
90,	≥ 45 days	≥ 45 days
92,	≥ 56 days	< 55 days
95.	≥ 45 days	≥ 45 days
L. salivarius IBNA 86,	< 45 days	< 45 days
87,	< 45 days	< 45 days
100.	nd*	nd*

*= not determinated

DISCUSSION

L. fermentum and *L. plantarum* are included in the itinerant lifestyle species group of lactobacilli. *L. fermentum* was isolated from plant material, mouth, milk products, sewage, humans faeces, and intestines of pig, rat, cattle, mouse and birds). *L. plantarum* was isolated from insects, vertebrate digestive tract, dairy products, plants, silage, outdoor environments. *L. salivarius* and *L. acidophilus* belongs to the vertebrate adapted lactobacilli group and were isolated from human, pigs, hamsters, horses and birds. *L. delbrueckii* ssp. *delbrueckii* is present in the human and animal intestinal tract (pig, mouse,

rat), and vaginal tract (Duar et al., 2017; Stoica and Sorescu, 2018; Sorescu et al., 2019; Sorescu et al., 2020).

The strains described here, isolated from intestinal content and faeces of 30-107 days old pigs, could be important for developing probiotic compounds for the same species because they are host-adapted and have a high ecological compatibility. This characteristic is relevant in the process of outcompeting the pathogens.

Differentiation of *Lactobacillus* strains was performed as described before by Sorescu, (2019), mainly on the basis of some morphological characters (aspect of bacilli and grouping of them), some cultural characters (colony size, smooth or rough type, colour and degree of transparency/opacity) and especially, biochemical characters (fermentation of L-arabinose, D-ribose, D-xylose, D-galactose, D-fructose, D-mannose, L-rhamnose, D-mannitol, D-sorbitol, N-acetylglucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-lactose, D-melibiose, D-trehalose, D-raffinose, starch, gentibiose). It can be noticed that strains of *Lactobacillus* isolated from pigs generally fermented more carbohydrates (26) than those from turkeys (15) (Sorescu et al., 2019) and chickens (21) (Sorescu et al., 2020), which may interfere with the absorption and metabolism of these carbohydrates in the host gut, if these strains are used in animal nutrition (Ciurescu et al., 2020).

As in turkeys (Sorescu et al., 2019) and chickens (Sorescu et al., 2020; Dumitru et al., 2020a) cases, in the intestinal cecum of weaned piglets (Dumitru et al., 2020b), the numbers of CFU lactobacilli/g were higher (10^7-10^9) than in the ileum (10^5-10^8), obviously especially in the case of isolation of the same species from both intestinal segments (*L. acidophilus* biotype 3, *L. fermentum*, *L. delbrueckii ssp. delbrueckii*). In faeces, the number of lactobacilli was similar to the cecum. *L. acidophilus* biotype 3, *L. delbrueckii ssp. delbrueckii* and *L. salivarius* strains had a relative higher presence (up to 10^9 CFU/g) than other lactobacilli (up to 10^8 CFU/g), which suggests a possible ecologic and, therefore, probiotic advantage for them. This fact is interesting, because the *L. acidophilus* and *L. salivarius* strains are adapted to vertebrate species.

As phenotypic identification systems, both software (apiwebTM and ABIS) proved to be appropriate, especially for L. acidophilus biotype 2 and *L. delbrueckii ssp. delbrueckii*, where the same taxonomic classification was obtained, but with different percentage results, the way of calculating them being different. Instead, for *L. acidophilus* biotype 1 and *L. fermentum* biotype 1, ABIS software is not yet refined enough for exact phenotypic identification.

Capability of probiotic strains to remain viable through storage and GI passage is an important trait during strains selection (Upadastra et al., 2011) and the resistance at 4°C and room temperature are applicable technologically characters of the strains, also. *L. fermentum* biotype 1 isolates resisted for the

longest period of time, up to 4 months at 4°C and up to 2.5 months at room temperature. Strains from other species of *Lactobacillus* were, also, resistant at least 45 days (*L. delbrueckii ssp*. *delbrueckii, L. acidophilus* biotype 3 and *L. acidophilus* biotype 2). These results are useful in screening the phenotypic characters of the candidate strains in order to formulate a probiotic product, involving resistance at least 45 days at 4°C. The commercially successful probiotics were based on their technological robustness, they retaining viability during product shelf-life (O'Toole et al., 2017).

Considering the quantitative level of the *Lactobacillus* strains present in the ecological niche, the resistance at 4°C and room temperature and the %ID/% SIM from identification systems, the *L. fermentum* biotype 1 (IBNA 71, 78), *L. delbrueckii ssp. delbrueckii* (IBNA 72, 77) and *L. acidophilus* biotype 2 (IBNA 91) strains only were selected for further testing of the probiotic characteristics.

CONCLUSIONS

The intestinal content (ileum and cecum) and faeces of twenty weaned piglets (30-107 days old) were used to isolate, phenotypically identify and preserve twenty-six strains of the genus *Lactobacillus* (*L. acidophilus* biotype 1-one strain, *L. acidophilus* biotype 2-one strain, *L. acidophilus* biotype 3-eleven strains, *L. plantarum*-one strain, *L. delbrueckii ssp. delbrueckii*-two strains, *L. fermentum* biotype 1-seven strains and *L. salivarius*-three strains). It was found that the number of lactobacilli in cecum content and faeces of weaned pigs is higher (10^7-10^9 CFU/g) than in the ileum (10^5-10^8 CFU/g) and *L. acidophilus* biotype 3, *L. delbrueckii ssp. delbrueckii* and *L. salivarius* strains had relative higher presence (up to 10^9 CFU/g) than other lactobacilli (up to 10^8 CFU/g). The *Lactobacillus* identification by apiwebTM API50CHL V.5.1, BioMerieux (France) software, and ABIS online software recovered similar results, especially for *L. acidophilus* biotype 2 and *L. delbrueckii* ssp. *delbrueckii*, where the taxonomic classification obtained was the same, but with different percentage results. L*. fermentum* biotype 1, *L. delbrueckii ssp. delbrueckii*, *L. acidophilus* biotype 3 and *L. acidophilus* biotype 2 isolates resisted for the longest period of time. Of the isolated Lactobacillus strains, those from *L. fermentum* biotype 1, *L. delbrueckii ssp. delbrueckii*, and *L. acidophilus* biotype 2 are technically and ecologically suitable as potential probiotics and worth continuing the testing of their probiotic qualities.

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