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The Potential Efficiency of *Lactobacillus farraginis* Isolated From Ruminants for Use as Animal Probiotics

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Abstract: Probiotics refer to microorganisms that exhibit a beneficial effect on the animals' health through intestinal microbial balance. This investigation intends to identify and define the probiotic characteristics of Lactobacillus strains isolated from the digestive systems of ruminants. Bacterial strains were isolated purified and characterized based on morphological and biochemical characteristics. The isolates were identified using the 16S rRNA gene partial sequencing method. Most of the strains exhibited a decrease in the growth by increasing the concentration of the bile salt, NaCl, increasing the temperature over 37ºC, and moving toward neutrality and alkalinity of the media. Strain *Lactobacillus farraginis* MD_A11 revealed the lowest decrease in the growth percentage when subjected to different bile salt concentrations of 5.96, 6.61, 6.85, 7.40, 7.53, and 7.64%, NaCl% concentrations of 1.40, 5.62, 6.48, 7.36, 7.39, 7.41, 7.42, 7.28, and 13.76%, raising the temperature over 37ºC being 4.19 & 3.945%, different pH levels as compared to control, and it recorded the lowest medium pH after the third day being 4.20 with titrable acidity of 0.32%. Strain *Lactobacillus farraginis* MD_A11 could be recommended as a probiotic feed additive for ruminants to improve their growth performance and productivity.

1 Introduction

Probiotic is a group of non-pathogenic microorganisms that exhibit beneficial effects for their host by improving the microbiological balance in the intestine (Islam et al 2016, Dhewa et al 2010, Schrezenmeir and de Vrese 2001). Lactic Acid Bacteria (LAB) is considered a major group of probiotic bacteria which consists of lactobacilli, lactococci and

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bifidobacteria which are regarded as health-benefiting bacteria (Tuohy et al 2003, Rolfe 2000).

Lactobacilli are thought to be the most significant probiotic group of microorganisms found in animal microbiota (Klein et al 1998). They are members of the lactic acid bacteria that can produce lactic acid as the main end-product of carbohydrate fermentation (Tham et al 2012) and have a diverse range of applications due to their known as "Generally Recognized as Safe" (GRAS) (Mattia and Merker 2008).

According to Vos et al (2009), the genus *Lactobacillus* spp. belongs to the Phylum *Firmicutes*, Class *Bacilli*, Order Lactobacillales, Family Lactobacillaceae**.** *Lactobacillus* spp. are gram-positive, non-spore-forming, and catalase-negative bacteria.

Since the discovery of PCR and DNA sequencing, the molecular methods based on 16S rRNA sequencing have been extensively used to identify bacteria at the species level as it is highly conserved within a species and among species of the same genus (You and Kim 2020, Stackebrandt and Goebel 1994). Nowadays, the most precise way to identify lactobacilli is the partial or complete sequencing of the 16SrRNA gene, as well as the general physiological characteristics of the genus and species (Salvetti et al 2012, Liu et al 2014).

Lactobacillus spp. normally present as native inhabitants in the gastrointestinal of animals as non-pathogenic commensals (Papadimitriou et al 2016, Liu et al 2014, Hammes and Hertel 2006). Therefore they are used as a health-promoting indigenous agent as they contribute to the balance in gut flora (Schrezenmeir and de Vrese 2001) and have several therapeutic functions (Curragh and Collins 1992).

Bacterial strains used as probiotic supplements for animals are supposed to resist the environmental conditions in which they live. As a result, probiotics found in the gastrointestinal environment (GIT) must be able to withstand low pH, variable temperature, high bile concentration, and high salt concentration (Collado and Sanz 2006, Mishra and Prasad 2005).

This study aims to isolate and characterize potential autochthonous *Lactobacillus* strains from ruminant gastrointestinal tracts as a local Egyptian strain and assess their characteristics to select the best strains as additives or supplements to ruminants in Egypt to improve their growth performance and productivity.

2 Methods

2.1 Experimental animals

The heifers and adult cows were housed at the Unit of Experimental and Agricultural Research, Faculty of Agriculture Ain Shams University, and were cared for according to the guidelines of Olsson et al (2016).

2.2 Samples collection

From July to November 2018, saliva, feces, and ruminal liquid samples were collected from heifers and adult cows. Each sample was taken in a sterile container and placed in a polyethylene bag before being transported to the laboratory under standard sample collection and transformation conditions.

2.2.1 Saliva samples

Five samples (10 mL for each) were collected from healthy heifers and adult cows using sterilized 15 mL screw tubes.

2.2.2 Feces samples

Five samples (10 mL for each) were collected from healthy heifers using sterilized 50 ml screw tubes.

2.2.3 Rumen liquid samples

Five samples (50 mL for each) were collected from healthy heifers and adult cows using sterilized 50 mL screw tubes.

