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Probiotic Characterization of Primate Origin *Lactiplantibacillus plantarum* LG138

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Abstract

This study was aimed to characterize previously isolated lactic acid bacteria *Lactiplantibacillus plantarum* LG138 from primate feces for its probiotic potential. The ability of the isolate to withstand different *in vitro* gastrointestinal stresses was assessed over a period of time i.e. at pH 2.0 and 3.0, bile salts (0.5, 1.0 and 2.0 %) and lysozyme (50,100, 150 mg/ml). Further the *L. plantarum* LG138 was tested for hydrophobicity, auto-aggregation and co-aggregation abilities, coexistence, exopolysaccharide production and hemolytic activity. The isolate demonstrated significant growth in the presence of different types of artificialdigestive conditions (low pH, bile and lysozyme).Furthermore, oxbiledid not affect the viability of *L. plantarum* LG 138 cells compared to the control. The isolate *L. plantarum*LG138 exhibited 65.7 ± 0.32 % auto-aggregation after 24 h incubation. The hydrophobicity test found the culture moderately hydrophobic (35 to 69 %) forhexadecaneand highly hydrophobic (70 to 100 %) for toluene and xylene. Moreover, it was observed to co-aggregate (66.13 ± 0.18 %) with a pathogen, *Shigellaflexneri*, without antagonizing other probiotic bacteria. *L. plantarum*LG138 was found to be able to produce exopolysaccharide and found to be non-hemolytic. These findings highlight the potential of *L. plantarum* LG138 as a promising probiotic candidate, suitable for incorporation into pelleted orgranulated animal feed formulations.

Keywords: Lactic acid bacteria, primate, animal, probiotics, characterization

Introduction

Lactic acid bacteria (LAB) existing in the gut microbiota are vital for the wellbeing of both animals and humans. When utilized as probiotics, they show potential in boosting growth performance in livestock agriculture (1).LABasGram-positive microaerophilic organisms are extensively studied amongst the gastrointestinal (GIT) microbiota. Jin et al. (2) reported that the main LABs present in the milk from rhesus monkeys are belonging to the genera of *Streptococcus* and *Lactobacillus.* Out of these, many LAB species like *L. johnsonii, L. animalis*and *L. brevis* along with *Bacillus* species are notreported in human milk as revealed by different metagenomic studies. These bacteria due to their presence as indigenous species in the host gut are well acclimatized and mostly present as host-specific LAB populations (3).In extensive livestock farming operations, the circumstances often lead to heightened stress and health problems among the animals. The disruption in the microbiota balance can indeed be a significant factor contributing to disease development in such settings (4). These bacteria, commonly utilized as probiotics, contribute to maintaining the equilibrium of the microbiota in gut by engaging in competitive interactions with pathogens to ensure their survival, enhance growth performance, improve feed conversion efficiency, optimize nutrient utilization, modification of gut microbiome and promote gut health (5).The interest in the study of microbial heterogeneity of human and nonhuman animals correlating their importance in the functioning of GIT and wellness of host is becoming the new area of investigation. In primates, gut bacterial communities are known to be species specific, but they fluctuatemostly with stage of development, social organization and

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nutrition with substantial loss of microbes in confinement(6).

The ideal probiotic microorganism should be able to withstand acidic conditions and tolerate bile without posing any risk of carcinogenicity or pathogenicity. Additionally, it should adhere to the host's epithelial tissue, enhance the gut microflora, decrease pathogen attachment, and produce secondary metabolites that combat pathogenic microorganisms (7-8). Modifying the microbial ecosystem can indeed result in improved livestock productivity. The introduction of live microbes as probiotics serves as a safe and effective substitute for antibiotics as growth supplements, as they carry no risk of toxicity in livestock products and leave behind no residues. In fact, the contact between LABs and gut microorganisms leads to an increase in propionate and total volatile fatty acids (VFAs) production. This beneficial interaction ultimately enhances feed utilization, improves growth performance, and reduces the diarrheal cases (9).

