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Isolation of Some Lactic Acid Bacteria from Poultry and Evaluation for Their Probiotic Features

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Abstract

The ban on the use of antibiotics as growth promoters for poultry production in many countries has led to increasing interest in using probiotics as a promising alternative. Thus, the aim of this study is to isolate, screen, and identify probiotics from poultry intestines, and to select probiotic candidates for subsequent in vivo experiments. A total of 60 lactic acid bacterial isolates were recovered from 21 to 42-day-old chickens onto De Man, Rogosa and Sharpe (MRS) agar plates, then screened for their probiotic features using eleven *in vitro* assays. Among 60 LAB isolates, only three (SI5, IL22, and CE55) showed excellent probiotic features such as cell surface hydrophobicity, pH tolerance, survival in bile salts, cholesterol assimilation, NaCl tolerance, auto-aggregation, co-aggregation, hemolytic activity, antimicrobial activity, and lysozyme resistance as well as antibiotic susceptibility tests. The FASTA homology showed that the 16S rRNA gene sequences of the selected isolates had 95.45, 99.88, and 100% nucleotide similarity with that of *Enterococcus faecium* NBRC 100486, *E. faecium* NBRC 100486 and *E. faecium* DSM 20477strains, respectively. Our results also suggest that the new three strains have potential for future application as probiotics in health-promoting foods and have the potential to enhance the immunity of infants against invading pathogenic microbes.

Keywords: Lactic acid bacteria, *Enterococcus*, poultry, antibiotic sensitivity, antimicrobial activity.

Introduction

Antibiotic promoters have been widely used to improve growth performance and protect poultry from pathogens. The overuse of antibiotics causes a lot of problems such as drug resistance in animals and drug residues in animal products, which jeopardize the sustainable development of humans and nature, and it has become a serious issue related to food security **(Rana *et al.*, 2019)**. In order to address this problem, many countries have passed laws prohibiting the use of antibiotics as growth promoters in feed **(Vieco-Saiz *et al.*, 2019)**. Consequently, the selection and use of growth promoters as a replacement for antibiotics has become a hot topic in feed research. Natural and healthful replacements to antibiotics had been developed at this point, such as probiotics, prebiotics, enzymes, and acidifiers as previously mentioned by **Khan *et al.*, (2016) and Zou *et al.*, (2022).**

Probiotics are live microorganisms (bacteria, fungi, or yeasts) that can be used as feed additives or supplements for livestock **(Jha *et al.*, 2020)**. *Bacillus, Bifidobacterium, Lactobacillus, Enterococcus, Pediococcus, Leuconostoc, Escherichia coli*, and *Streptococcus* are among the bacteria species types utilized as probiotics, whereas yeast species include *Saccharomyces cerevisiae* and other *Saccharomyces* **(Andrew Selaledi *et al.*, 2020** and **Derakhshan *et al.*, 2023).** In poultry, probiotics often known as direct-fed microbes, benefit the host gastrointestinal tract health (GIT) by reducing of the dominance of pathogenic bacteria, balancing of microbial populations, and stimulating of immune responses **(Jha *et al.*, 2020) *and* (Andretta *et al.*, 2021).** Probiotic supplements can enhance poultry growth performance, laying traits, bone strength, but there is little information about the effect of probiotics in improving liver health in chickens **(Anee *et al.*, 2021)** and **(Derakhshan *et al.*, 2023)**.

Enterococcus faecium is a lactic acid bacteria that mostly spreads in the intestines of humans and animals **(Krawczyk *et al.*, 2021)**. The supplementation of *E. faecium* to the diet promotes the growth of beneficial organisms in the intestine and inhibits harmful organisms, keeping a healthy gut and allowing the bird to achieve higher growth performance **(Krawczyk *et al.*, 2021)**. *E. faecium*

enhances the concentration of organic acids and bacteriocins, which are crucial for the digestive system due to their nutritive benefits for intestinal cells and pathogen inhibitory activities **(Liu *et al*., 2023)**. Several studies demonstrated that *E. faecium* improves the metabolism, feed conversion, growth performance, intestinal morphology, the immune response and inhibits pathogen proliferation **(Liu *et al*., 2023)**. Furthermore, *E. faecium* inhibits *E. coli*-induced intestinal diseases and manipulates cecal microbiota **(Hanifeh *et al*., 2021)** and **(Zhang *et al*., 2022)**. In this regard, the addition of probiotics to the diet was seen as a promising alternative to antibiotics **(Dev *et al*., 2020).** However, according to the physiological properties of probiotics, it is possible that adding probiotics to the diet of broilers may have a beneficial effect.

This study aimed to isolate and characterize some lactic acid bacteria (LAB) from different parts of the chickens' intestines as well as estimate their properties as probiotics. Finally, most potent isolates were identified to use them as novice probiotics strains.

Materials and Methods

1.1. Collection of samples

The samples were collected from different parts of the intestines of Moshtohor chickens (small intestine, caecum, and ileum), to isolate probiotic bacteria **(Figure 1)**. Six chickens were randomly selected at the age of 21 to 42 days old.

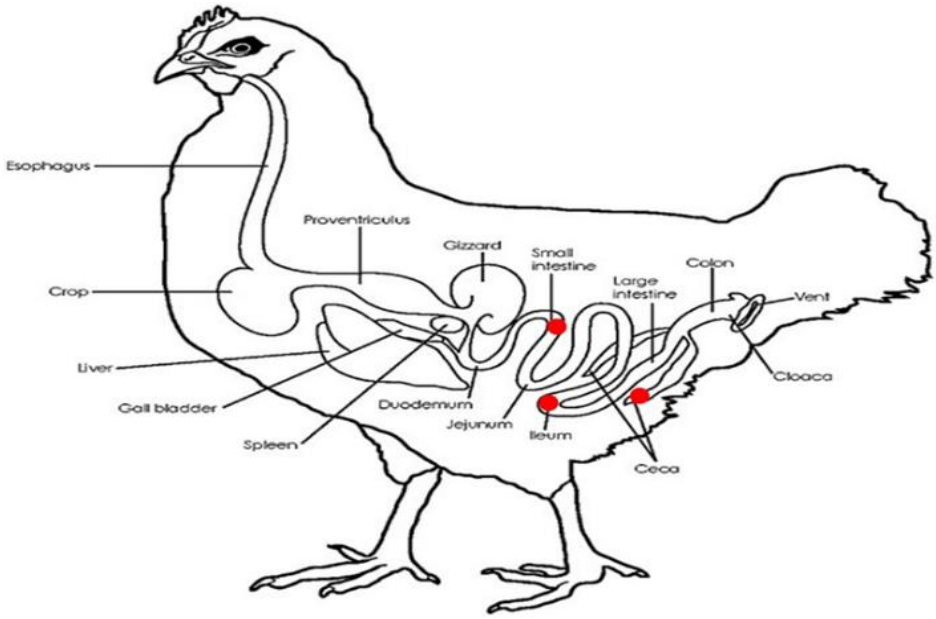


Figure 1. Diagram indicates parts of the intestines of chickens used for isolation.

1.2. Enrichment and isolation of LAB

Five grams of each sample were mixed with 100 ml of PBS buffer solution (0.1 *M*, pH 7.0). After that, 1.0 mL of each mixture was diluted up to 10^{-7}, then 0.1 mL spread onto the surface of De Man Rogosa Sharp **(**MRS) agar (HIMEDIA) (**DeMan *et al*. 1960**) and were incubated at 37°C for 48-72 h under anaerobic condition using anaerobic jar.

From the third subculture, an episode of culture is shed on Reinforced Petri plates of choice of MRS agar, incubated according to standards. One colony from each dish was isolated and purified three times before inoculation in MRS broth.

1.3 Primary screening of LAB isolates for probiotics features

For primary screening of LAB isolates, their ability to survive under gastrointestinal tract (GIT) conditions was evaluated using three tests, namely cell surface hydrophobicity **(Blajman *et al*., 2015),** acid tolerance **(Hassanzadazar *et al*., 2012),** tolerance of Bile salts **(Aziz *et al*., 2019).**

1.3. Secondary screening

To evaluate the most efficient LAB isolates selected from the primary screening, six tests namely auto-aggregation assay **(Kos *et al*., 2003),** co-aggregation assay **(Del Re *et al*., 2000),** hemolytic activity **(Maragkoudakis *et al*., 2009)**, NaCl tolerance **(Graciela and Maria, 2001)** and cholesterol assimilation **(Searcy and Bergquist, 1960)** were achieved. As well as, the antagonistic activity of LAB isolates against the chickens pathogenic bacteria was assessed using the agar well diffusion method described by **(De Vries *et al*., 2006)** using five pathogenic strains namely *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium*

ATCC 25566, *Klebsiella pneumoniae* ATTC 700603 and *Staphylococcus aureus* ATCC 25293 which obtained from Microbiology Department, National Research Center, Giza, Egypt.