2.3 Isolation of *Lactobacillus* spp.

One ml from each sample (saliva, feces, and ruminal liquid) was suspended in 9 mL sterilized water, vortexed for 1- min, and then a serial dilution was prepared from each sample. One mL of each dilution was inoculated on a plate. The MRS agar medium was poured onto plates using the double-layer technique and incubated at 37°C for 48-72 hr. Colonies with standard characteristics of *Lactobacillus* spp. were randomly picked from plates and re-inoculated into MRS liquid medium and incubated at 37°C without agitation. One mL from each enriched sample was inoculated on a Petri plate using the double-layer technique within MRS agar medium and incubated at 37°C for 48-72 hr. These steps were repeated several times to obtain pure cultures. Single colonies with standard morphological characteristics of *Lactobacillus* spp. were randomly selected from plates.

2.4 Morphophysiological and biochemical characteristics of *Lactobacillus* isolates

For primary identification of *Lactobacillus* isolates, fresh overnight pure culture of each isolate was subjected to Gram-staining, endospore test and examined microscopically for their morphology under the light microscope (Olympus BX 50, Japan) with a magnification of 1000x to determine cell shape, cell arrangements according to Bergey's Manual of Systematic Bacteriology Vos et al (2009).

2.4.1 Motility test

The hanging-drop wet method was performed on the selected isolates to examine their motility. The slide was observed under a light microscope (Olympus BX 50, Japan) with 40x magnification to check the motility of the bacteria.

2.4.2 Catalase test

A single colony from each overnight culture selected isolate was streaked on a sterile glass slide with a drop of 3% hydrogen peroxide (Merck, Germany). Catalase positivity was demonstrated by the production of bubbles or froth, whereas catalase negativity was indicated by the absence of bubbles or froth.

2.5 Molecular identification and phylogenetic analysis

The tested isolates were identified using the 16S rRNA gene partial sequencing method You and Kim (2020). A genomic DNA Extraction Kit (Intron, Biotechnology, Korea) was used to extract the genomic DNA from the tested isolates. PCR amplification of 16S ribosomal DNA (16S rDNA) was performed using; 27F-Forward primer 5′AGA GTT TGA TCC TGG CTC AG 3′ (20 mer) and 1492R-Reverse primer 5′CTA CGG CTA CCT TGT TAC GA 3′ (20 mer) universal primers. The PCR amplification was performed using a programmed thermal cycler (Thermo Fisher Scientific, USA). The produced amplicons of the tested isolate (PCR product) were tested for their quality by electrophoresis in a Bio-Rad submarine (8x12 cm) using agarose gel (1%) and compared with a 1kb DNA ladder (Intron Biotechnology, Korea). A UV-transilluminator (Thermo Fisher Scientific, USA) was used to visualize DNA banding patterns of 16S gene(s) amplicons under UV light. Gene JETTM genomic DNA purification kit was used to purify PCR products (Intron Biotechnology, Korea). Then sequenced using forward and reverse primers with an ABI 3730xl DNA sequencer (Macrogen Korea, comp.) After sequencing, the nucleotide bases of each DNA sample were identified and compared to comparable sequences derived from the GenBank database within the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/GenBank/index.html) using nucleotide Basic Local Alignment Search Tool (BLAST) Gene Sequences in the database website (http://www.ncbi. nlm.gov/BLAST/). The nucleotide sequences

of the 16S rRNA genes were deposited under accession numbers shown in **Table 1**. The phylogenetic analysis of sequences was created using MEGA-integrated software for sequence alignment and Molecular Evolutionary Genetics Analysis (http://www.megasoftware.net/). Based on the Tamura-Nei model, the statistical method revealed maximum likelihood, and the test of phylogeny is the Bootstrap method with no. of bootstrap replication equal to 500 based (Stecher et al 2020, Tamura and Nei 1993).

2.6 Bile salt resistance assay

The effect of bile salt on the growth of *Lactobacillus* spp. was examined using the method described by Mulaw et al (2019). A series of bile concentrations with a range of 0.05 to 0.3% were employed on the MRS broth medium and only media was used as a control. 100 µl of freshly prepared cultures were inoculated into the medium and incubated at 37°C for 48 hr. The total counts of all treatments were determined through 10-fold serial dilution in 0.1% peptone water using the pour plate method. The experimental analysis was carried out in triplicate and the count was determined as colony-forming units (CFU/mL).

$$\text{Percentage changes of the tested strains} = \frac{\log \text{CFU/mL of the treated media} - \log \text{CFU/mL of the control media}}{\log \text{CFU/mL of the control media}}$$

If the results it is pc
If the results it is negative, it is a decrease

2.7 Sodium chloride resistance test

All the tested strains were grown on MRS broth medium accompanied by various concentrations of NaCl (1-10%), and only media was used as a control. The MRS broth tubes were inoculated with 100 µl overnight culture of the tested strains and incubated at

37°C for 48 hr. The total counts of all the samples were determined using the pour plate method through 10-fold serial dilution in 0.1% peptone water. The experimental analysis was carried out in triplicate and the count was determined as colony-forming units (CFU/mL) (Mannan et al 2017).

