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Original paper

Isolation, identification, preservation and determinations of lactic acid bacteria from chicken's gut content at 26 days old

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Abstract Chichens's gastrointestinal (GI) tract presents a large bacterial diversity and can be a beneficial source in order to the isolation of probiotics candidates. In this study, twenty-three strains from *Lactobacillus* genus (one strain of *L. acidophilus* biotype 1, eight strains of *L. acidophilus* biotype 3, one of *L. crispatus*, five of *L. fermentum* biotype 1, two of *L. brevis* biotype 2, five of *L.* salivarius and one strain of *L. delbrueckii* subsp. delbrueckii) were isolated, cultured, and conserved from ileum and cecum digesta of sixteen chickens, 26 days old, based on their phenotypic characters. The strains confirmation was done by apiwebTM API 50 CHL V.5.1, BioMerieux (France) software, and ABIS online soft. The *Lactobacillus* strains (10^6-10^8 CFU/g intestinal content) level in the selective medium were determined. The growth of *Lactobacillus* strains conserved at 4°C (from 48 days to 7 months) and room temperature (from 48 to 90 days) was evaluated. This study showed that chickens can be a stable source for isolating possibly active probiotic lactic acid bacteria (LAB). From *Lactobacillus* strains isolated and identified, respectively *L. fermentum* biotype 1 and *L. brevis* biotype 2 are theoretically suitable for continual testing of probiotic properties.

Keywords *Lactobacillus* spp., gut, chicken, phenotypic identification, preservation.

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Introduction

Gastrointestinal (GI) health is a prime focus of interest in poultry nutrition and production. The gastrointestinal microbiota and their metabolic products play an important role in digestion, absorption, and metabolism of nutrients, and improve the overall health and growth performance of poultry (YADAV & JHA [1], SLIZEWSKA & al [2], RODRIGUES & al [3]). The composition of the GI microflora in animals is very relevant and may protect hosts from pathogens (BARBOSA & al [4], CIURESCU & al [5], DUMITRU & al [6]).

Lactobacillus species are one of the predominant bacterial genera in the GI tract of poultry, non-pathogenic with a record of harmless use as probiotic bacteria (AMIT-ROMACH & al [7], DUAR & al [8], WAITE & TAILOR [9], WEI & al [10], LU & al [11]). LABs are proposed to reestablish the ecological balance of the gut and mucosal membranes integrity (AAZAMI & al [12]). During the first 2 to 4 d post-hatch, streptococci and enterobacteria colonize the small intestine and cecum of chicks. After the first week, *Lactobacillus* becomes a significant component of the small intestine (AAZAMI & al [12], ZHU & al [13], SWIDA & BINEK [14], NOOHI & al [15]). AZIZ & al [16] affirmed that approximately 70% of the microbial flora from different sections of the chickens' GI vary; for example, ileum chickens are composed of autochthonous *Lactobacillus* spp., while the caecum segment becomes more conquered by lactobacilli as the animal ages.

Probiotics, or direct-fed microbials, have been defined as *"live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"* (PINEIRO & STANTON [17]). Administration of the probiotic based on *Lactobacillus* spp. in poultry feed progresses not only the feed digestion process, as well as nutrient absorption. Moreover, probiotics administration improves the growth of animal performance, increases the immune responses, and counterbalancing several enterotoxins (AL-KHALAIFA & al [18]). Furthermore, probiotics supplementation diminish the risk of GI colonization with foodborne pathogens, such as *Campylobacter* (GHAREEB & al [19], NEAL-McKINNEY & al [20], KHAN & al [21]), *Clostridium* (LI & al [22], DUMITRU & al [23]) and *Salmonella* (KIZERWETTER-SWIDA & BINEK, [24], TELLEZ & al [25], KOWALSKA & al [26]), and intensify the poultry diets security (GAGGÌA & al [27]).

Many strains of *Lactobacillus* have been selected as probiotics based on their *in vitro* inhibitory effects on chicken pathogenic bacteria (KOWALSKA & al [26], FENG & al [28], REUBEN & al [29]). Moreover, ZOU & al [30] demonstrated, by meta-analysis, that *Lactobacillus* elicit a differentiating effect on the chicken cecum microbiom, the majority of taxa displaying significant numbers of negative (*Lachnospiraceae, Ruminococcaceae*) or positive (*Lactobacilli*, *Bacteroides*, *Clostridiales* and *Christensenellaceae*) relationships with *Lactobacillus*, suggesting a major influential role for this genus.

This study aimed to isolate, characterize, and assess *Lactobacillus* spp. from the GI content of 26 days old broiler chickens with special attention to their properties as a possible probiotic candidate in poultry feed.

