Applied Biological Research 26(1): 117-124; (2024)
DOI: 10.48165/abr.2024.26.01.17

COMPARATIVE PERFORMANCE OF THREE PROBIOTIC ISOLATES OF *Lactobacillus* sp. ISOLATED FROM DAIRY PRODUCTS

M. Shashikala, Gampa Satish Kumar and Y. Aparna*

Department of Microbiology, Bhavan's Vivekananda College of Science, Humanities and Commerce, Sainikpuri, Secunderabad – 500 094, Telangana (India)
*****e-mail: *aparnachandra7673@gmail.com*

(Received 14 December, 2023; accepted 5 March, 2024)

ABSTRACT

The probiotics, when administered in adequate amounts, have the ability to confer health benefits to the host by positively influencing the composition and function of gut microbiota. The present study was aimed to isolate and characterize the probiotic bacteria from locally available curd and Yakult samples and compare them with Sporolac for probiotic properties. Three bacterial isolates were isolated and tentatively identified on morpho-cultural and biochemical characteristics as *Lactobacillus* sp. The isolates were named *Lactobacillus* sp. isolates PBC, PBY and SPO. *E coli* was used as negative control. The study revealed that the isolates exhibited auto-aggregation and co-aggregation activities, more in isolate SPO as compared to the isolates PBC and PBY. Biofilm and quorum quenching activities were high in *Lactobacillus* sp. isolate PBC and PBY as compared to *Lactobacillus* sp. isolate SPO. The other tests showed more or less similar properties as compared to the commercially available product. The study revealed that the probiotic bacterial isolates from curd and Yakult had more beneficial properties than the commercially processed probiotic bacteria.

Keywords: Auto-aggregation, biofilm, *Lactobacillus*, MATH test, probiotics

INTRODUCTION

Probiotics are living microorganisms that, when administered in adequate amounts, confer health benefits to the host. These microorganisms are known for their beneficial effects on human health, and have transcended their traditional role in the realm of nutrition and medicine to become a focal point of research across various disciplines. Human gut harbours a vast complex ecosystem of trillions of microbes, collectively known as 'gut microbiota'. These microbes play a crucial role in various aspects of our health by influencing the digestion, immunity, metabolism, and even mental well-being. Among these gut residents, probiotics stand as live microorganisms that confer health benefits when consumed in adequate amounts (Hill *et al*, 2014).

Recent research, has shed new light on the significance of probiotics in various aspects of human health. Probiotics reportedly improve gut barrier function, reduce inflammation, and alleviate symptoms of digestive disorders like irritable bowel syndrome and inflammatory bowel disease (Hu and Gubatan, 2023). Probiotics can modulate the immune system by enhancing its ability to fight pathogens and reduce the risk of allergies and autoimmune diseases. Emerging evidences suggest that gut microbiota play a vital role in mental health, with probiotics showing promise in managing conditions like anxiety and depression (Ansari *et al.*, 2020). The overuse of antibiotics has led to the emergence of antibiotic-resistant bacteria, posing a serious threat to global health. Probiotics offer a

potential solution by promoting the growth of beneficial bacteria that can compete with resistant strains (Velasco *et al*., 2019).

The advances in genomics and metabolomics have unravelled the genetic and metabolic diversity of probiotic strains. These studies have revealed the presence of specific genes related to probiotic functions (Latif *et al*., 2023). Understanding the genetic basis of probiotic properties allows the targeted selection and development of strains with desired characteristics (Latif *et al*., 2023). Some such significant properties of probiotics in research highlight auto-aggregation and co-aggregation abilities as key properties for selecting potent strains that help probiotics clump together, colonize the gut, and resist displacement (Wang *et al*., 2022). Bile salts aid digestion but can harm probiotics, therefore tolerance to bile salts ensures survival during transit. Beta-galactosidase enzymes break down lactose, so aid in digestion and potentially reduce bloating (De Angelis *et al*., 2020). Mucosal adhesion (MATH) test measures the probiotic's ability to adhere to gut lining which is crucial for colonization. Drop collapse test assesses the surface tension, linked to biofilm formation and potential immune benefits conferred by probiotic bacteria (Kosmina *et al*., 2020). By evaluating these properties through standard tests, one can select probiotics with true potential for human health benefit. The present study was undertaken to isolate, characterize and study the properties of potential probiotic bacterial strains from locally available curd and Yakult samples. Also, a comparative study was done using Sporolac, a proven probiotic as a reference.