2.8 Determination of optimum temperature

10 mL sterilized MRS broth tubes were inoculated by 100 µL of overnight grown tested strains and incubated at different temperatures ranging from 25 to 45ºC for 24 hr. After incubation, the bacterial growths were recorded by the plate count technique (Reuben et al 2019).

2.9 pH resistance test

To study the acid resistance of the tested *Lactobacillus* spp. 100 µl of freshly prepared tested strains were inoculated on MRS broth media containing different pH levels ranging from 2.5 to 8.5 and incubated at 37°C for 48 hr. and only media was used as a control. pH resistance was estimated after 48 hr. by measuring the growth of viable cells in all the tested treatments using the pour plate count method to determine viable cells as colony-forming units (CFU/mL) (Mannan et al 2017).

2.10 Quantification of produced organic acid and determination of the media's pH values

Organic acids produced by *Lactobacillus* spp. were quantified according to Hoque et al (2010). MRS broth complemented with 10% skim milk was inoculated with 100 µl overnight culture of the tested strains and incubated at 37°C for 72 hr. The fermented samples were gathered every 24 hr. for three consecutive days and filtration was used to separate the liquids of coagulated milk. The pH of the split liquid was measured using a digital pH meter electrode and the organic acid was quantified through titration with 0.1 N NaOH using phenolphthalein as a pH indicator.

3 Results and Discussion

3.1 Morphophysiological and biochemical characteristics of selected isolates

In this experiment, a total of 12 *Lactobacillus* spp. were identified based on the morpho-physiological and biochemical characteristics of the colony and the cells. The isolates were cultivated in deMan, Rogosa and Sharpe (MRS) medium. All the isolates were facultatively anaerobic and produced small, convex, and round shapes with shiny whitish cream-colored, consistency, and smooth colonies. All isolates were found as gram-positive, rod-shaped, non-spore-forming, occurring singly or in chains under a bright field microscope to observe their microscopic features. The isolates showed them nonmotile**.** Additionally, the isolates were catalase-negative. Based on the morpho-physiological and biochemical characteristics of selected isolates and according to Bergey's Manual of Systematic Bacteriology: Volume 3: The Firmicutes, the tested isolates were classified at the genus level to be quite similar to *Lactobacillus* spp. These characteristics are similar to the findings of (Mannan et al 2017, Salvetti et al 2012, Hoque et al 2010, Vos et al 2009).

3.2 Molecular identification and the phylogenic tree of the selected isolate

PCR amplification of the DNA samples from the tested 12 locale bacterial isolates generated (1500 bp) PCR product fragments. The 16S rRNA gene(s) of the selected 12 locale isolates were sequenced with 27F and 1492R primers in the forward and reverse directions. The NCBI database similarity percentages with reference strains are presented in **Table 1**. The highest similarity was 99.90 for the *Lactobacillus farraginis* MD_A11 strain under accession no. MW193050 with reference strain *Lactobacillus farraginis* strain NRIC 0676, While the lowest similarity was 85.6 for *Lactobacillus reuteri* MD_A3 under accession no. MW193049 with reference strain *Lactobacillus reuteri* strain NBRC 15892. All the alignments were with Zero E value.

Gene bank nucleotide database using the blast-n algorithm revealed significant matches with high max scores and, zero e-value, for all the 16s rRNA genes of the 12 selected strains this confirms the identification of the selected strains as *Lactobacillus* with deferent species as shown in **Table 1**. The phylogenetic tree **Fig 1** shows a high genetic relationship between the 12 selected strains and each other as Lactobacillus bacteria. The phylogenetic tree shows a range of possible nucleotides at each ancestral node based on their random probability at site 1. Initial heuristic trees were constructed using BioNJ algorithms and Neighbor.

Join to a matrix of pairwise distances using the Tamura-Nei model (Elnady et al 2022). Topology was chosen with a superior log-likelihood value and the analysis included 12 nucleotide sequences **Fig 1** confirming its identity in all the selected strains as shown in **Table 1**.

Table 1. Locale Isolated Strains and accession numbers with the identity percentage with gene bank reference strains