Materials and Methods

The experimental trial was agreed by the Ethical Committee of the National Research Development Institute for Biology and Animal Nutrition (IBNA Balotesti), in agreement with Romanian legislation (Law 305/2006, Directive 2010/63/EU) for supervision and safety of animals used for experimental purposes.

1. Isolation and phenotypic characterization of Lactic acid bacteria

The intestinal content from sixteen randomly selected healthy chickens (26 days old) was collected aseptically in sterile plastic tubes and processed for Lab's isolation. One gram of ileum and cecum digesta of each chicken was aseptically transferred and homogenized into 15 mL sterilized tube with 7 mL BHI broth (Brain Heart Infusion, Oxoid, Ltd., Hampshire, England) and 2 mL glycerol, followed by frozen at –20°C until testing. After defrost the method of MOUNTZOURIS & al [31] and completed by SORESCU & al [32] with Gram staining of smears from colonies morphology confirmation of *Lactobacillus* by microscopic examination was followed. The catalase test was done and CFU/g gut content was determined.

2. Identification of bacterial strains

Isolated bacteria were phenotypic identified based on Bergey's Systematic Manual Bacteriology, ABIS online, and APIweb™ API 50CHL soft BioMerieux (France), as described by several authors (SORESCU & al [32]; STOICA & SORESCU [33]; PELINESCU et al [34]).

3. Lactic acid bacteria preservation

Bacterial strains viability was tested after 48-90 days, 4 and 7 months. The preservation for an extensive period (2 years) was done at -80°C, with supplementation of 20% glycerol.

Results and Discussion

The isolated were pre-identified by Gram staining, catalase reaction, and taxonomically classified as fallow: morphological (Gram-positive bacteria with non-spore-forming rods), cultural (anaerobic conditions), and biochemical aspects (negative catalase result).

Based on biochemical properties, *Lactobacillus* spp. were identified with the application of API 50CHL test. Accordingly, twenty-three strains of the *Lactobacillus* genus belonging to 6 species (*L. acidophilus* biotype 1 IBNA 21, *L. acidophilus* biotype 3 IBNA 23, 26-28, 30, 35, 36, 40, *L. crispatus* IBNA 22, *L. brevis* biotype 2 IBNA 24, 31, *L. fermentum* biotype 1 IBNA 25, 32, 37, 42, 43, *L. delbrueckii* subsp. *delbrueckii* IBNA 38 and *L. salivarius* IBNA 29, 33, 34, 39, 41) were isolated on MRS medium, identified and preserved from sixteen chickens (26 days old) intestinal content (ileum and cecum).

Figures 1-3 illustrate smears (Gram staining, x 1000) from *L. acidophilus* biotype 3 IBNA 35, *L. fermentum* biotype 1 IBNA 37 and *L. salivarius* IBNA 33 cultures in MRS broth medium.

The taxonomic criteria for identified and selecting *Lactobacillus* strains to be used as probiotics in poultry feed is summarized in Table 1.

Table 1. Phenotypical characters of *Lactobacillus* strains isolated from chicken's tract (26 days old)

Tests	**Strains**						
	1	2	3	4	5	6	7
Morphological	a	a	c	c	a, c	a	b
Cultural	x	x	y	y	x, y	x	y
Catalase	0(1)*	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
Fermentation (API 50CHL)							
glycerol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
erythritol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-arabinose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
L-arabinose	0(1)	0(8)	1(1)	2(2)	3(5)	0(1)	0(5)
D-ribose	0(1)	0(8)	1(1)	2(2)	3(5)	0(1)	0(5)
D-xylose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
L-xylose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-adonitol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
Methyl-βD-xylopyran.	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-galactose	1(1)	3(8)	1(1)	2(2)	5(5)	0(1)	5(5)
D-glucose	1(1)	8(8)	1(1)	2(2)	5(5)	1(1)	5(5)
D-fructose	1(1)	8(8)	1(1)	2(2)	2(5)	1(1)	5(5)
D-mannose	1(1)	6(8)	0(1)	1(2)	1(5)	1(1)	5(5)
L-sorbose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
L-rhamnose	?(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
dulcitol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
inositol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	5?(5)
D-mannitol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	5(5)
D-sorbitol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
Methyl-αD-mannopyr.	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
Methyl-αD-glucopyr.	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
N-acetylglucosamine	1(1)	8(8)	1(1)	2(2)	0(5)	1(1)	5(5)
amygdalin	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
arbutin	?(1)	0(8)	1(1)	0(2)	0(5)	0(1)	0(5)
esculin	1(1)	8(8)	1(1)	2(2)	4(5)	1(1)	5(5)
salicin	1(1)	0(8)	1(1)	2(2)	0(5)	0(1)	0(5)
D-cellobiose	1(1)	1(8)	1(1)	2(2)	0(5)	0(1)	0(5)
D-maltose	?(1)	2(8)	1(1)	2(2)	4(5)	0(1)	5(5)
D-lactose	0(1)	0(8)	1(1)	2(2)	4(5)	0(1)	5(5)
D-melibiose	1(1)	0(8)	1(1)	2(2)	5(5)	0(1)	5(5)
D-saccharose	1(1)	8(8)	1(1)	2(2)	5(5)	?(1)	5(5)
D-trehalose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	5(5)
inulin	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-melezitose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-raffinose	1(1)	7(8)	1(1)	2(2)	5(5)	?(1)	5(5)
amidon (starch)	0(1)	0(8)	1(1)	1(2)	0(5)	0(1)	0(5)
glycogen	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
xylitol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
gentibiose	1(1)*	1(8)	0(1)	2(2)	0(5)	0(1)	0(5)
D-turanose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-lyxose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-tagatose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-fucose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
L-fucose	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
D-arabitol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
L-arabitol	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
potassium gluconate	0(1)	0(8)	0(1)	2(2)	2(5)	0(1)	0(5)
potassium 2-ketogluc.	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)
potassium 5-ketogluc.	0(1)	0(8)	0(1)	0(2)	0(5)	0(1)	0(5)