MATERIALS AND METHODS

Isolation of bacteria

The study was conducted in Bhavan's Vivekananda College, Hyderabad (India) in the year 2023. Curd and Yakult (manufactured by Yakult Danone Pvt. Ltd) samples were purchased from the local market and used for the isolation of probiotic bacteria. Sporolac-DS tablets, manufactured by JB Chemicals Ltd.) were purchased from a nearby medical store and used to isolate probiotic bacterium as a reference strain in the study. For isolation, 0.1 mL of serially diluted curd and Yakult samples were plated on MRS agar (HI-Media), and incubated at 37°C for 24 h. After incubation, the pure cultures of selected isolates were subjected to morpho-biochemical characterization including Gram staining, morphological identification on culture media and biochemical characterization like IMViC tests, catalase, oxidase, sugar fermentation tests, nitrate reductase test, motility tests, etc. as per the standard methods (Aneja, 2004). The bacterial characteristics observed were fed in ABIS online software tool (*https://www.tgw1916.net/bacteria_Lactobacillus_input.php*) so as to identify the bacteria. The pure cultures were preserved in a refrigerator and sub-cultured at weekly interval for maintenance throughout the study period. The bacterial isolates from curd, Yakult and Sporolac were used in the present study.

Probiotic properties characterization

Auto-aggregation assay: Bacterial cells were removed from an overnight culture by centrifugation (5,000 g, 20 min, 4°C), rinsed twice with phosphate-buffered saline PBS pH 7.1 (10 mM Na_2HPO_4, 1 mM KH_2PO_4, 140 mM NaCl, and 3 mM KCl), and suspended in the same buffer. The optical density of homogenized bacterial suspension was initially measured at 600 nm, and then again on the same suspension after it had been permitted to rest for 24 h at 37°C without being vortexed. The aggregation percentage was calculated from equation (Collado *et al*., 2008):

$$\text{Autoaggregation } (\%) = (1 - \frac{\text{AT}}{\text{A0}}) x\ 100$$

where AT stands for the combination's absorbance at 24 h, and A0 for the absorbance at time 0 of the mixture

Co-aggregation assay: Co-aggregation assay was done by suspending the bacterial isolates in equal volumes of pathogen cultures (lab isolates of *Staphylococcus* sp. and *Pseudomonas* sp.). The cultures

were incubated at 37°C for 24 h without agitation. The absorbance was measured in a UV visible spectrophotometer (Systronics, model 118) at 600 nm and co-aggregation calculated as per Kos *et al*. (2003).

Bile salt assay: Tolerance of bacterial isolates to bile salts and acidic pH was tested as per the method of Nami *et al*. (2019) on MRS agar purchased from Sigma Aldrich. Bile salts were added at a concentration of 5 and 10%, then autoclaved, cooled and plated. Aliquots of overnight grown cultures were then plated and incubated at 37°C for 72 h. Resistance to bile salts was indicated by presence of growth.

β-galactosidase activity: A qualitative test was performed to assess the presence of β-galactosidase enzyme. The isolates were cultured in MRS broth (HI-Media), incubated at 37°C for 24 h, then streaked onto MRS agar and incubated for 48 h. Single colony from each sample was put into test tubes with ONPG (O-nitrophenyl-β-D-galactopyranoside) broth purchased from HI-Media, and incubated at 37^0C for 24 h. The change in colour to yellow signified the release of o-nitrophenol (Aneja, 2004).

Drop collapse test: The drop collapse test was carried out as per Jain *et al*. (1991). Test isolates were grown in MRS at 37°C for 24 h. The supernatants (100 µL) were added to 96-well µL plates after centrifugation at 12,000 rpm for 5 min. Two types of oils (generator oil and crude motor oil) were used for testing the isolates. Water was used as negative control. When the drop diameter was at least 1 mm larger than that of deionized water, the result was considered positive for biosurfactant production.