1.4. Molecular identification of most potent LAB isolates

The selected isolates (SI5, IL22 and CE55) were grown in 5 ml MRS broth at 37ºC overnight. The DNA was extracted as per the protocol described by **Saito and Miura (1963)**. The DNA was amplified using primer 27 F (AGAGTTTGA TCMTGGCTCAG) and 519 R (GGATTACCG CGGCCGCTG). PCR amplification reactions were carried out in a 25µl reaction mixture. 1µl of the DNA was amplified with 2.5µl of PCR buffer, 2.5µl of 25 Mm $MgCl_2$, 2.0µl of 2 mM dNTPs, 1.0µl of 20 pmol primer 270 F, 1.0µLof 20 pmol primer 519 R, 0.125µl of LA Taq and PCR grade water up to 25µl. PCR conditions were as follows: initial denaturation at 94ºC for 4 min, followed by 30 cycles of denaturation at 94ºC for1 min, annealing for 30 sec., at 53ºC, extension at 74ºC for 2 min, followed by a final extension at 74ºC for 13 min. Amplified product was confirmed by agarose gel (1%). electrophoresis and documented using Syngene G-box gel documentation system.

The probable identify of each sequence was determined by performing a Basic Local Alignment Search Tool (BLAST) search at the National Center for Biotechnology Information (NCBI) website. The complete 16S rRNA sequence of each strain was aligned using MEGA6 software against corresponding sequences of representatives of appropriate bacteria strains retrieved from the Gene Bank and used to generate phylogenetic trees.

1.5. Additional probiotic features of identified LAB strains

1.5.1. Lysozyme resistance

Lysozyme resistance was executed according to the method by **Hossain *et al.* (2021)**. Overnight bacterial cultures were centrifuged at (10000 ×g, for 10 min) under cooling (4°C) then washed twice with PBS (pH 7.0), further the pellets were resuspended in 2 mL of Ringer solution (Sigma-Aldrich). 1.0% of bacterial suspension was inoculated into a sterile electrolyte solution supplemented with 100 mg/L lysozyme (Sigma-Aldrich). A bacterial culture in sterile electrolyte solution without lysozyme was used as a control. All treatments were incubated at 30°C for 2 h, then the viable bacterial cell counts were calculated after by the plate-count method on MRS agar plates incubated at 30°C for 48 h. the survival rate was calculated using the following equation:

$$\text{Survival rate (\%)} = \frac{\text{Final Log cfu/mL}}{\text{Initial Log cfu/mL}} \text{x } 100$$

1.5.2. Temperature tolerance

The isolates were tested for their ability to grow at various temperatures (25 - 30 - 37 and 42°C). For this, 1.0 ml of each LAB strain was inoculated in 10.0 ml MRS broth and incubated at the previous temperatures for 24-48 h. The appearance of turbidity in culture tubes was observed and result was recorded as positive or negative (**Vineetha *et al.*, 2016**).

1.5.3. Antioxidant Activity

The antioxidant activity of the identified LAB strains was estimated by measuring their DPPH free radical scavenging activity according to the method by **Mu *et al.* (2018)**. Preparation of samples was performed according to the method described by **Chen *et al.* (2014)**. Then the following formula was used for calculation of the antioxidant activity:

$$\text{Free radical scaving activity towords DPPH (\%)} = \frac{1 - (\text{A sample} - \text{A blank})}{\text{A control}} \times 100$$

Where: A_{sample} is the optical absorbance at 517 nm of the sample group, A_{blank} is the optical absorbance at 517 nm of the blank group, $A_{control}$ is the absorbance of the control group.

1.5.4. Antibiotics susceptibility test

This assay was executed following the method of **Zago *et al.* (2011)** and **Hossain *et al.* (2021)**. Whereas, antibiotic sensitivity or resistance of the selected isolates was determined by the standardized technique of diffusion of **Phillips *et al.* (1991)** and **Zhou *et al.* (2000)** using 18 commercials antibiotics discs: Amoxycillin + clavulanic acid (25/10 µg), polymyxin B (300 µg), Cefoperazone - sulbactam (105µg), Oxacillin (1µg), Gentamycin (10 µg), Norfloxacin (10µg), Levofloxacin (5 µg), Amikacin (30 µg) , Cefprozil (30 µg), Imipenem (10 µg), Amoxycillin (10 µg), Streptomycin (10 µg), Erythromycin (15 µg), Tylosin tartrate (30 µg), Neomycin (30 µg), colistin sulphate (10 µg), Ampicillin (10 µg) and Doxycycline(30 µg). The results were categorized as: resistant (R), intermediate (I), and susceptible (S), according to the levels proposed by **NCCLS (2002).**

1.6. Statistical analysis

Analysis of variance (ANOVA) was performed using CoStat version 6.400 (CoHort software, Monterey, CA, 93940, USA). Mean values among treatments were compared by the Duncan test at 5% level (p. value < 0.05) of significance and presented as the mean values ± standard deviation (SD).

Results and Discussion

1.7. Isolation of LAB

Sixty lactic acid bacteria were isolated from three different parts of chicken intestine (small intestine, illum and cecum). All recovered isolates were morphologically characterized by the colony characteristics, along with their Gram reaction and microscopic examination.

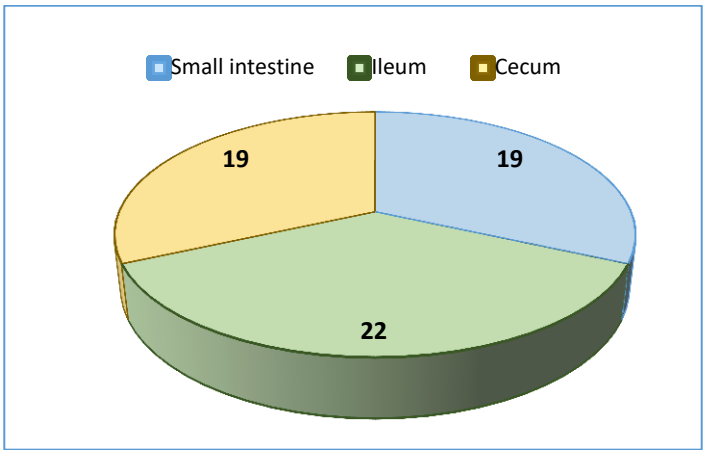


Figure 2. Number of bacterial isolates from different sources

Only Gram-positive isolates were selected for further experiments and coded as SI (1-19), IL (20-41), CE (42-60). Data illustrated by **Figure (2)** showed that 19, 22 and 19 isolates were isolated from the small intestine, ileum, and cecum, respectively. Similarly, **Reuben *et al.* (2019)** isolated 57 LAB strains from the gastrointestinal tract of healthy broiler chickens and selected based on their typical morphological characteristics (colony and cell-shaped), Gram reaction, certain enzymes (catalase and coagulase), and motility.

1.8. Survival under gastrointestinal tract conditions

1.8.1. Cell surface hydrophobicity

The ability of bacteria for attachment to the epithelium of the digestive tract is determined by the cell surface hydrophobicity test **(Kos *et al*., 2003)**. The isolates showed a wide range of hydrophobicity ranging from 1.91 to 78.90% **(Table 1)**.

Table 1. Cell surface hydrophobicity by bacterial isolates.

Isolate No.	Hydrophobicity (%)	Isolate No.	Hydrophobicity (%)
SI1	1.93±0.09	IL31	0
SI2	3.16±0.21	IL32	14.8±0.01
SI3	34.66±0.95	IL33	21.6±0.01
SI4	16.05±0.28	IL34	16.4±0.01
SI5	78.89±0.76	IL35	15.96±0.01
SI6	22.76±0.95	IL36	17.03±0.057
SI7	15.27 ± 15.3	IL37	0
SI8	15.86±1.01	IL38	0
SI9	29.85±0.56	IL39	39.4±0
SI10	40.42±0.56	IL40	5.52±0.22
SI11	2.92±0.57	IL41	2.17±0.01
SI12	6.48±0.54	CE42	15.97±0.01
SI13	12.52±0.49	CE43	73.69±0.01
SI14	24.70±0.01	CE44	37.40±0.01
SI15	27.60±0.01	CE45	0
SI16	0	CE46	0
SI17	12.56±0.58	CE47	10.73±0.30
SI18	5.47±0.45	CE48	20.53±0.46
SI19	36.36±0.58	CE49	33.60±0.34
IL20	43.9±0.01	CE50	14.44±0.54
IL21	6.15±0.37	CE51	9.42±0.57
IL22	39.76±0.77	CE52	3.09±1.01
IL23	39.39±0.01	CE53	29.59±1
IL24	48.49±0.52	CE54	0
IL25	0	CE55	45.20± 0.85
IL26	64.51±0.53	CE56	9.50± 0.02
IL27	57.38±0.68	CE57	55.80±0.99
IL28	2.22±0.01	CE58	64.5±0.99
IL29	51.86±1.05	CE59	27.02±0.58
IL30	38± 1	CE60	55.30±1.01

Values are the mean ± standard deviation of n=3

Out of sixty isolates, 12 isolates showed more than 40% cell surface hydrophobicity. Overall, among of the 60 isolates investigated, SI5, IL26, IL27, CE 43, CE57, CE58 and CE60 exhibited the highest percentage of hydrophobicity while SI1, SI16, IL25, IL37, IL38, CE45, CE46 and CE54 were the least hydrophobic producers. Probiotic strains must have the ability to extend to the host intestine and adhere to its wall, before exerting any functional impacts. Thus, cell surface hydrophobicity is crucial factor for assessment of potential probiotic strains **(Shokryazdan e*t al*., 2017)**. Several studies have documented that hydrophobicity tests **(Pessoa *et al*., 2017)** correlated with adhesion ability of probiotic bacteria to epithelial cells **(Botes *et al*., 2008).**

The cell surface of microorganisms contains hydrophobic compounds such as proteins, teichoic acids, and lipids, which make them attach to the surface of the intestinal epithelium through covalent bonds. The differences between the cell surface hydrophobicity of bacteria are influenced by several parameters, such as the chemical composition and structural properties of bacteria (type of amino acids, composition of proteins, polysaccharides, and lipid compounds in the bacterial cells), the growth phase of bacteria, and environmental factors **(Vasiee *et al*., 2020)**.