1=*L. acidophilus* biotype 1 IBNA 21; 2= *L. acidophilus* biotype 3 IBNA 23, 26-28, 30, 35, 36, 40; 3= *L. crispatus* IBNA 22; 4= *L. brevis* biotype 2 IBNA 24, 31; 5= *L. fermentum* biotype 1 IBNA 25, 32, 37, 42, 43; 6= *L. delbrueckii* subsp.*delbrueckii* IBNA 38; 7= *L. salivarius* IBNA 29, 33, 34, 39, 41;

a= Gram positive, non-spore forming rods, congregated in pairs or chains, filaments and, rarely, in palisade;

b= Gram positive short rods, with rounded end, non-spore forming, arranged in pairs, short chains or small, 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, up to 2.0 mm, smooth type, round, opaque, transparent or semi-transparent, whitish, grey or colourless on MRS agar plate;

y= large colonies, 2.0-4.0 mm in diameter, rarely smaller, smooth type, round, white, opaque;

*= number of positive strains from number of tested strains;

?= weekly, positive, dubious.

Figure 1. *L. acidophilus* biotype 3 IBNA 35

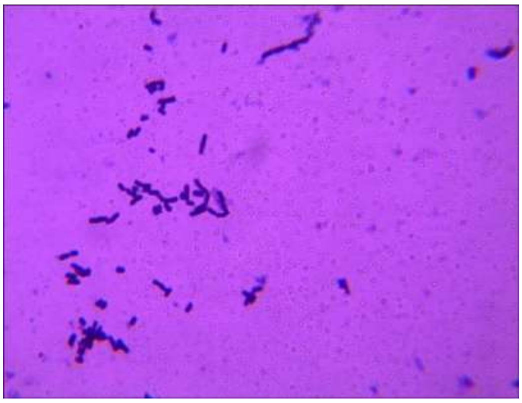

Figure 2. *L. fermentum* biotype 1 IBNA 37

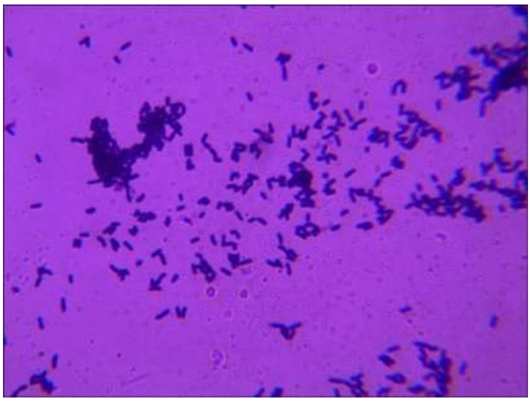

Figure 3. *L. salivarius* IBNA 33

A total of 23 LAB strains were isolated from the gut content of healthy broiler chickens (26 days old).

Morphological characters of bacilli, a grouping of them, cultural aspects (colony size, colour and degree of opacity/transparency) and specifically, biochemical properties (fermentation of L-arabinose, D-ribose, D-galactose, D-fructose, D-mannose, D-glucose, inositol, D-mannitol, N-acetylglucosamine, salicin, arbutin, esculin, D-cellobiose, D-maltose, D-melibiose, D-lactose, D-saccharose, D-raffinose, starch, gentibiose, and potassium gluconate) involve importantly differences between isolates cultures. Carbohydrate tests produced a blue color for a positive result or red-orange color for a negative result agreeing to manufacturer instructions. It can be remarked that *Lactobacillus* isolates from chickens ferment more carbohydrates (21) than those from turkeys (15) (SORESCU & al [32]), which may interfere with the absorption and digestion of these sugars in the host gut segment if they are used in diets of poultry.