MATH test: Bacterial cell surface hydrophobicity was assessed by measuring the adhesion to hydrocarbons (MATH) test as per the method of Kotzamanidis *et al*. (2010). The cultures were subjected to two washes in phosphate-buffered saline (PBS) and resuspended in 3 mL 0.1 M KNO_3 to attain a concentration of 10^8 CFU mL^{-1}. The initial absorbance of suspension was measured at 600 nm (A_o). To create a two-phase system, 1 µL xylene was added to the cell suspension, which was then vortexed for 2 min after allowing it to stand for 10 min at room temperature. After an additional 20 min incubation at room temperature, the aqueous phase was separated, and the absorbance recorded at 600 nm (A_1). The percentage of cell surface hydrophobicity (%) was calculated using the formula:

$$\text{Hydrophobicit (\%)} = (1 - \frac{\text{AT}}{\text{A0}})x\ 100$$

where A_o is the absorbance at initial time and A_1 the absorbance of mixture 20 min after incubation at room temperature.

Biofilm assay: The biofilm formation was assayed as per Borges *et al*. (2012). Diluted culture (100 µL) was dispensed into each well of sterile 96-well µL plate and incubated at 37°C for 24 h to allow biofilm formation. After incubation, the cells were removed carefully by gently washing the wells with sterile PBS. The biofilm was fixed in each well by adding 200 µL 0.1% crystal violet solution and incubated for 15 min at room temperature. The wells were gently washed with distilled water to remove excess crystal violet and 200 µL DMSO was added to each well to solubilize the crystal violet stain. The absorbance of each well was measured spectrophotometerically at 595 nm.

Antibiotic sensitivity: Antibiotic sensitivity was measured by disc diffusion assay (Aneja, 2004). Briefly, overnight grown bacterial cultures were spread on nutrient agar plates containing antibiotic discs (gentamycin, cloramphenicol, ciprofloxacin, penicillin G and trimethoprim of 10 µg mL^{-1}, purchased from Thermo Fischer Scientific) and incubated at 37°C for 24 h. The zone of inhibition around the antibiotic disc indicated the sensitivity of bacteria for a particular antibiotic.

Quorum quenching activity: Quorum quenching activity was tested by using *Chromobacterium violaceum* 12472 (purchased from MTCC) as an indicator organism. Briefly, the organism was pre-seeded on nutrient agar medium. Sterile discs were prepared and dipped in the supernatant of test

isolates, positive control and negative control. Blank was set as medium without inoculation. After incubation at 30°C the petri-plates were observed for the zone of discoloration (Zahin *et al.*, 2010).

Statistical analysis

All quantitative experiments were performed in triplicate in a completely randomized design. The mean averages were computed and graphs plotted using MS Excel, featuring error bars set at 5% confidence interval. The significance level was established at $p \leq 0.05$.

RESULTS AND DISCUSSION

Isolation and characterization of probiotic bacteria

The isolated bacteria were Gram positive rods. The morpho-cultural and biochemical characterization in ABIS online software (*https://www.tgw1916.net/bacteria_Lactobacillus_input.php*) revealed 92.9, 92.3 and 94.0% similarity with *Lactobacillus* sp. for isolates PBC (isolated from curd), PBY(isolated from Yakult) and SPO (isolated from Sporolac), respectively. Thus the bacteria isolated were tentatively identified as *Lactobacillus* sp. *Escherichia coli* was used as negative control.

Auto-aggregation assay

The results indicated notable differences in auto-aggregation among the test isolates of *Lactobacillus* sp. The *Lactobacillus* isolates PBC, PBY and SPO exhibited an auto-aggregation of 71.0, 55.0 and 85.9%, respectively, while the reference microbe *Escherichia coli* had an auto-aggregation of 37.5% (Fig. 1). *Lactobacillus* sp. isolate SPO exhibited highest auto-aggregation. The capacity for self-aggregation to achieve high cell density in gastro-intestinal tract, secure adherence to intestinal epithelial cells, and preventing the colonization of pathogens is fundamental for a microbe to be probiotics. Re *et al.* (2000) suggested auto-aggregation rate of >80% for robust isolates. Further study is needed to identify the specific isolates and their probiotic or health-related properties. In contrast, *E. coli* exhibited lower auto-aggregation which aligns with the expected behaviour of typical *E. coli* isolates, not known for strong auto-aggregation characteristics.