Samples sources		Samples Code	Isolated Strain	Accession number	Identity %	Gene bank reference Strain
Saliva	Heifers	A11	*Lactobacillus farraginis* MD_A11	MW193050	99.90	*Lactobacillus farraginis* strain NRIC 0676
	Adult cows	A12	*Lactobacillus farraginis* MD_A12	MW193048	98.28	*Lactobacillus farraginis* strain NRIC 0676
		A31	*Lactobacillus hilgardii* MD_A31	MW193051	98.26	*Lactobacillus hilgardii* strain NBRC 15886
		A32	*Lactobacillus farraginis* MD_A32	MW193071	98.80	*Lactobacillus farraginis* strain NRIC 0676
		A3	*Lactobacillus reuteri* MD_A3	MW193049	85.60	*Lactobacillus reuteri* strain NBRC 15892
Rumen	Heifers	R31	*Lactobacillus rhamnosus* MD_R31	MW193084	95.00	*Lacticaseibacillus rhamnosus* strain NBRC 3425
	Adult cows	R7	*Lactobacillus rhamnosus* MD_R7	MW193083	99.59	*Lacticaseibacillus rhamnosus* strain NBRC 3425
Feces	Heifers	B11	*Lactobacillus farraginis* B_11	MW193076	99.56	*Lactobacillus farraginis* strain NRIC 0676
	Adult cows	B21	*Lactobacillus farraginis* MD_B21	MW193080	99.47	*Lactobacillus farraginis* strain NRIC 0676
		B 1	*Lactobacillus farraginis* MD_B1	MW193072	99.58	*Lactobacillus farraginis* strain NRIC 0676
		B 2	*Lactobacillus rhamnosus* MD_B2	MW193073	99.12	*Lacticaseibacillus rhamnosus* strain NBRC 3425
		B3	*Lactobacillus rhamnosus* MD_B3	MW193093	98.00	*Lacticaseibacillus rhamnosus* strain NBRC 3425

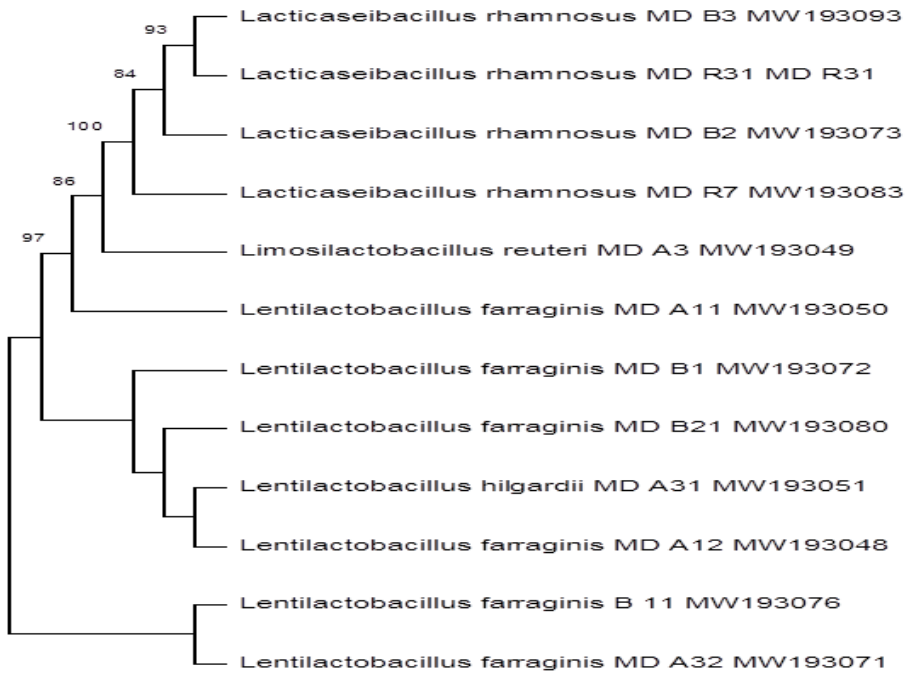

Fig 1. Phylogenetic tree of the selected 12 *Lactobacillus* strains

3.4 Physiological characteristics of *Lactobacillus* strains

The main factors influencing the growth and activity of ruminal microbial populations are bile salt, temperature, pH, buffering capacity, and concentration of NaCl.

A large body of evidence has highlighted the potentiality of using probiotic bacteria as feed supplements, in sustaining animal production systems for improving their performance and productivity. *Lactobacillus* strains are ingested alive, and they must survive the harsh conditions in the gastrointestinal tract, to reach the colon alive and in a desirable population to exert their function (Reuben et al 2019, Shehata et al 2016). Probiotic strains obtained from their native host are highly preferred since they are already familiar with the GIT and can proliferate spontaneously and express the desired beneficial effects better than strains isolated from other sources (Reuben et al 2019, Dowarah et al 2018).

Many microorganisms have been used as probiotics, but *Lactobacillus* strains seem to be the most potential probiotic agent in animal nutrition because of their tolerance to different stress conditions (Harnentis et al 2020) as they are allochthonous members in the GIT of the ruminants (Walter 2008).

3.5 Resistance of *Lactobacillus* strains to different bile salt concentrations

Data presented in **Fig 2** reveal clear variation in the growth of *Lactobacillus* strains at different bile salt concentrations. Generally, all the strains exhibited a decrease in the growth by increasing the concentration of the bile salt when compared to control media, except strain A31 which exhibited a slight increase in the growth at 0.05% and 0.1% being 12.65 and 11.53 log CFU/mL and these increases were calculated as 10.30, and 0.60% increase percentage in the growth compared to the growth of the strain in the control medium, respectively. Then started to exhibit a slight decrease in growth by increasing bile salt concentrations and the percentage of the growth decline was calculated as 8.90, 9.60, and 10.90% respectively when compared to the control.