1.8.2. Tolerance of pH by bacterial isolates

Acid tolerance assessment was performed at pH 2.5, pH 3.5 and pH 7.5. The results of the initial pH tolerance screening presented in **Figure (3)** showed that most of the isolates had the ability to survive at all tested pHs. Where 10 isolates and 50 isolates were able to survive at pH 2.5 and pH 3.5 respectively after 4 hours, and at the same time all isolates were able to survive at pH 7.2. The survival of LAB strains in the range of pH 2.5 to 3.5 is an essential factor to perform as potential probiotics **(Vasiee *et al*., 2020)**. The acidity test was performed on LAB isolate to see the ability of the isolates to grow in various conditions of acidity of the digestive tract of the broiler. The ability of bacteria to survive the condition of the acidity of the stomach is an important thing to consider for a bacterium to be used as a probiotic candidate in poultry.

Bacteria that enter the stomach will decrease the population, due to the influence of hydrochloric acid (HCl) on the stomach, which is about pH 2-3.5. HCl is a strong acid and a major component of gastric acid **(Hidayat *et al*., 2018)**. In this study, all isolates tested possessed varying growth abilities especially at low pH (2.5 and 3.5). According to **Siegumfeldt *et al*., (2000)** the ability of LAB to survive at low pH, because the intracellular pH can adjust to the decrease in extracellular pH, so as not to cause a large proton gradient. Further **Hutkins and Nannen (1993)**states that in addition to LAB experiencing slow growth at low pH, LAB cells can also be damaged due to acid and loss of viability. Each strain has different resistance to acid or low pH. According to **Cotter *et al*., (2003)** there are several possible mechanisms by which bacteria regulate the internal pH, but the most important is the proton translocation by the ATP-ase enzyme.

Potential probiotic strains must tolerate acidic environments and bile secretions to successfully pass through the stomach and small intestine. The pH of the gastric juice is around 2.0–3.0, which causes most ingested microorganisms to die **(Singh *et al*., 2012)**. The present findings are comparable to those reported by several researchers **(Blajman *et al*., 2015)** and **(Makzum *et al*., 2023)** showed that most bacterial strains isolated from the gastrointestinal tract. The chicken tract is tolerant to the pH of the chicken intestine. However, most of the probiotic bacteria showed lower to medium growth at pH 2.5 to 3.5.