In Table 2 are presented the origin and the quantitative level of the isolate's occurrence in chicken's digesta.

Table 2. *Lactobacillus* spp. strains existence in chickens' intestinal content (26 days old)

Strains types	**Source, sample number**	**CFU/g (log10)**
L. acidophilus biotype 1 IBNA 21	cecum content, 28.	7.845 + E
L. acidophilus biotype 3 IBNA 23, 26-28, 30, 35, 36, 40	ileum content, 29, 31; cecum content, 32; ileum content, 35; cecum content, 37; ileum content, 43; cecum content, 44, 46.	7.301 + E; 8.176 + E; 7; 6.698 + E; 8.477 + E; 7.079 + E; 8.845 + E; 8.903 + E
L. crispatus IBNA 22	ileum content, 29.	6
L. brevis biotype 2 IBNA 24, 31	cecum content, 30, 38.	8.845 + E; 7.778 +E
L. fermentum biotype 1 IBNA 25, 32, 37, 42, 43	ileum content, 31; cecum content, 41, 44, 49, 49.	7.698 + E; 8.447 + E; 8.301 + E; 7.875 +E; 8.544 + E
L. delbrueckii subsp. *delbrueckii* IBNA 38	cecum content, 45.	7.698 + E
L. salivarius IBNA 29, 33, 34, 39, 41	cecum content, 35, 41, 42, 45; ileum content 48.	8.477 + E; 8.361 + E; 8; 8.113 + E; 6.954 + E

Unlike turkeys where, at the corresponding serial dilutions in PBS buffer, *Lactobacillus* spp. developed as pure culture bacteria, with one exclusion (when co-exist *L. fermentum* IBNA 18 and *L. salivarius* IBNA 19, so two strains from nine isolated are mixed), in chickens ten isolates from twenty-three co-exist with strains from other or same species of *Lactobacillus* sp. *L. acidophilus* biotype 3 co-exist with *L. fermentum* biotype 1 or L. *crispatus*, *L. salivarius* with *L. fermentum* biotype 1 and, interesting, co-exist 2 strains of *L. fermentum* biotype 1, IBNA 42 and 43, which differ through some morphologically, culturally and biochemically characters. This polymorphism and variability are highlighted in Table 1.

In the cecum content, the CFU number of *Lactobacillus*/g is higher (7-8 $\log_{10}$) than in the ileum (6-7 $\log_{10}$).

Table 3 presents the results of LAB strains identification by APIweb^{TM} soft, API50 CHL V.5.1, BioMerieux (France), and ABIS online soft.

Table 3. Parallel LABs identification by APIweb^{TM} soft, API50CHL V.5.1, BioMerieux (France) and ABIS online

Strains	**API, % ID**	**ABIS, % SIM**
L. acidophilus biotype 1, IBNA 21	*L. acidophilus* 1, 91.7	*L. acidophilus*, 92
L. acidophilus biotype 3, IBNA 23 IBNA 26 IBNA 27 IBNA 28 IBNA 30 IBNA 35 IBNA 36 IBNA 40	*L. acidophillus* 3, 97.7 *L. acidophillus* 3, 95.6 *L. acidophillus* 3, 99.9 *L. acidophillus* 3, 95.6 *L. acidophillus* 3, 95.6 *L. acidophillus* 3, 97.7 *L. acidophillus* 3, 98.6 *L. acidophillus* 3, 98.1	*L. kunkeii*, 91 *L. curvatus*, 89 *L. acidophilus*, 81 *L. delbrueckii*, 92 *L. acidophilus*, 78 *L. curvatus*, 86 *L. acidophilus*, 78 *L. delbrueckii*, 92 *L. acidophilus*, 78 *L. delbrueckii*, 92 *L. acidophilus*, 78 *L. kunkeii*, 91 *L. curvatus*, 89 *L. acidophilus*, 81 *L. kunkeii*, 91 *L. curvatus*, 89 *L. acidophilus*, 81 *L. delbrueckii*, 89 *L. acidophilus*, 79
L. crispatus, IBNA 22	*L. crispatus* 90.2	*L. acidophilus* 92
L. brevis biotype 2: IBNA 24 IBNA 31	*L. brevis* 2, 94.6 *L. brevis* 2, 94.3	*L. oris*, 93 *L. brevis* (possibility of *L. buchneri*), 90 *L. brevis* (possibility of *L. buchneri*), 94
L. fermentum biotype 1: IBNA 25 IBNA 32 IBNA 37 IBNA 42 IBNA 43	*L. fermentum* 1, 96.9 *L. fermentum* 1, 88.3 *L. acidophilus* 3, 10.9 *L. fermentum* 1, 99.1 *L. fermentum* 1, 91.3 *L. fermentum* 1, 97	*L. similis*, 95 *L. fermentum* (possibility of *L. reuteri*), 92 *L. kefiranofaciens*, 94 *L. acidophilus*, 92 *L. fermentum* (possibility of *L. reuteri*), 85 *L. fermentum* (possibility of *L. reuteri*), 92 *L. kefiranofaciens*, 95 *L. fermentum* (possibility of *L. reuteri*), 85 *L. antri*, 91 *L. similis*, 91 *L. mucosae*, 90 *L. fermentum* (possibility of *L. reuteri*), 88
L. delbrueckii subsp. *delbrueckii*, IBNA 38	*L. delbrueckii* ssp. *delbrueckii*, 60.2	*L. delbrueckii* ssp. *delbrueckii*, 91
L. salivarius: IBNA 29 IBNA 33 IBNA 34 IBNA 39 IBNA 41	*L. salivarius*, 98.9 *L. salivarius*, 98.9 *L. salivarius*, low discrimination *L. salivarius*, low discrimination *L. salivarius*, 98.9	*L. salivarius*, 94 *L. salivarius*, 90 *L. salivarius*, 94 *L. salivarius*, 97 *L. salivarius*, 91