Strain A11 revealed the highest resistance to bile salt concentrations when compared to all tested strains as it exhibited the lowest decrease in the microbial growth percentage when compared to all tested strains being 5.96, 6.61, 6.85, 7.40, 7.53, and 7.64%, respectively.

The gastrointestinal systems of animals have varying concentrations of bile salt. These concentrations vary, depending mainly on the type of feed consumed. The capability of *Lactobacillus* spp. to tolerate bile salt is important in governing its effectiveness and growth in the gastrointestinal tract, particularly in the upper small intestine which may be critical for controlling the growth of intestinal pathogens entering the digestive system (Gilliland et al 1984). Therefore, resistance to bile toxicity is one of the main requirements for the selection of probiotic strains that may be able to grow, colonize and perform their metabolic activities effectively in the gastrointestinal tract. Bile concentrations ranging from 0.05% to 0.3% have been used for the selection of *Lactobacillus* strains considering that 0.3% is the average of intestinal bile concentration that could be used for screening the strains resistant (Aswathy et al 2008, Erkkilä and Petäjä 2000).

In the current study, it was observed that all the isolated strains revealed resistance to bile salts from 0,05 to 0.3% quite effectively with a variable decrease between the strains. A clear decrease in the microbial count of all the tested strains was found at 0.3% bile salt concentration. These variations in the resistance are consistent with those of Garriga et al (1998) who reported resistance of *Lactobacillus* spp. strains to a range of 1% - 4% bile salts, and Mulaw et al (2019) who stated a survival percentage ranging from ≈ 91 to 97% for *lactobacillus* spp. in the presence of 0.3% bile salt after 24 h of incubation. In the same consequence, Aswathy et al (2008) revealed that *Lactobacillus* spp. were able to maintain good growth and multiplication at 0.3, 0.5, and 0.8 % bile salt concentrations, and this growth was variable according to the tested strain.

3.6 Resistance of *Lactobacillus* strains to different NaCl concentrations

Data presented in **Fig 3**, shows that *Lactobacillus* strains revealed variable growth in the medium subjected to different NaCl concentrations. Generally, strains A3, R31, R7, B21, B1, B2, and B3 revealed a clear decrease in the growth for all NaCl concentrations when compared to the control.

Strains A31 and A32 revealed an increase in the growth within the concentrations 1, 2, and 3% NaCl. These increases were calculated as 13.47, 10.06, and 7.10% with strain A31 and 6.61, 6.96, and 3.81% with strain A32 respectively as an increasing percentage in

the growth. By increasing salt concentration, a clear decrease in the growth of both strains was recorded.

Strain A12 revealed an increase in the growth within concentrations 1, and 2 % NaCl followed by a decrease in the growth by increasing the NaCl% concentration. These increases were 14.65 and 14.07%.

Strain B11 revealed an increase of 6.95% in the growth only at 1% NaCl when compared to control, then revealed a decrease in the growth by increasing the NaCl%. While strain A11 showed a slight increase in the growth at 1% NaCl% equals 0.16 %, then revealed a decrease in the growth by increasing NaCl% concentrations. Concurrently, strain A11 exhibited the highest resistance to different NaCl% concentrations when compared to all tested strains as it revealed the lowest decrease in the microbial growth percentage in all NaCl concentrations being 1.40, 5.62, 6.48, 7.36, 7.39, 7.41, 7.42, 7.28, and 13.76%, respectively.

Resistance of *Lactobacillus* strains to high concentrations of NaCl is necessary for the selection of effective additives for the animal, as it is a scale to measure how much *Lactobacillus* strains can tolerate toxic and osmotic shock in the GIT (Śliżewska et al 2021, Shehata et al 2016). So, it is considered one of the main factors that could be used to distinguish among *Lactobacillus* strains to select the most resistant strain to be used as an animal additive. In the current study, variable reactions were observed in the selected strains among different salt concentrations. However, *Lactobacillus* strains isolated from saliva revealed the ability to tolerate and showed good growth at high NaCl concentrations. Hassan et al (2020) reported that *Lactobacillus* strains showed high growth at 1-4% NaCl concentrations, while bacterial growth decreased gradually with increasing in NaCl%, from 5 to 7%. While Chowdhury et al (2012) reported that *Lactobacillus* strains revealed good growth up to 3% NaCl and the growth decreased sharply with increasing salt concentration.

3.7 Resistance of *Lactobacillus* strains to a range of temperatures

Data presented in **Fig 4** show clear variation in the growth of the *Lactobacillus* strains at different temperatures. Generally, all the strains revealed a decrease in growth by increasing the temperature over 37ºC.

Strains A3, R31, B11, B2, and B3 revealed the highest growth at 37ºC as optimum temperatures of 13.66, 13.39, 12.61, 11.87, and 12.16 log CFU/ml along with a clear decrease in the growth by increasing or decreasing the temperature from 37ºC.