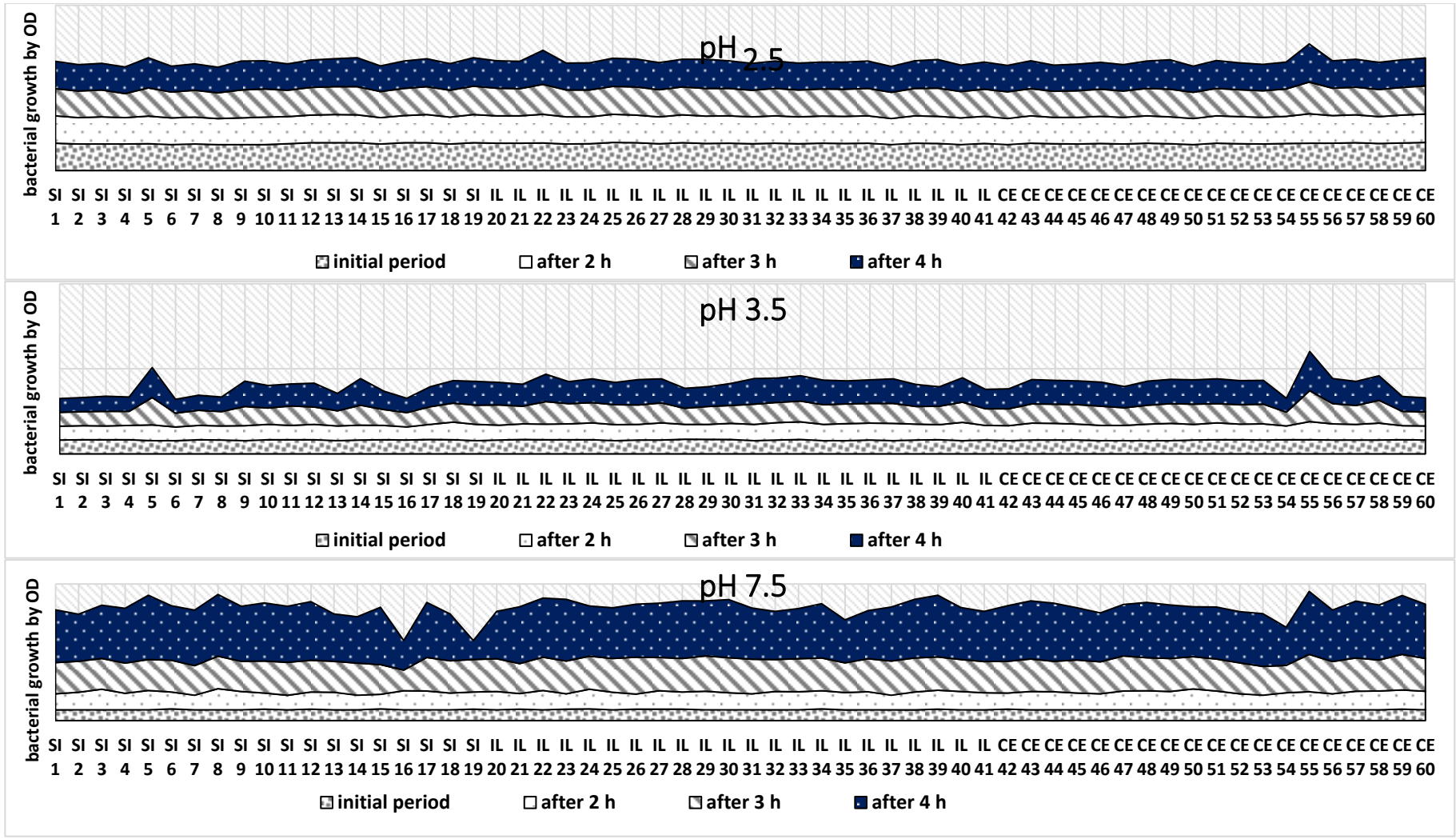


Figure 3. Effect of pH concentrations on growth of LAB isolates

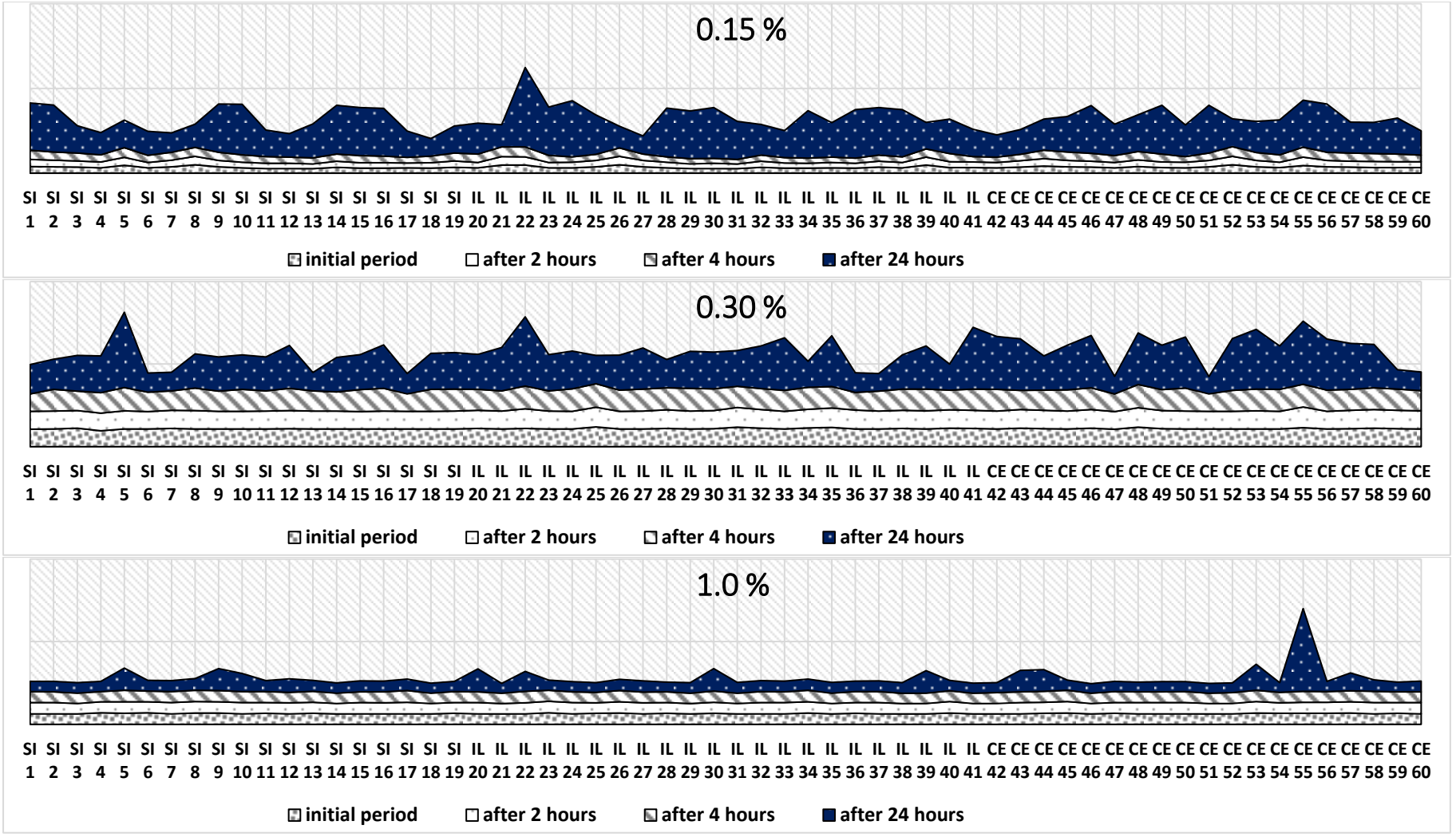


Figure 4. Effect of bile salts concentrations on growth of LAB isolates

Tolerance of bile salts by bacterial isolates

Resistance to bile salts is of great importance to survival and growth of bacteria in the intestine attract, and thus, is a prerequisite for bacteria to be used as probiotics **(Havenaar *et al*., 1992)**. All strains were able to survive in bile salt (0.15 and 0.3% in MRS broth, 24 h at 37°C). As illustrated in **Figure (4),** isolate IL22 showed the best survival rate (at 0.15% and 0.3% bile salt) while isolate CE55 showed the best survival rate at 1% bile salt). Also, other isolates are intermediate between the two, while at a concentration of 1% bile salt, most isolates are not able to survive after 24 h. The bile salts resistance of some strains varies significantly between lactic acid bacteria **(Xanthopoulos *et al*., 1997)**. Several studies have demonstrated that probiotics survive well at 0.3% bile concentrations **(Kobierecka *et al*., 2017)** and **(Rajoka *et al*., 2018)**. It should be mentioned that the typical bile content is around 0.3%, with a maximum of 2.0% during the first hour of digestion **(Gotcheva *et al*., 2002).** In this investigation, a significant proportion of isolates demonstrated their ability to survive in bile salts.

3.2.4. Auto-aggregation

Auto-aggregation measures the ability of bacterial strain to aggregate with each other, in a nonspecific manner, which is considered as prerequisite for colonization to allow the probiotic bacteria to exert its beneficial effects **(Del Re *et al*., 2000)** . In current study, the percentages of auto-aggregation during 4h and 24h of incubation at 37°C of the best 15 bacterial isolates based on their previous test are displayed in **Table (2)**. Date demonstrated a good percentage of auto-aggregation ranging from 11.9-83.2 % and 40.2 - 87.0% throughout 4h and 24h of incubation, respectively. Isolates CE55, SI5 and IL22 presented the highest auto-aggregation percentage of 87.0%, 83.2% and 82.8%, respectively, out of the 15 isolates. In the present study, auto-aggregation results differed significantly ($P < 0.05$) during incubation time.

Our results coincide with other works which have exhibited that auto-aggregation rate increase with incubation time **(Gil-Rodríguez *et al*., 2015).** Several studies have documented that auto-aggregation tests correlated with adhesion ability of probiotic bacteria to epithelial cells **(Botes *et al*., 2008)**.

3.2.5. Coaggregation

The aggregation between bacteria of variant species and/or strains known as coaggregation where the probiotics are active **(Piwat *et al*., 2015).** The pathogenic adhesion to mucosa can be inhibited by defensive barrier which is formed via direct aggregation of probiotic **(Vidhyasagar and Jeevaratnam, 2013).** Many researchers have reported that coaggregation in the presence of gut pathogens will reinforce probiotic properties and cell colonization of LAB. Data in **Table (2)** displays the results of coaggregation of investigated bacterial isolates in the presence of two pathogenic bacteria namely *E. coli* ATCC 25922 and *S. aureus* ATCC 25293 individually at 4 h and 24 h of incubation at 37°C.

Table 2. Auto-aggregation and Co-aggregation by the selected LAB isolates.

Isolates No.	Auto-aggregation (%)		Co-aggregation (%)			
			E. coli		*Staph. aureus*	
	4h	24h	4h	24h	4h	24h
SI 5	70.5±1.21^{c}	83.2±0.11^{b}	79.82±0.06^{b}	81.55±0.58^{c}	77.08±1^{c}	87.66±0.35^{a}
SI 9	20.6±0.35^{l}	45.7±0.32^{j}	35.68±0.43^{i}	50.56±1.35^{i}	25.52±0.30^{m}	39.58±0.14^{i}
SI 10	60.5±0.47^{f}	75.4±0.60^{c}	63.55±0.56^{d}	68.53±0.79^{e}	74.78±0.04^{d}	79.47±0.07^{b}
IL 20	21.6±0.59^{l}	50.6±0.33^{i}	35.44±0.32^{i}	40.47±0.32^{m}	29.67±0.15^{l}	33.04±0.59^{l}
IL 22	77.2±0.84^{b}	82.8±0.86^{b}	81.89±0.25^{a}	86.19±0.56^{b}	79.81±0.43^{b}	87.81±0.91^{a}
IL 23	40.9±0.50^{h}	55.5±0.48^{h}	43.58±0.07^{g}	56.58±0.08^{g}	66.84±0.13^{e}	75.75±0.11^{c}
IL 29	18.75±0.56^{m}	40.7±0.32^{l}	38.51±0.23^{h}	43.59±0.03^{k}	34.77±0.62^{j}	37.70±0.13^{j}
IL 30	65.7±0.75^{d}	72.6±0.54^{d}	47.57±0.13^{f}	61.55±0.13^{f}	55.57±0.22^{h}	70.43±0.05^{d}
IL 39	35.2±0.08^{j}	59.4±0.14^{g}	65.32±0.53^{c}	69.36±0.24^{d}	46.71±0.18^{i}	50.50±0.09^{g}
CE 43	11.9±0.24^{n}	40.2±0.32^{l}	19.50±0.69^{k}	25.50±0.27^{n}	15.79±0.14^{n}	18.57±0.10^{m}
CE 44	25.0±0.17^{k}	65.9±0.84^{f}	21.64±0.70^{j}	48.22±0.47^{j}	31.72±0.81^{k}	48.64±0.13^{h}
CE 49	37.8±1.01^{i}	41.8±1.08^{k}	19.64±0.46^{k}	42.64±0.27^{l}	15.75±0.52^{n}	35.78±0.08^{k}
CE 53	64.5±0.53^{e}	75.6±0.39^{c}	53.60±0.38^{e}	61.71±0.12^{f}	59.69±0.49^{g}	63.68±0.56^{f}
CE 55	83.2±0.78^{a}	87.0±0.11^{a}	81.52±0.61^{a}	87.63±0.03^{a}	82.21±0.30^{a}	87.63±0.51^{a}
CE 57	52.3±0.46^{g}	69.8±0.50^{e}	38.67±0.51^{h}	55.69±0.18^{h}	62.83±0.70^{f}	67.74±0.61^{e}

Values are the mean ± standard deviation of n=3

Means with a different superscript litter in the same column are significantly different at ($P<0.05$)

Accordingly, all bacterial isolates showed good capabilities to co-aggregate toward the two pathogens **(Table 2)**. Bacterial isolates showed higher co-aggregation toward *S. aureus* during the first 4 h compared with *E. coli*. Whereas when incubation time prolonged to 24 h this trend was changed, as the 15 bacterial isolates tested showed the higher rate of coagulation against *E. coli* and *S. aureus*, with a high rate of (87.63%) and (87. 81%), respectively. In general, bacterial isolates IL22,

CE55, and SI5 showed higher coagulation rates compared with the other bacterial isolates.