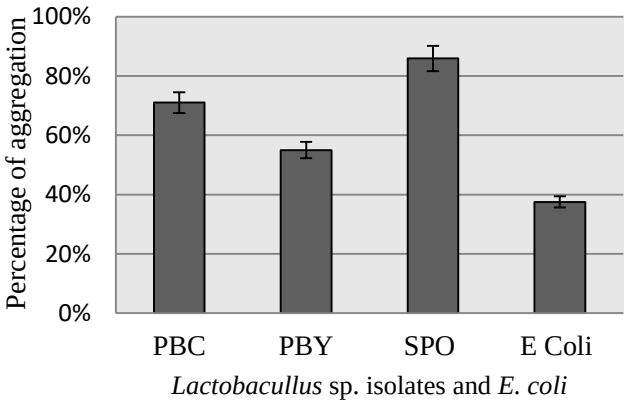

Fig. 1: Auto-aggregation of *Lactobacillus* isolates from curd (PBC), Yakult (PBY), Sporolac (SPO) in comparison to negative control (*E. coli*)

Co-aggregation assay

Co-aggregation is a phenomenon in which bacterial cells of different species come together and form aggregates. This process has implications for microbial community dynamics and biofilm formation. This is an important property of probiotics for their co-aggregation ability with pathogens. This ability of isolates prevents pathogens from colonizing in GI tract, thereby help to constitute an important defense mechanism against infection. As per Gomez *et al.* (2014) co-aggregation values of < 20% correspond to the isolates that show weak co-aggregation ability. In present study, co-aggregation with pathogens was observed in all the test isolates in strain-pathogen combination-dependent manner. The isolate PBC displayed moderate co-aggregation (55%) with *Staphylococcus*, and notably higher co-aggregation (81%) with *Pseudomonas* (Fig. 2). This suggested that isolate PBC has strong tendency to aggregate with *Pseudomonas*, indicating a potential ecological interaction between these

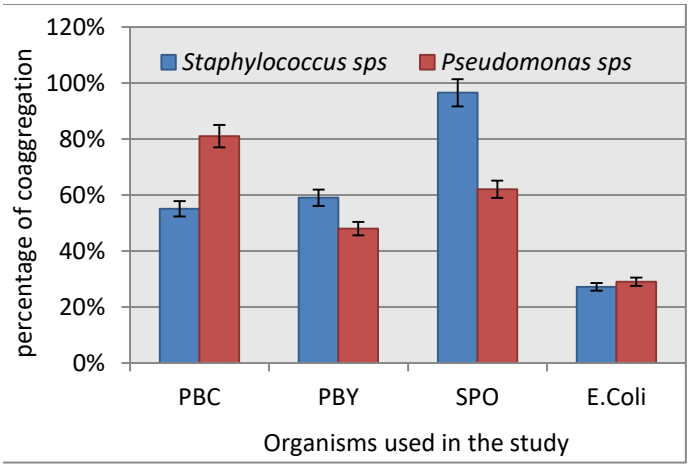

Fig. 2: Co-aggregation of *Lactobacillus* isolates tested with *Staphylococcus* sp. and *Pseudomonas* sp. against negative control *E. coli*

two species. The isolate PBY exhibited a similar trend, with co-aggregation of 59% with *Staphylococcus* and 48% with *Pseudomonas*. Though the co-aggregation percentages are not higher than those of isolate SPO, still they have affinity for co-aggregation with both the bacterial species. In case of *Lactobacillus* isolate SPO, the co-aggregation was notably higher revealing a strong propensity to co-aggregate with *Staphylococcus* (96.5%) and *Pseudomonas* (62.0%). This suggests that isolate SPO may have specific interactions or mechanisms that promote co-aggregation with these bacterial species. Conversely, *E. coli* showed lower co-aggregation with *Staphylococcus* (27.2%) and *Pseudomonas* (29.0%) which could be attributed to the differences in surface properties or adhesion mechanisms as compared to the other test organisms.