Despite the emergence of transgenic mice, nonhuman primates are still widely regarded as the premier laboratory animal due to their close evolutionary relationship to *Homo sapiens*. The rhesus monkey, in particular, is extensively used as a nonhuman primate model in medical research because of its close evolutionary proximity to humans (2). To ensure the most effective use of primates as models, it is essential for pathologists participating in study design and interpretation to have a thorough understanding of their histological anatomy, physiology, natural history, associated disease and place of primate origin (10). Kang et al. (11) suggested that lactobacillus strains derived from primates are more effective for intestinal health in primates compared to human-derived lactobacillus and other lactic acid strains.There is increasing support for the notion that gut microflora play a crucial role in regulating digestive health and the immune system. This presents a promising avenue for reducing idiopathic chronic diarrhea (ICD) and improving overall health and well-being in nonhuman primates. Probiotics provide an alternative strategy for reducing the diarrheal incidences in captive nonhuman primates by bolstering the indigenous microflora in the gut.

Primates, in terms of anatomy, physiology, genetics, and immunology, closely resemble humans and are considered the preclinical animal species that most closely resemble us. They are extensively employed in various biotechnology research domains across the globe and kept in wild, semi-captive, and captive. Idiopathic chronic diarrhea, gastric dilatation, and rupture of unknown origin are the primary factors leading to spontaneous mortality in captive primates utilized primarily for experimental research.These conditions pose significant veterinary challenges, contributing to a multitude of health issues (12). Diverse factors have been considered as potential triggers for intestinal disorders, such as antibiotic consumption, stress, and *Clostridium perfringens* infection. However, a definitive mechanism has yet to be pinpointed. As a result, safeguarding the intestinal well-being of experimental primates, commonly utilized in studies related to brain and infectious diseases, holds significant importance not just in terms of veterinary care but also for ensuring experimental reproducibility (11).

Up to now, there has been a very few research studies focusing on primate probiotics. The aim of our research is to fill this gap in knowledge by presenting pertinent data. There is only one commercially available nonhuman primate–specific live probiotics named as Bio-Serv'sPrimiOtic and PrimiOtic Plus. The product contains *Lactobacillus reuteri*, a probiotic bacterium sourced from nonhuman primates which is a type of bacteria that naturally inhabits the digestive system of nonhuman primates. Its presence aids in establishing the bacteria in the primate gut, resulting in the beneficial impacts of probiotics on enhancing and maintaining gastrointestinal health (12). Primate facilities commonly depend on human lactic acid bacteria products to carry

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out indoor breeding experiments with primates. Hence, it is imperative to develop a probiotic strain exclusively tailored for primates to enhance their health and combat illnesses.

Currently, the ongoing practice involves the screening of new probiotics, particularly from underexplored species. The screening LAB strains from wild primates, inhabiting their natural unexplored gut environments, presents a promising opportunity for isolating novel species with excellent characteristics and developing potent probiotics to enhance production in animal-related industries. Besides, isolating and identifying lactic acid bacteria (LAB) strains with favorable probiotic traits from wild primates holds significant potential for their practical utilization as starter, adjunct, and protective cultures in improving animal food and feed products. Moreover, performing technological characterization of these strains can enhance their efficacy in diverse applications. The study was aimed to assess the probiotic characteristics of the isolated LAB strain. This involved conducting a series of tests to evaluate their acid tolerance, bile tolerance, lysozyme resistance, non-hemolytic activity, auto-aggregation, co-aggregation capability, coexistence compatibility, and antibiotic susceptibility etc.

Materials and Methods

The organism and storage conditions

The organism was previously isolated by Kumari et al. (13) from feces of primates of Shimla region in Himachal Pradesh.

Growth in low pH

The survival of the isolate acidic conditions was analyzedfollowing the procedure of Maragkoudakis et al. (14). The isolatewastreatedwithdifferentlow pH conditions (pH 2, pH 3 and pH 7).Then the isolate was kept at 37 ºC for 3 h and its survival was calculated and expressed as log cfu/ml.

Tolerance to bile salts

The bile tolerance ability of the given isolate was performed as per previously given methodbyGilliland et al. (15). The isolate was put into sterile MRS broth (9 ml) containing different Ox-bile concentrations (0.5 %, 1 % and 2 %). Then the isolate was kept at 37 ºC for 3 h and its survival expressed as log cfu/ml.

Lysozyme resistance test

The lysozyme tolerance was evaluated according to method of Zago et al. (16). 100 µl of freshly prepared LAB cultures was inoculated into the 10ml of MRS broth supplemented with different lysozyme concentration (50,100,150 mg/ml) and kept at 37ºC for 24 h. The survival of the isolate was recordedas of log cfu/ml.