Identification by APIweb is show the % ID (% identification), respectively % SIM for ABIS (% similarity).

L. acidophilus biotype 1, *L. delbrueckii* ssp. *delbrueckii*, *L. salivarius* and *L. brevis* biotype 2 were obtained a similar systematic classification, sometimes even with identical percentage results. Instead, for *L. acidophilus* biotype 3, which is biochemically close to *L. fermentum*, and for *L. fermentum* biotype 1, ABIS soft is not yet advanced enough for precise identification.

CATO & al [35]) reported that *L. crispatus* is similar to *L. acidophilus* group A2 because the strains of *L. crispatus* and *L. acidophilus* group A2 involve 100% DNA homology. So, the *L. crispatus* IBNA 22 strain was identified as *L. acidophilus* by the ABIS software, *L. crispatus* species being not registered in the ABIS database.

Lactobacillus types viability which are conserved at 4°C and room temperature are shown in Table 4.

Table 4. Viability of *Lactobacillus* spp. conserved at 4°C and room temperature

Strains	**4°C**	**Room temperature**
L. acidophilus biotype 1		
IBNA 21	< 48 days	< 48 days
L. acidophilus biotype 3:		
IBNA 23	< 48 days	< 48 days
IBNA 26	48 days	48 days
IBNA 27	< 48 days	< 82 days
IBNA 28	< 48 days	< 90 days
IBNA 30	< 48 days	< 90 days
IBNA 35	51 days, a single passage	51 days, a single passage
IBNA 36	60 days	< 51 days
IBNA 40	< 51 days	< 51 days
L. crispatus IBNA 22	48 days	< 90 days
L. brevis biotype 2:		
IBNA 24	7 months, a single passage	< 60 days
IBNA 31	4 months, a single passage	66 days, a single passage
L. fermentum biotype 1:		
IBNA 25	7 months	< 3 months
IBNA 32	60 days	60 days
IBNA 37	90 days	51 days, a single passage
IBNA 42	90 days	60 days
IBNA 43	6 months, a single passage	90 days
L.delbrueckii ssp. delbrueckii, IBNA 38	< 48 days	< 48 days
L. salivarius:		
IBNA 29	48 days, a single passage	48 days, a single passage
IBNA 33	< 48 days	< 48 days
IBNA 34	< 51 days	< 51 days
IBNA 39	< 48 days	< 48 days
IBNA 41	< 51 days	< 51 days

L. fermentum strains survived 2-7 months at 4°C and 51-90 days at room temperature, and *L. brevis* strains lived 4-7 months at 4°C (a single passage), and 66 days at room temperature (a single passage), one strain from two tested. Most of *L. acidophilus*, *L. delbrueckii*, *L. salivarius* and *L. crispatus* isolates not survive 48 days at 4°C and room temperature.

The origin of *L. salivarius* and *L. acidophilus* as bacterial source from humans, hamsters, horses, pigs, turkeys, and chickens, included them in the vertebrate adapted lifestyle lactobacilli group (DUAR & al [8], SORESCU & al [32]). DUAR & al [8] affirmed that *L. fermentum* makes part from nomadic species group of lactobacilli and generally, was found in different sources as: milk-based products, fermenting vegetable material, sewage, mouth and humans' faeces, and intestines of pig, cattle, rat, mouse and birds, including turkeys.