Bile salt tolerance and β-galactosidase activity

The bile salt tolerance test and β-galactosidase activity showed positive result positive results for across the test *Lactobacillus* isolates PBC, PBY, & SPO, and *E. coli* which indicated that these isolates possess tolerance to bile salt. The tolerance to bile salt is a crucial trait, particularly for the microbes inhabiting gastro-intestinal tract, as the bile salts are natural detergents produced by human body and are frequently encountered by the microbes in digestive system. Tolerance to bile salt is often associated with the ability of these isolates to survive and thrive in presence of bile salts. It allows probiotic organisms to resist antimicrobial action of bile salts and continue to function effectively in gut, where they play essential roles in digestion or other functions. Tolerance to acidic conditions and bile salts is both a species and strain-dependent property (Romero *et al.*, 2019). The studies on β-galactosidase activity revealed the ability of these strain to produce β-galactosidase enzyme. β-galactosidase enzyme plays a crucial role in the hydrolysis of lactose into glucose and galactose and is often used as an indicator enzyme in the identification of probiotic bacteria.

Drop collapse test

The drop collapse test revealed that all the test *Lactobacillus* sp. isolates had the ability to create biosurfactant. The drop collapse test was positive for all the isolated strains. In both engine oil and generator oil tests, the *Lactobacillus* sp. isolates SPO, PBC and PBY proved to be strong biosurfactant producers than *E. coli* (Table 2). The drop collapse test serves as a useful initial screening tool in identifying the potential probiotic isolates of industrial application.

Table 1: Drop collapse test results of *Lactobacillus* isolates

Organisms	Drop collapse (diameter in cm)	
	Generator oil diameter	Engine oil diameter
PBC	1.4	1.3
PBY	1.2	1.0
SPO	1.2	1.6
E. coli	1.0	1.2

Microbial adhesion to hydrocarbon test (MATH)

The microbial adhesion to hydrocarbons (MATH) assay is done to comprehensively assess the cell surface hydrophobicity. The assay revealed that *Lactobacillus* sp. isolates PBO and SPO exhibited notably higher degree of cell surface hydrophobicity as compared to the isolate PBY and *E. coli* (Fig. 3). Recent research findings have indicated that variations in cell concentration may, at times, impact the

extent to which microbial cells adhere to hydrocarbon surfaces. This phenomenon may be attributed to the mutual interaction of cells, leading to a more effective stabilization of hydrocarbon droplets. The MATH assay has proven valuable in elucidating the surface properties that either heighten (hydrophobins) or diminish (hydrophilins), the hydrophobic characteristics of the outermost cell surfaces of certain microorganisms.

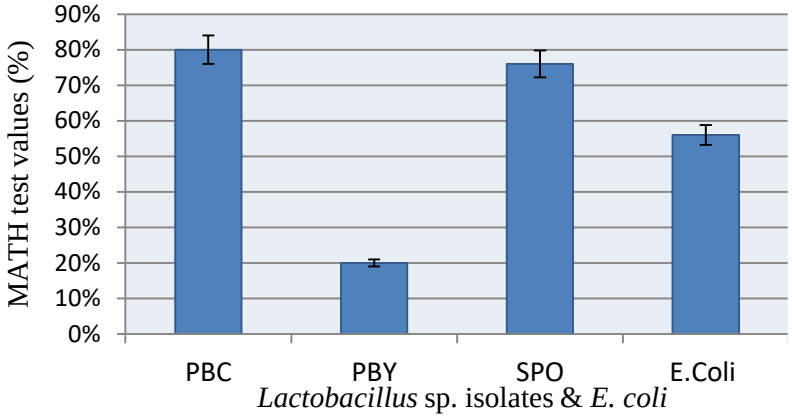

Fig. 3: Microbial adhesion to hydrocarbon (MATH) test. Cell surface hydrophobicity of *Lactobacillus* isolates as compared to the negative control *E. coli*