Our results demonstrated that bacterial isolates presented considerable coaggregation properties and comparable to findings have been reported by **Li *et al*. (2020)**. The capability of present bacterial isolates to co-aggregate with pathogens may be imputed to cell surface components. Furthermore, the presence of interactions among proteinaceous components and carbohydrate-lectin on the cell surface may be considered **(Tareb *et al*., 2013).**

3.2.6. Determination of antimicrobial activity

LAB can serve as microbial barrier against intestinal pathogen through competitive exclusion of pathogen binding, modulation of host's immune system, production of antimicrobial compounds such as organic acids (e.g., lactic acid, acetic acid, propionic acid) and proteinaceous compounds such as bacteriocins. The antimicrobial activity properties of the tested LAB were very variable as shown in **Table (3).** Antibacterial activity of 15 selected isolates was tested against *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. LAB isolates (SI5, SI9, SI10, IL20, IL22, IL23, IL29, IL39, CE43, CE53, CE55, CE57) showed antibacterial activity against all the tested foodborne showed in with inhibition zone 5 mm to 19 mm. Isolates SI9, IL29 and CE43 showed good inhibitory activity against most of the pathogenic bacterial strains.

Moreover, the isolate SI5 showed the highest inhibition zone diameter with *Staphylococcus aureus*, *Salmonella typhimurium* while CE55 showed the highest inhibition zone diameter with *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

Obtained results are in harmony with findings of **François *et al.* (2013**) who used the neutralized cell free supernatant to deactivate the acids and to exclude the activity due to organic acids. Hence, the activity may be due to bioactive substances such as bacteriocin-like inhibitory substances, biosurfactants and other relevant molecules.

LAB offers various advantages as potential probiotics and can be considered as alternatives to antibiotics during food-animal production. LAB are safe microorganisms with abilities to produce different inhibitory compounds such as bacteriocins, organic acids (as lactic acid), hydrogen peroxide, diacetyl, and carbon dioxide. LAB can inhibit harmful microorganisms with their arsenal, or through competitive exclusion mechanism based on competition for binding sites and nutrients (**Vieco-Saiz *et al*., 2019**). All the selected lactobacilli showed varying antimicrobial activities against *S. aureus* and *L. monocytogenes*. The isolates showed considerable antibacterial activity against Gram-negative and Gram-positive bacteria which are major food borne pathogens and of special concern with regard to food safety (**Mostafa *et al*., 2018)**. The antibacterial activity differed among all the LAB strains, where certain LAB showed activity against specific indicator strains.

Table 3. Antagonistic activity of LAB isolates' supernatants against pathogenic bacteria.

Isolates No.	G^+ bacterium (*Staph. aureus*)	G^- bacteria			
		Ps. aeruginosa	*k. pneumoniae*	*E. coli*	*S. typhimurium*
	Inhibition zone diameter (mm)				
SI 5	19.0±0.2^{a}	15.0±0.2^{b}	13.0±0.2^{b}	9.0±0.2^{e}	16.0±0.2^{a}
SI 9	13.0±0.2^{c}	9.0±0.2^{e}	10.0±0.2^{e}	10.0±0.2^{d}	5.0±0.2^{g}
SI 10	9.0±0.2^{g}	6.0±0.2^{h}	9.0±0.2^{f}	8.0±0.2^{f}	6.0±0.2^{f}
IL 20	11.0±0.2^{e}	8.0±0.2^{f}	11.0±0.2^{d}	10.0±0.2^{d}	7.0±0.2^{e}
IL 22	18.0±0.2^{b}	15.0±0.2^{b}	14.0±0.2^{a}	12.0±0.2^{b}	15.0±0.2^{b}
IL 23	9.0±0.2^{g}	6.0±0.2^{h}	8.0±0.2^{g}	9.0±0.2^{e}	5.0±0.2^{g}
IL 29	12.0±0.2^{d}	9.0±0.2^{e}	11.0±0.2^{d}	12.0±0.2^{b}	7.0±0.2^{e}
IL 30	8.0±0.2^{h}	6.0±0.2^{h}	0±0^{i}	9.0±0.2^{e}	5.0±0.2^{g}
IL 39	10.0±0.2^{f}	10.0±0.2^{d}	12.0±0.2^{c}	11.0±0.2^{c}	6.0±0.2^{f}
CE 43	11.0±0.2^{e}	11.0±0.2^{c}	10.0±0.2^{e}	13.0±0.2^{a}	7.0±0.2^{e}
CE 44	9.0±0.2^{g}	6.0±0.2^{h}	0±0^{i}	8.0±0.2^{f}	0±0^{h}
CE 49	8.0±0.2^{h}	7.0±0.2^{g}	8.0±0.2^{g}	7.0±0.2^{g}	0±0^{h}
CE 53	11.0±0.2^{e}	10.0±0.2^{d}	10.0±0.2^{e}	10.0±0.2^{d}	8.0±0.2^{d}
CE 55	13.0±0.2^{c}	19.0±0.2^{a}	14.0±0.2^{a}	13.0±0.2^{a}	11.0±0.2^{c}
CE 57	8.0±0.2^{h}	8.0±0.2^{f}	7.0±0.2^{h}	8.0±0.2^{f}	6.0±0.2^{f}

Values are the mean ± standard deviation of n=3

Means with a different superscript litter in the same column are significantly different at ($P<0.05$)

All tested LAB isolates exhibited the antimicrobial activity against *S. aureus* with inhibition zone ranged between 8.0-19.0 mm in diameter, *Salmonella typhimurium* with inhibition

zone ranged between 5.0-16.0 mm (except CE44 and CE49), *E. coli* with inhibition zone ranged between 7.0-13.0 mm, *Klebsiella pneumonia* with inhibition zone ranged between 7.0-14.0 mm (except IL30 and CE44) and *Ps aeruginosa* with inhibition zone ranged between 6.0-19.0 mm. The possible mechanisms of bactericidal action include diminished pH levels, competition for substrates, the production of substances with a bactericidal or bacteriostatic action, including bacteriocins and bacteriocin-like substances **(Pan *et al*., 2009)**. The outcomes of this study revealed that antimicrobial activity different among LAB due to variation between species and strains, this result in agreement with several works **(Rajoka *et al*., 2018)**.

3.2.7. Cholesterol assimilation

Some of the microorganisms can reduce the cholesterol levels naturally and show anti-cholesterol activity. **Table (4**) shows the results of cholesterol reduction by LAB isolates in the presence of 0.3% bile salt. Screening for cholesterol-lowering properties, *in vitro*, has become an important criterion in the selection of bacterial strains for *in vivo* probiotic investigations. This experiment investigated fifteen LAB isolates, selected from previous tests, for their ability to assimilate cholesterol. Initially, all the bacterial isolates were shown to successfully assimilate cholesterol but with high variability across the tested isolates. The obtained results recorded cholesterol assimilation ranged from 4.63 -74.16%.

Isolates SI5, IL22, CE53 and CE55 had cholesterol assimilation greater than 60% showed in **Table (4)**. The highest assimilation of cholesterol showed with isolate CE55. Moderate assimilation ranged from 42.60 to 65.10 % showed in S10I, IL20, IL30 and CE53. The lowest assimilation showed with isolate CE57. The previous studies have demonstrated cholesterol assimilation in the same range **(Bordoni *et al*., 2013)**.

3.2.8. Hemolytic activity

The obtained data **in Table (4)** recorded that none of all examined isolates exhibited α and β-haemolytic activity. The fifteen isolates with the best results were tested for their non-pathogenic character by streaking them on blood agar plates (HIMEDIA). Tested strains showed no haemolysis (γ-haemolysis). Evaluation of haemolytic activity is an important safety requirement frequently used to assess potential probiotic strain. The selected isolates were non haemolytic, not virulent, and further qualifying them as potential probiotic candidates. Absence of haemolytic activity is considered as safety criterion for the selection of probiotic strains. The obtained results are similar with previous observations by **Pisano *et al.* (2014)** who reported that all of the tested strains of LAB are γ-haemolytic activity.

Table 4. Cholesterol assimilation and Hemolytic activity types by LAB isolates.