Surface properties

The cell surface hydrophobicity of the isolatewasdeterminedaccording to the previously reported modified method of Rosenberg et al. (17). The cells of the freshly grown isolate were harvested (10,000 rpm, 10 min at 4ºC) by centrifugation.Then suspended in sterile normal saline after three washing with saline and optical density ($O.D._{600}$) was adjusted at 1.0. The bacterialcell suspension and different solvents (n-hexadecane, xylene and toluene) were taken in equal amounts and incubated at room temperature for 1 h.

The adherence of the isolate to hydrocarbons was calculated as:

Hydrophobicity (%) = $[(A_1-A_2)/A_1] \times 100$

Whereas, A_1 and A_2 are absorbance before and after mixing with solvents at 600 nm.

Auto-aggregation Test

Auto-aggregation ability was assessedfollowing the method described by Colladoet al (18) withsome modifications. The cell suspension was prepared similarly to cell surface hydrophobicity test.The cells were incubated at 37ºC for 24 h and $O.D._{600}$ was measured at 1,3,24 h using a UV-Vis

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spectrophotometer. Auto-aggregation % was measured as:

Auto-aggregation % = 1- (A_x/A_y) ×100,

Where A_x represents the absorbance at time t=1,3, 24 h and A_y the absorbance at t=0h (i.e.1.0).

Co-aggregation Test

This assay was performed by according to Handley et al. (19). The isolate and the indicator organism *Shigellaflexneri*were grown inMRS and nutrient broth at 35 ºC for 24 h. The cell suspension of both was prepared similarly to cell surface hydrophobicity test. The % co-aggregation wascalculated using Handley's equation as:

$$\text{Coaggregation (\%)} = \frac{[A_{Path} + A_{LAB}\ 2] - A_{Mix}}{A_{Path} - A_{LAB}/2]} \times 100$$

Where A_{path}represents the absorbance of the pathogen, $A_{LAB}$is the absorbance of theisolate and $A_{mix}$is the absorbance of the mixture.

Coexistence Test

The selected isolate was checked for its compatibility with other LAB isolates using 'cross streak method' as given by Guoet al. (20). The freshly grown cultures of all the isolates were streaked perpendicular to each other on MRS agar plates. The plates were observed for presence or absence of antagonism after incubation at 37 ºCfor 24 h.

Antibiotic sensitivity test

The test was conducted following the guidelines given by Clinical and Laboratory Standards Institute (21).The 100 µl of freshly grown culture was swabbed on the Mueller Hinton Agar plates and allowed to dry. Then antibiotic discs were placed on the agar surface and incubated at 35ºC for 24 h. After incubation, the plates were observed for formation of zones of inhibition and their diameters were recorded.

Antagonistic activity

The antimicrobial activity was evaluated using agar well diffusion method as given by Mishra and Prasad (22). The CFS (cell free supernatants) was prepared by centrifuging (1000 rpm for 10 min) the culture grown overnight in MRS medium and then screened against eight food spoilage causing bacteria (*S. aureus* MTCC 96, *L. monocytogenes* MTCC 657, *S. flexneri, B. cereus* MTCC 1272, *P. aeruginosa* MTCC 424, *E. coli* MTCC 118, *A.hydrophilla*and*S. typhi*).The fresh cultures of indicators were swabbed on agar surface and 100 µl of CFS was poured into the wells prepared in the agar plates.The zones of inhibition were observed and recorded after 24 h incubation at optimum temperature.

Exopoysaccharide (EPS) production

The qualitative evaluation for EPS production was done following the method given by Kersaniet al.(23). The 24 h old LAB culture was streaked on the plates of ruthenium red milk agar plates.The plates were observed for the formation of white color colonies against pink background. Overnight grown LAB cultures were streaked on the surface of plates containing ruthenium red milk agar medium. After incubation at 37 ºC for 24 h, exopolysaccharide producers appeared as white colonies and were selected for further studies.

Hemolytic activity

Hemolytic activity of the isolate wasassessed according to the method of Lombardi et al. (24).The 24 h old bacterial culture was streaked on blood agar plate and observed for presence of hemolysis after overnight incubation at 37 ºC.

Statistical Analysis

Each experimental trial was conducted three times, and the results were presented as mean ± standard deviation (SD). Statistical analysis was conducted using SPSS version 27.0.1 (SPSS Inc.111., USA). The significance level ($p < 0.05$) was determined through analysis of variance (ANOVA).