As a host-adapted, *Lactobacillus* strains from chicken's intestinal tract, have high natural suitability, and so will be beneficial for evolving a probiotic multifarious in the poultry field. Moreover, higher fitness is relevant to outcompete pathogens.

SORESCU & al [32] reported that the numbers of lactobacilli in turkey's cecum are higher (10^7-10^8) compared to the ileum digesta (10^6-10^7). Unlike the turkeys (perhaps due to the age interims-73 versus 26-and the species), *L. fermentum* has relative same presence to other lactobacilli in the GI content. This detail is motivating, because the *L. fermentum* is supposed to be nomadic species, while the *L. salivarius* and *L. acidophilus* is adapted to species of vertebrate. Besides, a representative level of *Lactobacillus* colonies in animal digesta can propose a better adaptation to the ecological niche and can naturally proliferate and involve better effects than other types of *Lactobacillus* strains isolated from other sources.

As phenotypical confirmation, APIweb$^{\text{TM}}$ and ABIS system demonstrated to be advantageous, expressly for *L. acidophilus* biotype 1, *L. delbrueckii* ssp. *delbrueckii*, *L. salivarius* and *L. brevis* biotype 2, where was obtained the identical identification.

The resistance at 4°C is a relevant strain property. An elongated resistance involves a positive strains property through their selection. *L. fermentum* biotype 1 and *L. brevis* biotype 2 isolates have resisted for the longest time. To prepare a probiotic product, the present results are suitable in screening the phenotypic characters where the resistance at least two months at 4°C is very important to analyze.

From this aspect, *L. fermentum* and *L. brevis* strains will be carefully chosen for testing several probiotic properties. Turkey strains of *L. fermentum* were previously selected for the same reason (SORESCU & al [32]). In the literature data, this specific information was not found maybe of the benefices of probiotic products of industrial manufacturers. Anyway, it is known that the capability of probiotics to live during culture storage and to tolerate the passage through the stomach and to resist in the small intestine area important criterion of *in vitro* selection (UPADRASTA & al [36]). PAPADIMITRIOU & al [37] noticed that, generally, the strain viability, and the growth phase of the probiotic cell wall, seem to involve significant interaction with the host, so on its performance.

Conclusions

In conclusion, our results indicate that the cecum segment involves a higher number of lactobacilli (10^7-10^8 CFU/g) than in the ileum (10^6-10^7 CFU/g), but unlike the turkeys, *L. fermentum* has relative same presence to other lactobacilli in the intestinal content. From isolated *Lactobacillus* strains, those from *L. fermentum* biotype 1 and *L. brevis* biotype 2 are technically apt for continual *in vitro* testing of potential as probiotic source for poultry.

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Conflict of Interest

The authors have no conflict of interest to report.