Isolates No.	Cholesterol assimilation		Hemolytic activity		
	(µg/ml)	(%)	α	β	γ
SI 5	0.525±0.00^{b}	72.36±0.21^{b}	-	-	+
SI 9	0.163±0.00^{j}	22.48±0.14^{j}	-	-	+
SI 10	0.402±0.00^{e}	55.35±0.07^{e}	-	-	+
IL 20	0.309±0.00^{g}	42.60±0.22^{g}	-	-	+
IL 22	0.505±0.00^{c}	69.65±0.21^{c}	-	-	+
IL 23	0.284±0.00^{h}	39.17±0.14^{h}	-	-	+
IL 29	0.114±0.00^{l}	15.72±0.14^{l}	-	-	+
IL 30	0.381±0.00^{f}	52.55±0.13^{f}	-	-	+
IL 39	0.252±0.00^{i}	34.75±0.13^{i}	-	-	+
CE 43	0.114±0.00^{l}	15.72±0.14^{l}	-	-	+
CE 44	0.095±0.00^{m}	13.08±0.17^{m}	-	-	+
CE 49	0.034±0.00^{n}	4.63±0.15^{n}	-	-	+
CE 53	0.472±0.00^{d}	65.10±0.13^{d}	-	-	+
CE 55	0.538±0.00^{a}	74.16±0.14^{a}	-	-	+
CE 57	0.151±0.00^{k}	20.82±0.14^{k}	-	-	+

Values are the mean ± standard deviation of n=3

Means with a different superscript litter in the same column are significantly different at ($P<0.05$), $_{2}$a (-) no haemolysis

3.2.9. NaCl concentrations

The ability of fifteen LAB isolates to grow on 0, 1, 3, 5, and 7% NaCl was done to determine salt tolerance of these isolates **(Table 5)**. The current results showed that LAB isolates were able to tolerate all tested concentrations of NaCl and good growth was observed at 1.5 – 3.0 % NaCl with some differences as the growth was decreased when concentration of NaCl was increased from 3.0 to 7.0%. In contrast, at 5.0 % NaCl, only 4 isolates (SI5, IL22, IL39 and CE55) showed higher growth than the other isolates. LAB group needs salt for growth at concentrations of moderate and extremely halophilic (5-3%). Each genus of LAB has different tolerances to growing on media with different concentrations of NaCl salt. NaCl is an inhibitory

substance which may inhibit growth of certain types of bacteria **(De Vos *et al.*, 2009)**. The obtained results have the similarities with the findings of **(Pancheniak and Soccol, 2005)** and **(Reale *et al.*, 2015).** They reported that Lactobacilli isolated from gastrointestinal tract of swine were tolerable to 4-8% NaCl.

Table 5. Tolerance of various NaCl concentrations after 24 h of incubation by the selected LAB isolates

Isolates No.	NaCl concentrations (%)				
	0	1	3	5	7
SI 5	1.7605±0.05^{d}	1.5968±0.09^{b}	1.3856±0.10^{c}	0.9545±0.10^{b}	0.4890±0.01^{a}
SI 9	1.9024±0.05^{b}	1.1661±0.09^{f}	0.9545±0.09^{f}	0.3756±0.01^{hi}	0.2432±0.01^{i}
SI 10	1.5348±0.01^{g}	1.2866±0.10^{e}	0.8536±0.1^{g}	0.6351±0.01^{f}	0.4550±0.01^{b}
IL 20	1.7393±0.01^{d}	0.9540±0.21^{h}	0.4293±0.01^{j}	0.3283±0.01^{i}	0.2193±0.01^{j}
IL 22	1.8388±0.01^{c}	1.5733±0.08^{b}	1.4853±0.04^{b}	0.8424±0.01^{c}	0.3894±0.01^{d}
IL 23	1.5344±0.01^{g}	1.1869±0.03^{f}	0.7355±0.04^{h}	0.3726±0.01^{hi}	0.2066±0.01^{j}
IL 29	1.8488±0.01^{c}	1.2945±0.01^{e}	0.8365±0.04^{g}	0.3756±0.01^{hi}	0.2957±0.01^{g}
IL 30	1.4236±0.01^{h}	0.9784±0.01^{h}	0.5936±0.01^{i}	0.3956±0.04^{h}	0.2694±0.01^{h}
IL 39	1.5728±0.01^{f}	1.3864±0.60^{d}	1.0397±0.03^{e}	0.9559±0.07^{b}	0.4251±0.01^{c}
CE 43	1.6342±0.01^{e}	1.0652±0.02^{g}	0.6481±0.01^{i}	0.4843±0.01^{g}	0.3084±0.01^{f}
CE 44	1.7520±0.01^{d}	1.4440±0.01^{cd}	1.1958±0.13^{d}	0.7947±0.01^{d}	0.3969±0.01^{d}
CE 49	1.5292±0.01^{g}	1.2862±0.01^{e}	0.8638±0.01^{g}	0.6365±0.01^{f}	0.2171±0.05^{j}
CE 53	1.7350±0.01^{d}	1.4880±0.01^{c}	1.1419±0.01^{d}	0.7495±0.01^{e}	0.4967±0.01^{a}
CE 55	1.9772±0.01^{a}	1.7541±0.01^{a}	1.5849±0.01^{a}	1.1970±0.02^{a}	0.4281±0.03^{c}
CE 57	1.5396±0.01^{g}	1.3863±0.01^{d}	0.9689±0.01^{f}	0.7502±0.01^{e}	0.3296±0.01^{e}

Data were recorded as $OD_{600nm,}$ 0% NaCl mean Control

Values are mean ± standard deviation of n=3

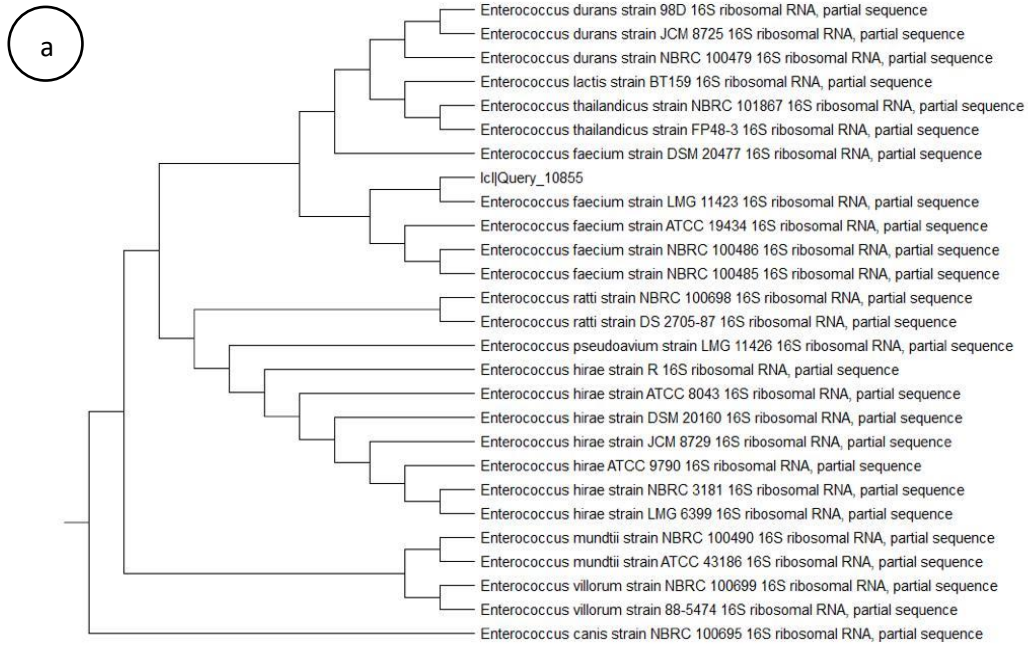
Means with a different superscript litter in the same column are significantly different at ($P<0.05$)

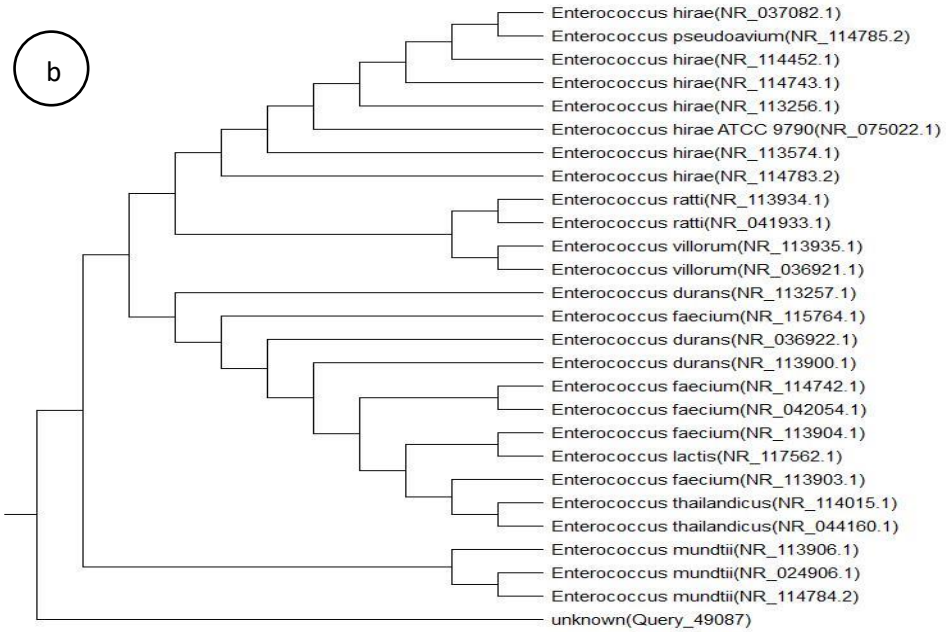
1.1. Identification of most potent LAB isolates using 16S rRNA sequences

The most potent isolates were chosen and identified by 16S rRNA gene sequence analysis to ascertain their taxonomic positions **(Table 6 and Figure 5 a, b and c)**. Sequencing results were registered in NCBI database and analysis of the obtained sequence via the Vecscreen database showed no contamination with vector sequence. The FASTA homology showed that the16S rRNA gene sequences of the selected isolates had 95.45, 99.88 and 100% nucleotide similarity with that of *Enterococcus faecium* NBRC 100486, *E. faecium* NBRC 100486 and *E. faecium* DSM 20477strains and deposited in GenBank under accession numbers (NR 113904.1, NR 113904.1 and NR114742.1), respectively.