While strains A11, A12, A31, A32, R7, B21, and B1 revealed an increase in the growth by decreasing the temperature below 37ºC. Strains A12, A31, R7, B21, and B1 exhibited the highest growth at 30ºC (13.50, 13.36, 12.42, 13.81, and 13.64 log CFU/mL) which was increased by 18.72, 16.50, 0.60, 28.37, and 3.93%, respectively.

While strains A11 and A32 exhibited the highest growth at 35ºC (10.80, 13.71 log CFU/mL) which was increased by 13.36 and 8.53%. Additionally, strains A11 and A32 revealed the highest resistance toward increasing temperature over 37ºC, as they revealed the lowest decrease in growth percentage when compared to control (37ºC) being 4.19 & 3.94%, for strain A11 and 3.44 & 5.23% for strain A32.

The temperature of the gastrointestinal tract of rumens is considered one of the main factors that influence the growth and activity of ruminal microbial populations. The temperature of the rumen is maintained in the range of 39 to 39.5°C (Wahrmund et al 2012) and can rise up to 41°C instantly after feeding the animal because the fermentation process generates heat (Brod et al 1982). Therefore, the selected strains were subjected to a range of temperatures between 30 – 45°C. The obtained results are in agreement with other studies stating that all LAB strains examined grew optimally at 37 °C after 24- 48 h of incubation and the growth of LAB strains is reduced by reducing or increasing the temperature out of this range (Reuben et al 2019).

3.8 Determination of optimum pH for *Lactobacillus* spp.

Clear variations were recorded between the growth of tested *Lactobacillus* strains at different pH concentrations and different optimum pH was recorded for the tested strains (**Fig 5)**. Generally, moving toward neutrality and alkalinity in the media (more than 6.5) revealed a growth decrease in all tested strains. Strains A12, A31, and A32 revealed an increase in the growth by decreasing the pH of the medium, and the highest growth was obtained at pH 5 (13.31 log CFU/mL), pH 5.5 (13.45 log CFU/mL), and pH 6 (12.96 log CFU/mL), which were increased by 19.81, 16.84, 0.46%, respectively.

Strains A11, A3, R31, R7, B11, B21, B1, B2, and B3 recorded decreases in the growth by changing the pH of the media over pH 6.5. Strain A11 exhibited the highest tolerance to different tested pH levels as it revealed the lowest decrease in growth percentage.

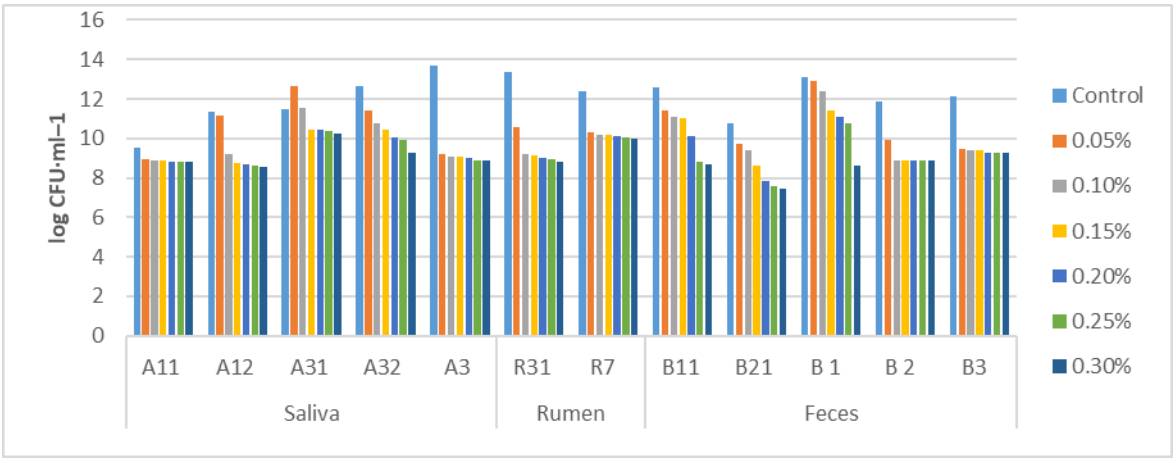

Fig 2. Resistance of *Lactobacillus strains* to different bile salt concentrations