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Results and Discussion

Survival rate at low pH

To examine the survival rate of probiotic strains based on their ability to withstand low pH levels is an important probiotic characteristic.Probiotic bacteria need to endure the stomach environment (highly low pH), tolerating pH values as low as 2.0 and become colonized to show beneficial effects on the host (25).Based on our results, the isolate survived under different acidic conditions and shown ability to survivewhich was not unexpected because these bacteria are well known to be an indigenous flora of the gastrointestinal tract of animals.In our study, with a small amount of viability loss (1.04 log cycles at pH 2.0 and 0.4 log cycles at pH3.0), (Table1). In a study carried out by Zielinska et al. (26), it was discovered that *Lactobacillus* probiotic strains demonstrated a survival rate varying from 30% to 100% upon exposure to gastric juice with a pH of 3.*L. plantarum* uses several strategies to withstand the stress of acid and bile salt. Huang et al. (27) illustrated that *L. plantarum* ZDY2013 possesses the ability to remove protons from the intracellular environment, contributing to the maintenance of pH homeostasis.

Bile tolerance

Another critical attribute of probiotics is their ability to tolerate bile salts, as these substances can break down lipids of membranes leading to cell death due to leakage of its contents. In this study, the log value of the population after incubation for 3 h without 0.3% oxbile was 9.5, but it was 7.46 at 0.5% oxbile, followed by 7.45 and 7.40 1and 2 % oxbileconcentration respectively.This shows a better survival at different bile salt concentration owing to previously conditioning in primate intestinal tract.Another study has demonstrated that *Lactobacillus plantarum* and *L. paracasei*exhibit acceptable survival(6.19 and 6.0 log cfu/ml) in a bile salt environment even in a high concentration (0.3%) (28). Choi et al. (29) reported that bile salt concentration was increased from 0.3 to 1%, population of *L. plantarum* GBL16 was reduced by 0.7–2.1 log cfu/ml while that of GBL17 was reduced by 0.7–1.4 log cfu/ml, similar to the reduction in population of commercial *L. plantarum* (KCCM40399) which was decreased by 1.3–2.4 log cfu/ml with increasing of bile salt concentrations. The resistance to bile salt of the strains might be induced by potential presence of some proteins.The differing levels of bile tolerance observed lactic acid strains were linked to six proteins (GshR1, Bsh1, Cfa2, GshR4, AtpH and OpuA). These proteins are believed to be pivotal in the response to /and adaptation to bile salts in *L. plantarum.*

Lysozyme resistance test

The initial prerequisite for potential probiotic bacteria is to possess resistance against lysozyme found in the saliva. This is crucial because the lysozyme present in the

Table1: Effect of pH (2.0, 3.0 and 7.0) and bile salt concentrations (0.5, 1.0 and 2.0%) on viable count of LAB isolates

Isolates	Acid tolerance (log cfu/ml)			Bile tolerance (log cfu/ml)			
	pH 2.0	pH 3.0	pH 7.0	Control	0.5 %	1 %	2 %
L. plantarum LG 138	8.53 ± 0.15	9.17 ± 0.25	9.57 ± 0.09	9.50±0.10	7.46±0.02	7.45±0.02	7.40±0.04
L. rhamnosus LGG	7.47 ± 0.12	7.97 ± 0.21	8.67 ± 0.09	8.7±0.20	7.33 ± 0.09	7.10 ± 0.21	6.83 ± 0.2
(Note: Values represented as mean ± standard deviation (SD) of triplicate analysis)							

*LactiplantibacillusPlantarum*LG138

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oral cavity has the ability to lyse Gram-positive bacterial cells.Our results as shown in the Figure 1indicated thatdue to their gut origin the isolate showed good lysozyme resistance (8.63, 8.40 and 8.20 log cfu/ml at concentration of 50,100 and 150mg/ml respectively) as compared to initial 8.83 log cfu/ml in control after 3 h incubation.Nandha and Shukla (30) studied the growth of LAB isolates in the presence of 100 mg/ml of lysozyme which reveals that the decline in LAB growth in the presence of lysozyme ranged from 0.23 to 3.80 logarithmic units after the 60-min incubation period. This outcome agrees with the results obtained for isolates from vegetables, camel milk, and fermented foods(31).