Table 6. Molecular identification of the selected isolates (SI5, IL22 and CE55).

Isolates code	Closest relatives in NCBI	Accession number	Similarity %
SI5	*Enterococcus faecium strain* (NBRC 100486)	(NR 113904.1)	95.45
IL22	*Enterococcus faecium strain* (NBRC 100486)	(NR 113904.1)	99.88
CE55	*Enterococcus faecium strain* (DSM 20477)	(NR114742.1)	100


a
Enterococcus durans strain 98D 16S ribosomal RNA, partial sequence
Enterococcus durans strain JCM 8725 16S ribosomal RNA, partial sequence
Enterococcus durans strain NBRC 100479 16S ribosomal RNA, partial sequence
Enterococcus lactis strain BT159 16S ribosomal RNA, partial sequence
Enterococcus thailandicus strain NBRC 101867 16S ribosomal RNA, partial sequence
Enterococcus thailandicus strain FP48-3 16S ribosomal RNA, partial sequence
Enterococcus faecium strain DSM 20477 16S ribosomal RNA, partial sequence
lcl|Query_10855
Enterococcus faecium strain LMG 11423 16S ribosomal RNA, partial sequence
Enterococcus faecium strain ATCC 19434 16S ribosomal RNA, partial sequence
Enterococcus faecium strain NBRC 100486 16S ribosomal RNA, partial sequence
Enterococcus faecium strain NBRC 100485 16S ribosomal RNA, partial sequence
Enterococcus ratti strain NBRC 100698 16S ribosomal RNA, partial sequence
Enterococcus ratti strain DS 2705-87 16S ribosomal RNA, partial sequence
Enterococcus pseudoavium strain LMG 11426 16S ribosomal RNA, partial sequence
Enterococcus hirae strain R 16S ribosomal RNA, partial sequence
Enterococcus hirae strain ATCC 8043 16S ribosomal RNA, partial sequence
Enterococcus hirae strain DSM 20160 16S ribosomal RNA, partial sequence
Enterococcus hirae strain JCM 8729 16S ribosomal RNA, partial sequence
Enterococcus hirae ATCC 9790 16S ribosomal RNA, partial sequence
Enterococcus hirae strain NBRC 3181 16S ribosomal RNA, partial sequence
Enterococcus hirae strain LMG 6399 16S ribosomal RNA, partial sequence
Enterococcus mundtii strain NBRC 100490 16S ribosomal RNA, partial sequence
Enterococcus mundtii strain ATCC 43186 16S ribosomal RNA, partial sequence
Enterococcus villorum strain NBRC 100699 16S ribosomal RNA, partial sequence
Enterococcus villorum strain 88-5474 16S ribosomal RNA, partial sequence
Enterococcus canis strain NBRC 100695 16S ribosomal RNA, partial sequence


b
Enterococcus hirae(NR_037082.1)
Enterococcus pseudoavium(NR_114785.2)
Enterococcus hirae(NR_114452.1)
Enterococcus hirae(NR_114743.1)
Enterococcus hirae(NR_113256.1)
Enterococcus hirae ATCC 9790(NR_075022.1)
Enterococcus hirae(NR_113574.1)
Enterococcus hirae(NR_114783.2)
Enterococcus ratti(NR_113934.1)
Enterococcus ratti(NR_041933.1)
Enterococcus villorum(NR_113935.1)
Enterococcus villorum(NR_036921.1)
Enterococcus durans(NR_113257.1)
Enterococcus faecium(NR_115764.1)
Enterococcus durans(NR_036922.1)
Enterococcus durans(NR_113900.1)
Enterococcus faecium(NR_114742.1)
Enterococcus faecium(NR_042054.1)
Enterococcus faecium(NR_113904.1)
Enterococcus lactis(NR_117562.1)
Enterococcus faecium(NR_113903.1)
Enterococcus thailandicus(NR_114015.1)
Enterococcus thailandicus(NR_044160.1)
Enterococcus mundtii(NR_113906.1)
Enterococcus mundtii(NR_024906.1)
Enterococcus mundtii(NR_114784.2)
unknown(Query_49087)

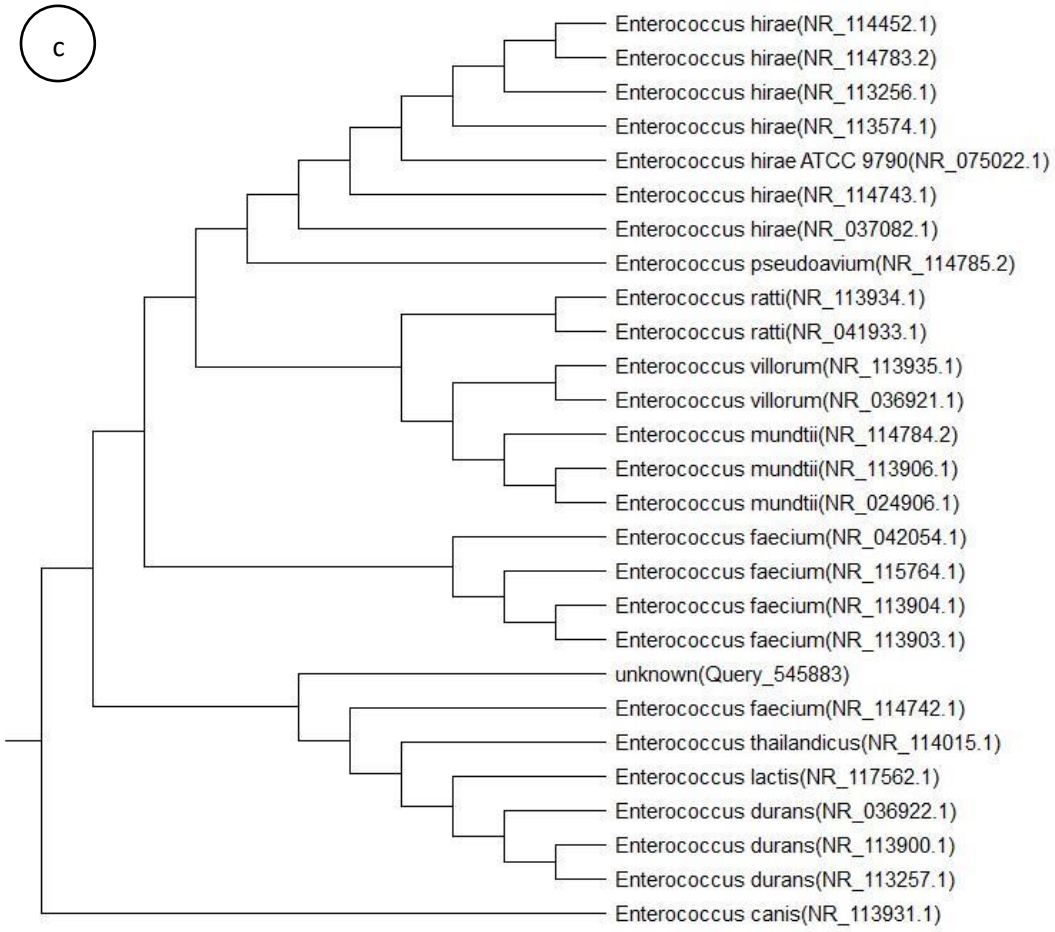


Figure 5. Phylogenetic trees recovered from maximum likelihood analyses of the 16S rRNA gene Partial sequences.

1.2. Additional probiotic features of identified strains

1.2.1. Antioxidant activity

DPPH is a common abbreviation for the organic chemical compound 2,2-diphenyl-1-picrylhydrazyl which relatively stable organic radical and has been widely used in the determination of antioxidant activities of cell free extracts of bacteria. *E. faecium* strains with antioxidant activity were screened by measuring DPPH free radical scavenging activity **(Table 7).** The DPPH radical scavenging activities of culture filtrate of the *E. faecium* were near and over 60% showed in **(Table 7)**. The maximum antioxidant activity was observed with *E. faecium* NBRC 100486 showing 75.53% followed by *E. faecium* D SM 20477 with 71.67%. On the other hand, *E. faecium* NBRC 100486 showed antioxidant activity of 68.68 %. Increase of the antioxidant capacity by optimizing the environmental factors makes it possible to obtain useful industrial materials. **Yang *et al.*, (2014)** reported that there is a significant positive correlation among the antioxidant activities and the metabolite of *E. faecium*. It is considered that purification of the filtrate could exhibit a higher activity. This study provides support for the formulation of novel probiotic foods or supplements that can play a role in the prevention of oxidative stress and related diseases.