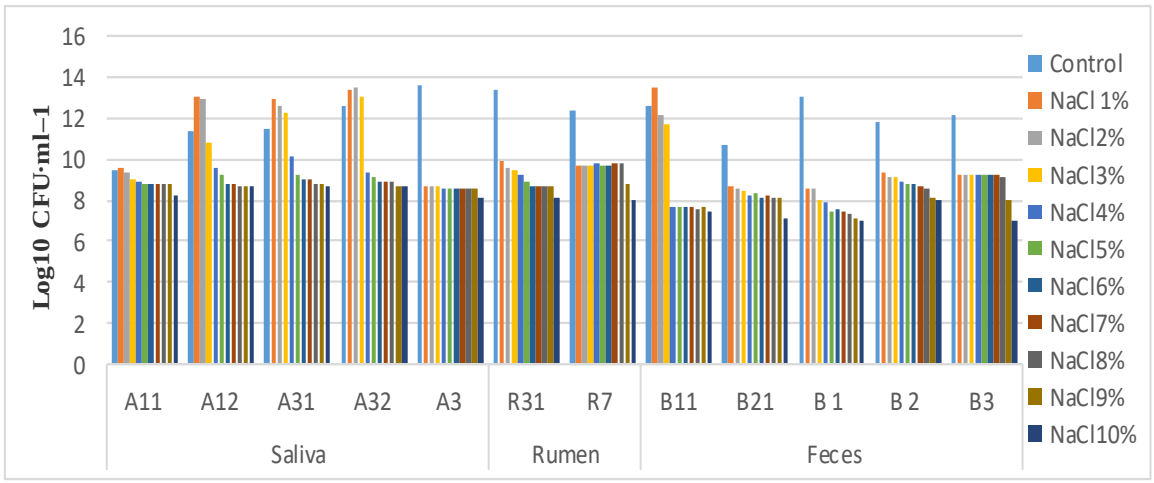

Fig 3. Resistance of *Lactobacillus strains* to different NaCl concentrations

In ruminants, different regions of the gastrointestinal tract have varying acid levels and this pH varies irregularly between the different parts of the system (Walter 2008). Therefore, the essential criteria for *Lactobacillus* spp. to grow and profitably perform its function in the gastrointestinal tract is to be resistant to different pHs as they encounter harsh conditions during passing through different parts of the gastrointestinal tract with different pHs (Walter 2008).

In this study, all the strains were tested in both acidic and alkaline conditions at a range of pH 2.5 to pH 8.5, and the survivability of *Lactobacillus* spp. was accomplished. The growth pattern of tested *Lactobacillus* spp. at the pH values specifying that these bacteria exhibited survival in both highly acidic and moderate alkaline conditions with less growth in high alkaline conditions with the preference to grow in neutral and acidic pH. Similar results were observed by Pyar and Peh (2014), and Hoque et al (2010).

3.9 Quantification of Organic Acid and Determination of pH Value

Data presented in **Fig 6** show the pH of the medium and the titratable acidity % of the supernatant during three subsequent days for each tested strain. Although the variation recorded between the tested *Lactobacillus* strains, there is a consistent tendency was observed for all strains in the measured values during the incubation period. The pH of the medium recorded a gradual decrease by increasing the incubation period while the tithable acidity % increased by increasing the incubation period. Strain A11 recorded the lowest medium pH after the third day being 4.2 with tithable