References

1. S. YADAV and R. JHA. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. *J Anim Sci Biotechnol* 10, 2 (2019). https://doi.org/10.1186/s40104-018-0310-9
2. K. SLIZEWSKA, P. MARKOWIAK-KOPEC, A. ZBIKOWSKI, and P. SZELESZCZUK. The effect of symbiotic preparations on the intestinal microbiota and her metabolism in broiler chickens. *Sci Rep* 10, 4281 (2020). https://doi.org/10.1038/s41598-020-61256-z
3. D.R. RODRIGUES, W. BRIGGS, A. DUFF, K. CHASSER, R. MURUGESAN, C. PENDER, S. RAMIREZ, L. VALENZUELA, and L. R. BIELKE. Comparative effectiveness of probiotic-based formulations on cecal 3 microbiota modulation in broilers. *PLoS One* 15, e0225871 (2020). https://doi.org/10.1371/journal.pone.0225871
4. BARBOSA, V. BALAGUÉ, F. VALERA, A. MARTÍNEZ, J. BENZA, M. MOTAS and C. PEDRÓS-ALIÓ. Age-related differences in the gastrointestinal microbiota of chinstrap penguins (*Pygoscelis antarctica*). *Plos One* 11(4), e0153215 (2016). doi:10.1371/journal.pone.0153215
5. C. CIURESCU, M. DUMITRU, A. GHEORGHE, A. UNTEA, and R. DRAGHICI. Effect of *Bacillus subtilis* on growth performance, bone mineralization, and bacterial population of broilers fed with different protein sources. *Poult Sci* 99(11), 5960-5971 (2020). https://doi.org/10.1016/j.psj.2020.08.075
6. M. DUMITRU, M. HĂBEANU, N. LEFTER and A. GHEORGHE, 2020. The effect of *Bacillus licheniformis* as direct-fed microbial product on growth performance, gastrointestinal disorders and microflora population in weaning piglets. *Rom Biotechnol Lett* 25(6), 2060-2069. doi: 10.25083/rbl/25.6/2060.2069
7. E. AMIT-ROMACH, D. SKLAN, and Z. UNI. Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. *Poult Sci* 83, 1093-1098 (2004).
8. R.M. DUAR, X.B. LIN, J. ZHENG, M.E. MARTINO, T. GRENIER, M.E. PEREZ-MUNOZ, F. LEULIER, M. GANZLE and J. WALTER. Lifestyles in transition: evolution and natural history of the genus *Lactobacillus. FEMS Microbiol Rev* 30(41), 27-48 (2017).
9. D.W. WAITE and M.W. TAILOR. Characterizing the avian gut microbiota: membership, driving influences, and potential function. *Front Microbiol* 5, article 223, 1-12, (2014).
10. S. WEI, M. MORRISON, and Z. YU. Bacterial census of poultry intestinal microbiome. *Poult Sci* 92(3), 671-683 (2013).
11. J. LU, U. IDRIS, B. HARMON, C. HOFACRE, J.J. MAURER and M.D. LEE. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Appl Environ Microbiol* 69(11), 6816-6824 (2003).
12. N. AAZAMI, G.S. JOUZANI, Z. KHODAEI, A. MEIMANDIPOUR, M. SAFARI and M. GOUDARZVAND. Characterization of some potentially probiotic *Lactobacillus* strains isolated from Iranian native chickens. *J. Gen. Appl Microbiol* 60, 215-221 (2014).
13. X.Y. ZHU, T. ZHONG, Y. PANYA and R.D. JORGER. 16S rRNA-based analysis of microbiota from the cecum of broiler chickens. *Appl Environ Microbiol* 68, 124-137 (2002).
14. M. KIZERWETTER-SWIDA and M. BINEK. Assessment of potentially probiotic properties of *Lactobacillus* strains isolated from chickens. *Pol J Vet Sci* 19, 15-20 (2016).
15. N. NOOHI, G. EBRAHIMIPOUR, M. ROHANI, M. TALEBI and M. POURSHAFIE. Phenotypic characteristics and probiotic potentials of *Lactobacillus* spp. isolated from poultry. *Jundishapur J Microbiol* 7:e17824 (2014).