Antibiotic sensitivity test

The antibiotic sensitivity test revealed significant variations in the susceptibility of *Lactobacillus* sp. isolates to the tested antibiotics. The isolate PBC appeared susceptible to ciprofloxacin and gentamicin, while isolate PBY and SPO were susceptible to all the antibiotics tested, except penicillin-G (Table 3). *E. coli* showed susceptibility to ciprofloxacin but was resistance to chloramphenicol, penicillin-G, gentamicin and trimethoprim. Antibiotic resistance is a natural survival strategy of all

Table 3: Antibiotic sensitivity reaction in terms of zone of inhibition (cm) of different isolated probiotic *Lactobacillus* isolates and *E. coli* as per disc diffusion method

Bacterial isolates	Trimethoprim	Ciprofloxacin	Gentamicin	Chloramphenicol	Penicillin-G
Lactobacillus PBC	1.70 ± 0.42	3.45 ± 0.38	1.80 ± 0.28	1.50 ± 0.28	0
Lactobacillus PBY	2.35 ± 0.07	2.65 ± 0.49	2.20 ± 0.35	2.65 ± 0.35	0
Lactobacillus SPO	2.25 ± 0.35	2.75 ± 0.35	2.25 ± 0.21	2.45 ± 0.21	1.15 ± 0.07
E. coli	0.75 ± 0.35	2.75 ± 0.35	1.10 ± 0.07	1.15 ± 0.07	0

± values represent standard deviation. The organisms are classified as sensitive or resistant based on the scale: Resistant - ≤ 1.4 cm; Moderately sensitive - 1.5 cm to 1.7 cm; and Sensitive - ≥ 1.8 cm

organisms including probiotic bacteria. However, the antibiotic resistance exhibited by *Lactobacillus* sp. isolates need further understanding regarding their nature of resistance (intrinsic or extrinsic). Hence, it is naïve to conclude whether it is advantageous or disadvantageous to the isolated bacteria.

Biofilm assay

The isolated three *Lactobacillus* sp. demonstrated the capacity to form biofilm. This character holds significance for probiotic strains as biofilm formation is implicated in facilitating the colonization and persistence of lactic acid bacteria within the host mucosa. Such persistence serves to deter the colonization of pathogenic bacteria, thereby contribute to the host health (Juárez Tomás *et al.*, 2011). Subsequent analysis confirmed that

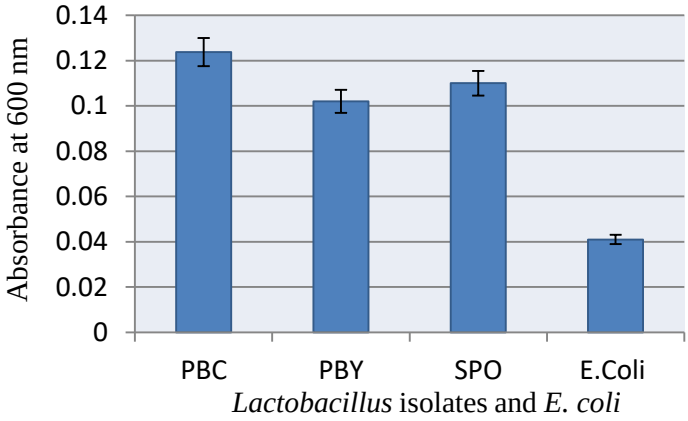

Fig. 4: Biofilm production by *Lactobacillus* isolates

all the isolates possessed the biofilm producing ability. The results elucidated that the *Lactobacillus* isolates PBC, PBY, and SPO exhibited good biofilm producing capabilities, whereas *E. coli* comparatively showed weak biofilm producing tendencies. The higher biofilm forming potential in *Lactobacillus* isolates may have implications for their probiotic applications and host interactions.

Quorum quenching activity against biofilm forming bacteria

In the realm of microbial regulatory mechanisms, quorum sensing (QS) serves as a pivotal system for monitoring the concentration of signal molecules autonomously produced and released by bacteria. QS orchestrates the coordinated behaviour of microbial populations. In contrast, quorum quenching signifies the intriguing process of disrupting the QS system, effectively impeding the formation of virulence factors.