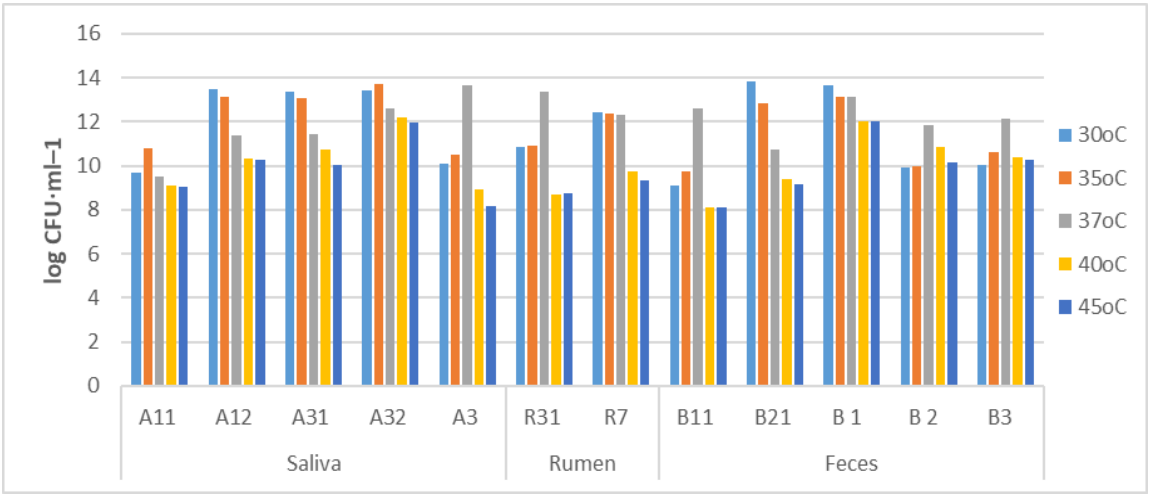

Fig 4. Resistance of *Lactobacillus* strains to range of temperatures

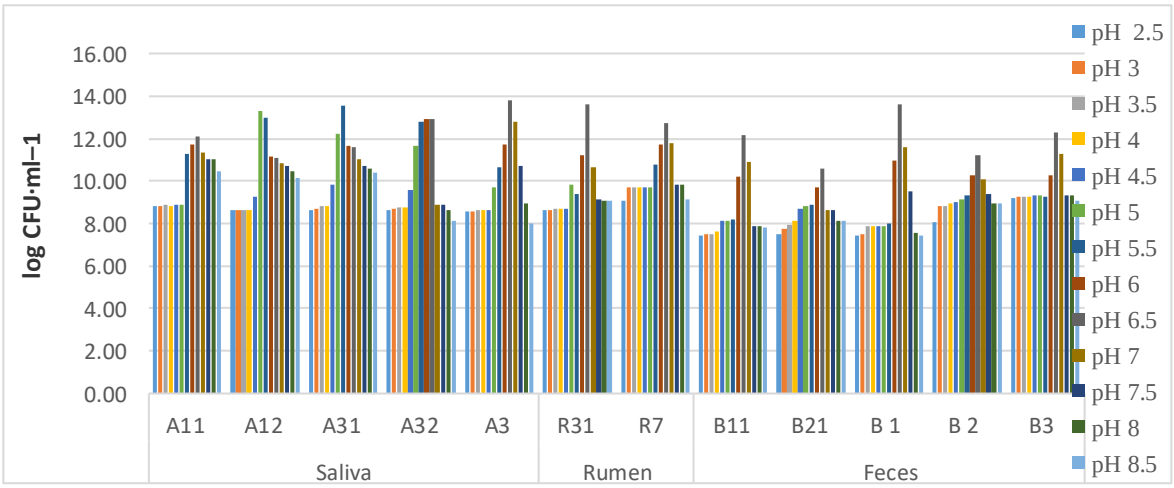

Fig 5. Growth of *Lactobacillus* spp. at different pH

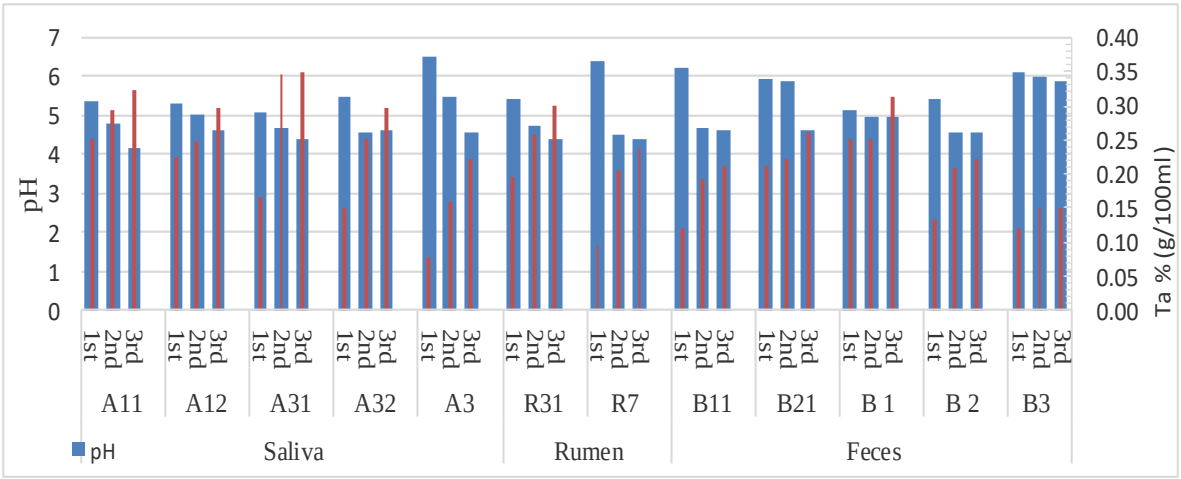

Fig 6. Quantification of Organic Acid and Determination of pH Value

acidity of 0.32%. While strain B3 recorded the highest medium pH after the third day being 5.9 with a tithable acidity of 0.15%. Strain A32 recorded the highest titratable acidity after the third day is 0.35% with pH 4.4 for the medium.

The results of the pH and the titratable acidity % of the supernatant during three subsequent days for each tested strain are in line with those of Chowdhury et al (2012) who reported the capability of *Lactobacillus* spp. to produce lactic acid during the growth which decreases the pH of the medium by increasing the incubation period. As well they stated minor variations in organic acid quantities by Lactobacilli due to their regional variation.

4 Conclusion

This study revealed that most of the tested strains showed a reduction in growth by increasing the concentration of the bile salt, NaCl, increasing the temperature over 37°C, and moving toward neutrality and alkalinity in the media, whereas strain A11(*Lactobacillus farraginis* MD_A11) revealed the highest resistance to various bile salt concentrations, NaCl% concentrations, increased temperature over 37°C, and different pH levels as it illuminated the lowest decrease in the growth percentage when compared to control. This study assumes that utilizing (*Lactobacillus farraginis* MD A11) as a local Egyptian strain is the best strain to propose as a feed additive probiotic for Egyptian ruminants to boost their growth performance and productivity.

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