Surface hydrophobicity

Following the hydrophobicity criteria given by Tyfa et al. (56)i.e.strongly hydrophobic (>50%), moderately hydrophobic (20–50%) and hydrophilic (<20%), the strain was found to be strongly hydrophobic for xylene (75.87 ± 0.22 %) followed by n-hexadecane (65.10 ± 0.17 %) and toluene (70.30 ± 0.12 %), demonstrating more hydrophobic than hydrophilic cell surface of isolate (Figure 2).The results of LAB hydrophobicity in this investigation were higher than LAB isolates as reported by El-Deeb et al. (32)from dromedary camels showing 49.6 ± 0.6% hydrophobicity for hexadecane, followed by xylene (44.3 ± 0.5%) and toluene (41.6 ± 0.6%).Isolates with a substantial presence of surface proteins and lipoteichoic acids generally display higher hydrophobicity compared to isolates containing a significant amount of hydrophilic polysaccharides.Cell surface hydrophobicity plays a critical role in indicating a bacterium ability to adhere to human intestinal cells, which is essential for probiotic effectiveness. This hydrophobic property is believed to facilitate bacterial adhesion to epithelial tissue, thereby aiding in their colonization and survival within the gastrointestinal tract(33).

Auto-aggregation ability

The interaction of cellular surface components like soluble proteins, lipoteichoicacidand carbohydrates result in cell aggregation.Following 24 h incubation at 37ºC, *L.plantarum* LG138 exhibited a co-aggregation ability of 65.7 ± 0.32%, as shown in Table 2 reflecting its higher potential of colonization in the gut epithelium. Auto-aggregation is an essential probiotic trait that contributes to the formation

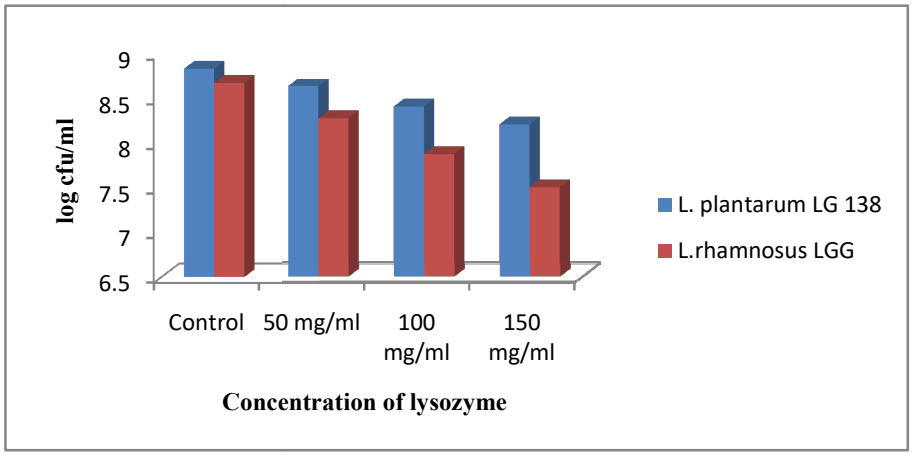

Fig.1: Lysozyme tolerance of LAB isolates in different concentrations (50,100,150 mg/ml) of lysozyme

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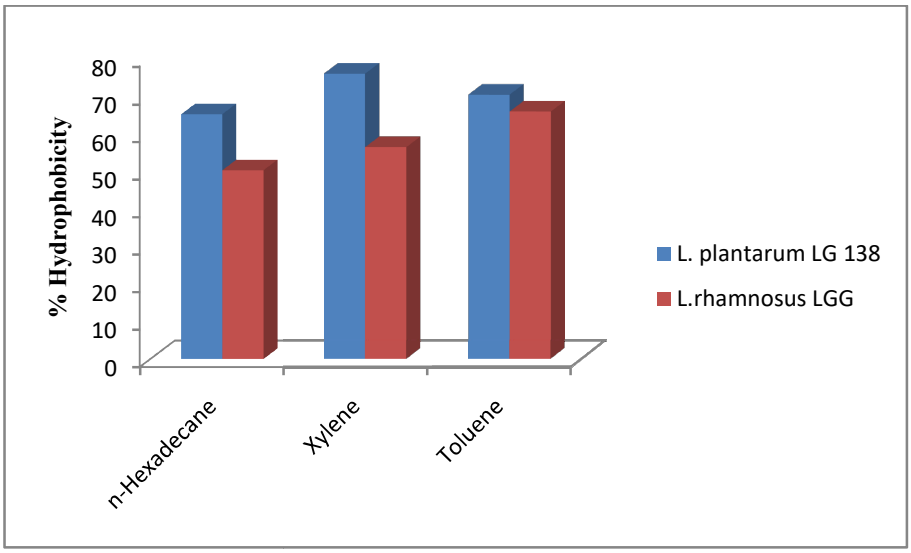