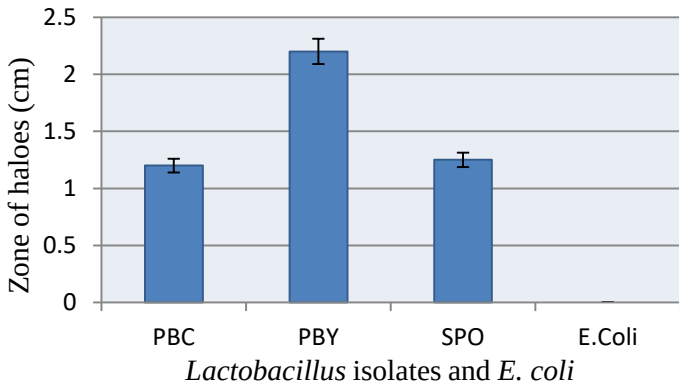

Fig. 5: Quorum quenching activity of *Lactobacillus* isolates tested against indicator organism *Chromobacterium violaceum* 12472

Lactobacillus sp. isolate PBY exhibited notably elevated quorum quenching activity, signifying its potent capacity to curtail the QS system and hinder virulence factor formation. In contrast, *Lactobacillus* isolates PBC and SPO exhibited less robust quorum quenching profile. Notably, *E. coli* demonstrated a lack of quorum quenching activity, indicating its inability to disrupt the QS system effectively. These findings illuminate diverse quorum quenching abilities of the test isolates, highlighting the potential implications for their roles in modulating microbial behaviour and virulence factor production.

Conclusion: The study concludes that the probiotic bacteria available in natural curd have more potential characters as compared to the commercially available strains. Auto-aggregation and co-aggregation assays showed good activity in *Lactobacillus* isolate SPO as compared to the isolates PBC and PBY.

Acknowledgements: The authors thank the Principal and Management of the Bhavan's Vivekananda College of Science, Humanities and Commerce for their constant support and encouragement.

REFERENCES

Aneja K.R. 2004. *Experiments in Microbiology, Plant Pathology and Biotechnology* (4th edn.). New Age International Publishers, Delhi, India.

Ansari, F., Pourjafar, H., Tabrizi, A. and Homayouni, A. 2020. The effects of probiotics and prebiotics on mental disorders: A review on depression, anxiety, Alzheimer, and autism spectrum disorders. *Current Pharmaceutical Biotechnology*, **21**(7): 555-565.

Kos, B., Savai, S., Vukovi C., Koviai, J.F. and Matosi, C.S. 2003. Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of Applied Microbiology*, **94**: 981-987.

Borges, S., Silva, J. and Teixeira, P. 2012. Survival and biofilm formation by group B streptococci in simulated vaginal fluid at different pHs. *Antonie Van Leeuwenhoek*, 101: 677-682.

Collado, M.C., Grześkowiak, Ł. and Salminen, S. 2007. Probiotic strains and their combination inhibit *in vitro* adhesion of pathogens to pig intestinal mucosa. *Current Microbiology*, **55**: 260-265.

De Angelis, M., Piccolo, M., Russo, P., Franchieri, G., Decaro, G., Campana, R. and Gobbetti, M. 2020. Beta-galactosidase activity and lactose digestion potential of probioticbacteria. *Microorganisms*, **8**(6): 909. [*https://doi.org/10.3390/microorganisms8060909*].

Gómez, N.C., Ramiro, J.M., Quecan, B.X. and de Melo Franco, B.D. 2016. Use of potential probiotic lactic acid bacteria (LAB) biofilms for the control of *Listeria monocytogenes, Salmonella typhimurium*, and *Escherichia coli* O157:H7 biofilms formation. *Frontiers in Microbiology*, **7**: 863. [*https://doi.org/10.3389/fmicb.2016.00863*].

Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B. and Sanders, M.E. 2014. Expert consensus document: The International Scientific Association for Probiotics and Prebiotic's consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology*, **11**(8): 506-514.