From the above results we could conclude that values of antioxidant activities of probiotic bacteria depend mainly on the way of antioxidant determination and type of probiotic bacteria. The results of the present study are in accordance with the results indicated by **Al Kalbani, (2018)** who showed that *E. faecium* have high antioxidant activity. Oxidative stress is defined as the imbalance between prooxidants and antioxidants and is regarded as one of the most critical stressors in poultry production. When ROS surpasses the ability of the antioxidant system of an organism to remove them, oxidative stress occurs **(Surai, 2003)**. The body is not able to synthesize the enzymes needed to destroy ROS or repair the damage. Oxidative stress damages cell proteins, lipids, and DNA, and reduces energy generation efficacy. Moreover, oxidized molecules can take electrons from other molecules, resulting in a chain reaction. If not controlled, this reaction can cause extensive tissue damage. Oxidative stress can cause losses in the productive performance, as well

as losses in both nutritional and organoleptic quality of the products derived from them. Antioxidants in the diet are thought to play a protective role against oxidative damage **(Nimalaratne *et al.*, 2015)**.

1.2.2. Lysozyme resistance

Lysozymes are antimicrobial enzymes found in saliva, tears, human milk, mucus, neutrophil granules and egg white **(Rada *et al.*, 2010)**. This enzyme can inhibit microbial growth because it can hydrolysis the β- (1,4) N-acetylglucosamine and N-acetylmuramic bonds in bacterial cell wall components and Gram-positive bacteria are more susceptible to lysozyme than Gram-negative bacteria **(Ogundele, 1999).** Resistance to lysozyme is an important criterion for probiotics because probiotics must survive until colonization in the intestine and provide health effects for the host. **Table (7)** shows the survival of bacteria after exposure to lysozyme for 120 minutes, *E. faecium* NBRC 100486 showed a high resistance to 100 mg/L lysozyme (87%) under simulated conditions of saliva in addition, *E. faecium* NBRC 100486 and *E. faecium* DSM 20477 were 84 and 82%, respectively. All the tested strains were identified as resistance to lysozyme (100 mg/L). The differences in lysozyme tolerances among *Enterococcus* spp. isolates may be attributed to variations in layers and cell wall structures. The results of lysozyme tolerances in current study are in agreement with results found by **Hossain *et al.*, (2021).** Furthermore, **Rajoka *et al.*, (2018)** examined the effect of lysozyme (100 µg/mL) on probiotic LAB, and they showed that the viability of all of the isolates was only slightly affected by lysozyme treatment, which is in accordance with our observations.

1.2.3. Temperature range

Selective strains could survive from 25 to 42◦C. The optimum temperature for all selective strains was 37◦C (**Table 7**). The obtained results have the similarities with the findings of **Kathade *et al.* (2020).** This ability of selective strains will enable them to survive under various temperatures during processing, storage, and transport **(Cabello-Olmo *et al.*, 2020).** Furthermore, the ability of selective strains to grow at high temperature is a desirable trait as it could translate to increased rate of growth and lactic acid production. At the same time, a high fermentation temperature reduces contamination by other microorganisms **(Wouters *et al.*, 2000)**. To be described as an industrial probiotic, it is preferable that the strain exhibits ability to resist heat. According to our results, alteration in the structure of cell wall among *Enterococcus* spp. may explain the variations in heat tolerances in our potential probiotics **(Al Kalbani, 2018)**.

Table 7. Scavenging ability on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, lysozyme resistance and tolerance of various temperatures of selective strains.

LAB Strains	Antioxidant activity as % of DPPH	Lysozyme resistance	Temperature range (°C)			
			25	30	37	42
***E. faecium* NBRC 100486**	68.68±1.16^{c}	84±2.64^{a}	+	+	++	+
***E. faecium* NBRC 100486**	75.53±1^{a}	87±2.0^{a}	+	+	++	+
***E. faecium* DSM 20477**	71.67±0.29 ^{b}	82±2.64^{a}	+	+	++	+

Values are mean ± standard deviation of n=3

a–b Mean values in the same column with different uppercase superscripts differ significantly ($p < 0.05$)

1.2.4. Susceptibility of LAB to antibiotic

The antibiotic resistance of potential probiotic bacteria is a main safety aspect for choosing these bacteria as probiotic organisms and starter culture due to possibility hazard of horizontal transmission of bacteria resistance to non-resistant bacteria including pathogens **(Demirbaş *et al.*, 2017)**. *E. faecium* susceptibility to different antibiotic Gentamicin, Amoxicillin + clavulanic acid, polymyxin B, Cefoperazone-sulbactam, Oxacillin, Norfloxacin, Levofloxacin, Amikacin, Cefprozil, Imipenem, Amoxycillin, Streptomycin, Erythromycin, Tylosin tartrate, Neomycin, colistin sulphate, Ampicillin, Doxycycline) is shown in **Table (8).** *E. faecium* strains are resistant to many antibiotics. Antibiotic susceptibility tests showed that *E. faecium* isolates were sensitive to (Tylosin tartrate) and were resistant to (Gentamicin, Amoxicillin + clavulanic acid, polymyxin B, Cefoperazone-sulbactam, Oxacillin, Norfloxacin, Levofloxacin, Amikacin, Cefprozil, colistin sulphate and Doxycycline). This antibiotic profile study clearly demonstrated a broad spectrum of antibiotic resistance proficiency was acquired by the isolated LAB strains. *E. faecium* NBRC 100486 was sensitive to Imipenem but *E. faecium* NBRC 100486 and *E. faecium* DSM 20477 were resistant to this antibiotic. *E. faecium* DSM 20477 was resistant to (Streptomycin, Erythromycin, Neomycin).

Although most of the antibiotics used in this study are not common antibiotics used in poultry production, this trend in the antibiotic resistance pattern of the LAB isolates could be attributed to the routine use of antibiotics in poultry production.

Antibiotics are routinely added in sub-therapeutic doses to the diet (drinking water or feed) of birds as treatment measures, control of diseases as well as for their growth-promoting effects. This regular practice tends to consistently expose the natural gut microflora to traces of antibiotics which accumulate with time. Enteric bacteria tend to develop resistance to the antibiotics used due to constant exposures. Similar trend in antibiotic susceptibility pattern has also been reported by **Oloyede *et al*. (2013)** and **Acurcio *et al*. (2014).**

Table 8. LAB isolates susceptibility to different antibiotics.

Antibiotics	Concentration (µg/disc)	*E. faecium* NBRC 100486	*E. faecium* NBRC 100486	*E. faecium* DSM 20477
		Diameter of inhibition (mm).		
Gentamicin	10	2±0.2	6±0.2	5±0.2
Amoxycillin + clavulanic acid	25/10	R	R	R
polymyxin B	300	R	R	R
Cefoperazone – sulbactam	105	R	R	R
Oxacillin	1.0	R	R	R
Norfloxacin	10	12±0.2	15±0.2	12±0.2
Levofloxacin	5	R	R	R
Amikacin	30	R	R	R
Cefprozil	30	R	R	R
Imipenem	10	11±0.2	25±0.2	15±0.2
Amoxycillin	10	10±0.2	R	R
Streptomycin	10	10±0.2	10±0.2	25±0.2
Erythromycin	15	10±0.2	15±0.2	30±0.2
Tylosin tartrate	30	25±0.2	40±0.2	25±0.2
Neomycin	30	10±0.2	R	35±0.2
colistin sulphate	10	10±0.2	R	R
Ampicillin	10	10±0.2	35±0.2	R
Doxycycline	30	8±0.2	R	R

R: resistant S: sensitive I: intermediate
Values are mean ± standard deviation of n=3

The resistance against antibiotics may be attributed to the lack of target site of the certain antibiotic in LAB cell. These findings are almost in conformity with those documented by **Noohi *et al*. (2014) and Oyewole *et al*. (2018)** who had tested antibiotic activity for LAB isolated from Poultry**.** The antibiotic resistance of potentially probiotic bacteria is controversial and various opinions have been stated so far. For instance, resistance to specific antibiotics might be desirable for some probiotic strains that are involved in antibiotic-induce diarrhea **(Temmerman *et al*., 2003)**. On the other hand, LAB as probiotics enter intestines in large numbers and are able to interact with the intestinal microbiota and therefore, they have the potential to transfer genes to other bacteria, even to pathogenic ones **(Mathur and Singh, 2005).** For safety reasons, the resistance observed to specific antibiotics has to be chromosomally encoded and not inducible or transferable.

Conclusion

Gastrointestinal tract is a good source of lactic acid bacteria. In this study, 15 LAB strains having probiotic potential were isolated from healthy poultry intestine. All the tested isolates had high potential to adhere and pass through the gastrointestinal tract. Generally, the isolates SI5, IL22and CE55 were good to tolerate to pH, bile salt, cholesterol assimilation, NaCl tolerance, antimicrobial activity, resistance of lysozyme, antioxidant activity and antibiotic sensitivity. Using16S rRNA gene sequences, LAB isolate SI5, IL22 and CE55 that exhibited excellent probiotic characteristics were identified as *Enterococcus faecium* and were recorded under accession number (NR 113904.1, NR 113904.1 and NR114742.1) respectively. Our results also suggest that new three strains have potential for future application as probiotics in health promoting foods and have the potential to enhance the immunity of infants against invading pathogenic microbes. Overall, our study indicated that poultry gut is a good resource to isolate lactic bacteria with good characteristics as probiotics. However, more *in vitro*, and *in vivo* investigations are still needed to confirm the beneficial role of the obtained isolates in this study to animal and human health.

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