Fig.2: Hydrophobicity of LAB isolates towards various hydrocarbons (n-hexadecane, xylene and toluene)

Table 2: Auto-aggregation (%) and co-aggregation (%) of the LAB isolates at time interval of 1 h, 3 h and 24 h

Isolates	Auto-aggregation ability (%)			Co-aggregation ability (%)		
	1 h	3 h	24 h	1 h	3 h	24 h
L. plantarum LG 138	20.9 ± 0.26	30.3 ± 0.25	65.7 ± 0.32	20.3 ± 0.26	24.87 ± 0.18	66.13 ± 0.18
Lactobacillus rhamnosus LGG	18.7 ± 0.31	28.17 ± 0.2	69.97 ± 0.24	19.9 ± 0.25	22.67 ± 0.3	61.53 ± 0.18

(Note: Values represented as mean ± standard deviation (SD) of triplicate analysis)

of biological niches, particularly within the gut of the organism. The clustering of bacteria relies on the auto-aggregation ability and hydrophobicity of the microbe. Similarly Jang et al. (34) described that *L. brevis* KU15153 and LGG showed 21.44% and 22.68% auto-aggregation abilities, respectively after 4 h incubation. After 24 h incubation, auto-aggregation of *L. brevis* KU15153 (52.55%) was higher than that of LGG (44.70%). *Lactobacillus plantarum strain GCC_19M1 exhibited 29.60% auto-aggregation, suggesting a reduced capacity to colonize and attach to the intestinal epithelium*(35).The test results suggest that the combined influence of auto-aggregation and hydrophobicity could potentially boost the adherence capability of probiotic strains.

Co-aggregation Ability

Co-aggregation assesses the level of bacterial adherence between the test strain and the enteric fever pathogen. The % co-aggregation (66.13 ± 0.18%) as depicted in

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the Table 2 demonstrates that *Lactobacillus plantarum* LG138 effectively prevented the growth of *Shigellaflexneri* within 24 hours of incubation in the pathogen exclusion study.Liu et al. (36) reported the results of co-aggregation of *Lactobacillus* isolates in the presence of target pathogens. The highest co-aggregation rates to *S. flexneri*, *S. paratyphi* B, and *E. coli* were obtained for isolate 115-4 (37.19, 45.93, and 28.19%, respectively). In the co-aggregation process, LABs release antimicrobial substances which effectively protect the surrounding environment from foodborne infections and this serves as an essential defense mechanism for the host (37). This co-aggregation ability of LAB isolates with pathogens is likely due to the presence of proteinaceous components on the cell surface and interactions between carbohydrates and lectins.

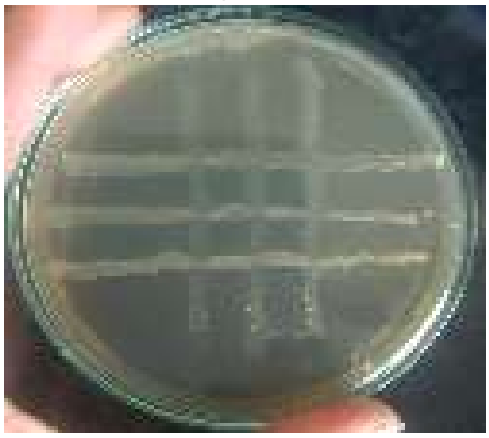

Fig.3: Cross streak test observed no antagonism among LAB isolates

Co-existence Test

The compatibility of the studied *Lactobacillus* strain with other lactic acid bacteria is ascertained to guarantee their co-existence with other probiotic bacteria in the products and subsequently in the intestinal environment of the host.The lactobacilli have been reported as antimicrobial-producing strains, and thus, antagonism amongst them they may potentially inhibit other strains and result in diminished efficacy in multispecies probiotic formulations(38).The examination of compatibility between isolated bacteria was conducted using the "cross-streak" method (Figure 3). Through this method, it was determined that there is no antagonistic effect among the selected bacterial strains. These findings align with the research conducted by Mahmoudi et al. (39), which reported that strains of *Bifidobacterium* and *Lactobacillus*, having anti-inflammatory and anti-obesity properties, were able to grow symbiotically. A contradictory result was revealed by Sabo et al. (40)the isolate *L. lactis* subsp. *lactis* C173 and *L. lactis* subsp. *lactis* C195 revealed their antagonistic effect against *E. faecium* C43.