Hu, K.A. and Gubatan, J. 2023. Gut microbiome-based therapeutics in inflammatory bowel disease. *Clinical and Translational Discovery*, **3**(2): e182 [*https://doi.org/10.1002/ctd2.182***]**.

Jain, D.K., Collins-Thompson, D.L., Lee, H. and Trevors, J.T. 1991. A drop collapsing test for screening surfactant-producing microorganisms. *Journal of Microbiological Methods*, **13**(4): 271-279.

Juárez Tomás, M.S., Saralegui Duhart, C.I., De Gregorio, P.R., Vera Pingitore, E. and Nader-Macías, M.E. (2011) Urogenital pathogen inhibition and compatibility between vaginal *Lactobacillus* strains to be considered as probiotic candidates. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, **159**(2): 399-406.

Kos, B. V. Z. E., Šušković, J., Vuković, S., Šimpraga, M., Frece, J., & Matošić, S. 2003. Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92. *Journal of Applied Microbiology*, **94**(6): 981-987.

Kosmina, J., Tožic, N., Čadež, N., Košuta, M., Petrovič, L. and Žgur-Bertok, D. 2020. Droplet collapse test: A novel method for studying surface properties of probiotic bacteria. *Journal of Microbiological Methods*, **177**: 106080. [*https: //doi. org/10.1016/j.mimet.2020.106080*].

Kotzamanidis, C., Kourelis, A., Litopoulou-Tzanetaki, E., Tzanetakis, N. and Yiangou, M. 2010. Evaluation of adhesion capacity, cell surface traits and immunomodulatory activity of presumptive probiotic *Lactobacillus* strains. *International Journal of Food Microbiology*, **140**: 154-163.

Latif, A., Shehzad, A., Niazi, S., Zahid, A., Ashraf, W., Iqbal, M. W., Rehman, A., Riaz, T., Aadil, R.M., Khan, I.M., Özogul, F., Rocha, J.M., Esatbeyoglu, T. and Korma, S.A. 2023. Probiotics: Mechanism of action, health benefits and their application in food industries. *Frontiers in Microbiology*, **14**: 1216674. [*https://doi.org/10.3389/fmicb.2023.1216674*].

Nami, Y., Vaseghi Bakhshayesh, R., Mohammadzadeh Jalaly, H., Lotfi, H., Eslami, S. and Hejazi, M.A. 2019. Probiotic properties of *Enterococcus* isolated from artisanal dairy products. *Frontiers in Microbiology*, **10**: 300. [*doi: 10.3389/fmicb.2019.00300*].

Re, B., Sgorbati, B., Miglioli, M. and Palenzona, D. 2000. Adhesion, auto-aggregation and hydrophobicity of 13 strains of *Bifidobacterium longum*. *Letters in Applied Microbiology*, **31**(6): 438-442.

Romero-Luna, H.E., Hernández-Sánchez, H., Ribas-Aparicio, R.M., Cauich-Sánchez, P.I. and Dávila-Ortiz, G. 2019. Evaluation of the probiotic potential of *Saccharomyces cerevisiae* strain (C41) isolated from Tibicos by *in vitro* studies. *Probiotics and Antimicrobial Proteins*, 11: 794-800.

Velasco, M., Requena, T., Delgado-Iribarren, A., Peláez, C. and Guijarro, C. 2019. Probiotic Yogurt for the prevention of antibiotic-associated diarrhea in adults: A randomized double-blind Placebo-controlled trial. *Journal of Clinical Gastroenterology*, **53**(10): 717-723.

Wang, S., Zeng, X., Ai, W., Li, H. and Lin, S. 2022. Probiotics in food: Health benefits and food applications. *Comprehensive Reviews in Food Science and Food Safety*, **21**(3): 1055-1084.

Zahin, M., Hasan, S., Aqil, F., Khan, M.S., Husain, F.M. and Ahmad, I. 2010. Screening of certain medicinal plants from India for their anti-quorum sensing activity. *Indian Journal of Experimental Biology*, **48**(12): 1219-1224.