16. G. AZIZ, H. FAKHAR, S. UR. RAHMAN, T. MUHAMAMMAD, and A. ZAIDI. An assessment of the aggregation and probiotic characteristics of *Lactobacillus* species isolated from native (desi) chicken gut. *Journal of Applied Poultry Research* 28(4), 846-857 (2019). doi:10.3382/japr/pfz042
17. M. PINEIRO, and C. STANTON C. Probiotic bacteria: legislative framework-requirements to evidence basis. *J Nutr* 137(3), 850S–853S (2007).
18. H. AL-KHALAIFA H, A. AL-NASSER, T. AL-SURAYEE, S. AL-KANDARI, N. AL-ENZI, T. AL-SHARRAH, G. RAGHEB, S. AL-QALAF, and A. MOHAMMED. Effect of dietary probiotics and prebiotics on the performance of broiler chickens. *Poult Sci* 98(10), 4465-4479 (2019).
19. K. GHAREEB, W.A. AWAD, M. MOHNL, R. PORTA, M. BIARNES, J. BÖHM and G. SCHATZMAYR. Evaluating the efficacy of an avian-specific probiotic to reduce the colonization of *Campylobacter jejuni* in broiler chickens. *Poult Sci* 91(8), 1825-1832 (2012).
20. J.M. NEAL-MCKINNEY, X. LU, T. DUONG, C.L. LARSON, D.R. CALL, D. H. SHAH and M.E. KONKEL. Production of organic acids by probiotic lactobacilli can be used to reduce pathogen load in poultry. *PloS One* 7, e43928 (2012). https://doi.org/10.1371/journal.pone.0043928
21. M. KHAN, A.A. ANJUM, M. NAWAZ, A.R. AWAN, and M.A. ALI. Effect of newly characterized probiotic lactobacilli on weight gain, immunomodulation and gut microbiota of *Campylobacter jejuni* challenged broiler chicken. *Pak Vet J*, 39(4): 473-478. (2019). http://dx.doi.org/10.29261/pakvetj/2019.051
22. Z. LI, W. WANG, D. LIU and Y. GUO. Effects of *Lactobacillus acidophilus* on gut microbiota composition in broilers challenged with *Clostridium perfringens*. *PloS One* 12, e0188634 (2017). https://doi.org/10.1371/journal.pone.0188634
23. M. DUMITRU, M. HABEANU, N. LEFTER. Effects of direct-feed microbial *Bacillus* supplementation on piglet's microbiota. *Scientific Bulletin. Series F. Biotechnologies* 24(2), 154-160 (2020). ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364.
24. M. KIZERWETTER-SWIDA, and M. BINEK. Protective effect of potentially probiotic *Lactobacillus* strain on infection with pathogenic bacteria in chickens. *Pol J Vet Sci* 12(1), 15-20 (2009).
25. G. TELLEZ, C. PIXLEY, R. WOLFENDEN, S. LAYTON, and B. HARGIS. Probiotics/direct fed microbials for *Salmonella* control in poultry. *Food Res Int* 45, 628-633 (2012).
26. J. D. KOWALSKA, A. NOWAK, K. ŚLIŻEWSKA, M. STAŃCZYK and J. DASTYCH. Anti-*Salmonella* potential of new *Lactobacillus* strains with the application in the poultry industry. *Pol J Microbiol* 69(1), 5-18 (2020).
27. F. GAGGÌA, P. MATTARELLI, and B. BIAVATI. Probiotics and prebiotics in animal feeding for safe food production. *Int J Food Microbiol* 141, S15-S28 (2010).
28. J. FENG, L. WANG, L. ZHOU, X. YANG and X. ZHAO. Using in vitro immunomodulatory properties of lactic acid bacteria for selection of probiotics against *Salmonella* infection in broiler chicks. *PLoS One* 11, e0147630 (2016). https://doi.org/10.1371/journal.pone.0147630
29. R.C. REUBEN, P.C. ROY, S.L. SARKAR, R.U. ALAM and I.K. JAHID. Isolation, characterization, and assessment of lactic acid bacteria toward their selection as poultry probiotics. *BMC Microbiol* 19, 253 (2019). https://doi.org/10.1186/s12866-019-1626-0
30. A. ZOU, S. SHARIF and J. PARKINSON. *Lactobacillus* elicits a 'Marmite effect' on the chicken cecal microbiome. *Nature partner journals*, *Biofilms and Microbiomes* 4(27), 1-5 (2018). doi: 10.1038/s41522-018-0070-5
31. K.C. MOUNTZOURIS, P. TSIRTSIKOS, E. KALAMARA, S. NITSCH, G. SCHATZMAYR and K. FEGEROS. Evaluation of the efficacy of a probiotic containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. *Poult Sci* 86, 309-317 (2007).
32. I. SORESCU, M. DUMITRU and G. CIURESCU. *Lactobacillus* spp. and *Enterococcus faecium* strains isolation, identification, preservation and quantitative determinations from turkey gut content. *Rom Biotechnol Lett* 24(1): 41-49 (2019). doi: 10.25083/rbl/24.1/41.49
33. C. STOICA and I. SORESCU. ABIS online-Advanced Bacterial Identification Software, an original tool for phenotypic bacterial identification. Regnum Prokaryotae- www.tgw1916.net, september 1 (2017).
34. D.R. PELINESCU, E. SĂSĂRMAN, M.C. CHIFIRIUC, I. STOICA, A.M. NOHIT, I. AVRAM, F. SERBANCEA, and T.V. DIMOV. Isolation and identification of some *Lactobacillus* and *Enterococcus* strains by a polyphasic taxonomical approach. *Rom Biotechnol Lett* 14 (2), 4225-4233 (2009).
35. E.P. CATO, W.E.C. MOORE and J.L. JOHNSON. Synonymy of strains *of Lactobacillus acidophilus* Group A2. (Johnson et al., 1980) with the type strain of *Lactobacillus crispatus* (Brygoo and Aladame, 1953) Moore and Holdeman 1970. *Int J Syst Bacteriol* 33 (2), 426-428 (1983).
36. A. UPADRASTA, C. STANTON, C. HILL, G. FITZGERALD, and R.P. ROSS. Improving the stress tolerance of probiotic cultures: recent trends and future directions. In: E. TSAKALIDOU and K. PAPADIMITRIOU (eds.), Stress Responses of Lactic Acid Bacteria, Springer, New York, 395-438 (2011).
37. K. PAPADIMITRIOU, G. ZOUMPOPOULOU, B. FOLIGNE, V. ALEXANDRAKI, M. KAZOU, B. POT and E. TSAKALIDOU. Discovering probiotic organisms: *in vitro*, *in vivo*, genetic and omics approaches. *Front Microbiol*, *Food Microbiol* 6(58), 1-28 (2015). doi: 10.3389/fmicb.2015.00058