Antibiotic sensitivity

The isolate showed the relatively high susceptibility to cotrimoxazole (22 mm), co-trimazine (20 mm) and oxacillin (20 mm), as clear from Figure 4. In addition, the

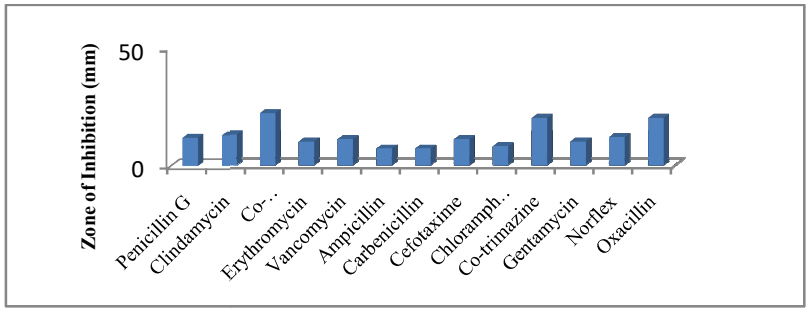

Fig.4: Antibiotic resistance profile of lactic acid bacteria

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absence of transferable resistance genes is an imperative requirement for approval of probiotics.The inherent resilience of probiotic strains enhances the therapeutic and preventive advantages when combined with antibiotics, facilitating the restoration of intestinal microbiota.Furthermore, it has been reported that *Lactobacillus* lacks the transfer of streptomycin, kanamycin, and ciprofloxacin resistance (16). Several strains displayed resistances to tetracycline, ampicillin, and cefotaxime, in line with earlier studies (41) and in agreement with the majority of commercially available probiotics.Probiotic varieties might encounter antibiotics within the digestive system of animals when antibiotics are employed to maintain animal health. Therefore to show their effect as probiotics, the strains need to have nontransferable antibiotic resistance ensuring their safety and survival in the host (42).

Antagonistic activity

The examined isolate displayed inhibitory effects (15.97 ± 0.18 to24.60 ± 0.15 mm diameter) against specific Gram-positive and Gram-negative pathogenic bacteria (Figure 5). Lactobacilli can counteract pathogens through various mechanisms, including the production of antimicrobial substances like lactic acid, acetic acid, hydrogen peroxide, and bacteriocins. Additionally, they compete for resources andco-aggregate with pathogens (43).The antagonistic activity was of *Lactobacillus plantarum* LG138 was found higher than *Lactobacillus plantarum* isolated from Raw cow milk which exhibited weak antibacterial activity (range of 0- 5mm diameter) against *Staphylococcus aureus, Staphylococcus* epidermidis, *Pseudomonas aeruginosa* and *E. coli.* (44). Contrary, The filtered supernatant of *L. plantarum*was found to be strongly inhibiting the growth of*E. coli* and *B. subtilis* ,*P. aeruginosa* and *S. hominis* having mm 29 and 27 ,38 ,36 ,zones of inhibition respectively as reported byQasim and Jafta 45 .(In addition to the findings of Kos et al. (46), probiotic strains have also been found to exhibit antagonistic activity against prevalent pathogens such as *S. aureus*, *P. aeruginosa*, *E. coli*, *Y. enterocolitica*, and *L. monocytogenes*. Kang et al. (11) investigated the antibacterial activity of monkey and human origin LABs against monkey origin enteric bacteria by the agar disc diffusion test and broth culture inhibition assay and found the higher antimicrobial properties of monkey origin LABs against homogenous enteric bacteria although humans and monkeys were phylogenetically similar species.

Exopoysaccharide Production

The screening of the isolatefor EPS production is greatly sought after because of the numerous health advantages it provides, including immunomodulation, pathogen inhibition, and the capacity to reduce

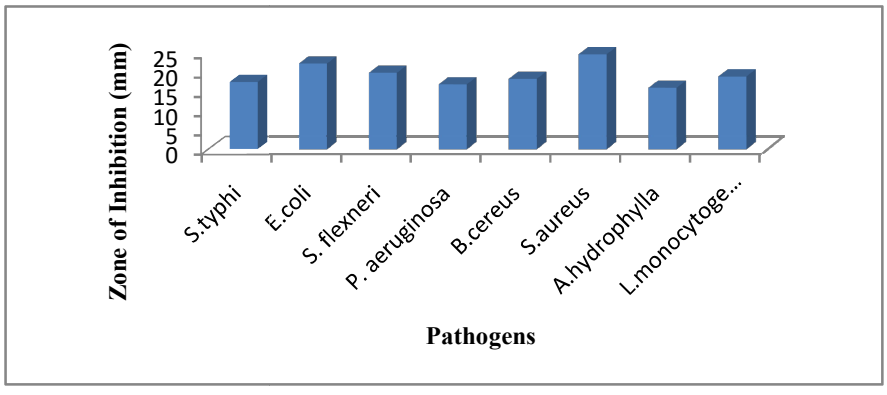

Fig.5: Antimicrobial activity of LAB isolates against food pathogens *LactiplantibacillusPlantarum*LG138

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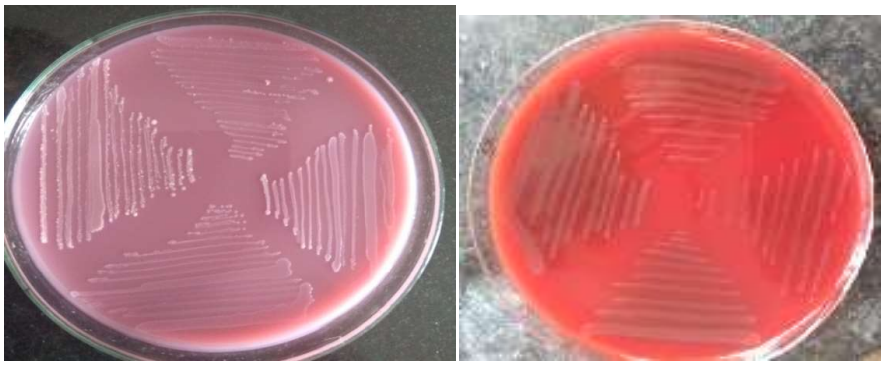

Fig.6: a) Exopolysaccharide production b) Hemolytic activity shown by the isolate

cholesterol levels.The white colony growth against pink background was observed when the isolate was streaked onto ruthenium red skim milk agar during the study indicates its ability to produce EPS. This is because the generation of either capsular EPS or secreted EPS would preventbacterial cell getting stained by the dye present in the medium(47). The isolate producing exopolysaccharide on skimmed milk-ruthenium red plates are shown in Figure 6a.The production of EPS using this medium was also previously investigated by NandhaandShukla (30) and our result are in line with them.

The estimation of hemolysis activity serves as a crucial probiotic assay, ensuring the safety of food products produced using these organisms. The non-toxic nature of *L. plantarum* LG 138 is demonstrated by its ability to induce γ-hemolysis, safeguarding both the environment and individuals (Figure 6b). Islam et al. (25) reported gamma-hemolytic potentiality of *Lactobacillus plantarum* DMR14 from oats indicated that it is not toxic to the environment or to individuals. Similar observations have been reported byMaragkoudakis et al. [11] in which *Lactobacillus* species isolated from dairy products have been shown to be non-hemolytic. On the contrary, another study reported LAB isolateswhich have shown hemolytic activity (48).

Conclusion

Primates have been extensively employed in worldwide research as preclinical models for various severe conditions, such as infectious, neurological, and metabolic disorders. The demand for primate research has significantly increased, particularly in infectious disease studies like COVID-19, leading to a global scarcity of primate resources. Indeed, preventing intestinal diseases in primates is crucial in veterinary medicine to safeguard and sustain resources for indoor breeding research of these animals. In the present investigation, the probiotic characteristics of LAB derived from primates were thoroughly investigated. The results are deemed valuable research data that could facilitate the development of new lactic acid strains beneficial for primates. The study reveal potential probiotic of primate origin *Lactiplantibacillus plantarum* LG138, due to survival in artificial conditions of the GI tract, γ-hemolytisis, antibiotic resistance, and strong inhibitory activity against food pathogens.The tested isolate of *L. plantarum* LG138 appeared to endowed with features similar to a probiotic organism.This isolate may be further subjected to*in vivo* studies with lab animals andtheassessment of their health benefits will encourage the utilization of the strain in bothfeed and pharmaceutical industry.

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Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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