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ORIGINAL ARTICLE **OPEN ACCESS**

Aggregation, co-aggregation and adhesion properties of probiotic bacteria collected from different supplements

Nisha Mavani[1]and Arti Raval[2].
Department of Microbiology, Arts, Science & Commerce College Kholwad, Kamrej Cross Road, Surat, Gujarat-394185, India.
Corresponding author's email: nishamavani54@gmail.com

ABSTRACT
LABs(lactic acid bacteria) are normal residents of the human gastrointestinal tract and play a very important role in maintaining the microbial ecosystem of the large intestine. Aggregation is one of the most important criteria for selecting good probiotic candidates. In addition, the aggregation ability of probioticsis increased. It has been characterized as a key factor related to the colonization of the intestinal epithelium. Studies have shown that certain lactic acid bacteria can prevent pathogens from attaching the intestinal mucosa to form a barrier through self-aggregation or aggregation with pathogens. We were collected different probiotic supplements from the different pharmacy stores. And get seven different probiotic bacterial species like Lactobacillus spp, Clostridium butyricum, Bacillus spp, Streptococcus faecalis, etc. These bacteria were then analyzed for aggregation and adhesion properties. We determined that Lactobacillus sporogenes gave the highest bacterial adhesion and co-aggregation with S aureus whereas; Bacillus mesenteric us gave the highest auto-aggregation.
Keywords: *Probiotics, Auto-aggregation, Co-aggregation, Hydrophobicity, etc.*

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INTRODUCTION

Probiotics are beneficial microorganisms in foods and dietary supplements, which are beneficial to human and animal health [1].In recent years, there has been great interest in the field of probiotic research and the verification and characterization of health benefits associated with the use of probiotics [2]. The accumulation of hydrocarbons and bacterial adhesion test (BATH test) showed the cell surface characteristics of the tested commercial probiotic strains [3]. Aggregation was first observed in bacteria isolated from human dental plaque. When bacteria are suspended or a certain type of bacteria adheres to the surface, aggregation may occur. Cohesion is a process in which the adhesion of one microorganism can promote the subsequent adhesion of other microorganisms [4].Auto-aggregation ability test together with cell-surface hydrophobicity and co-aggregation could be used for preliminary screening identifying potentially adherent bacteria with properties suitable for commercial purposes [5].Lactic acid bacteria have beneficial effects on the human body because they have health-promoting properties, such as inhibiting the invasion of pathogens and improving the epithelial barrier function. [6].
In vitro adhesion test has been successfully done using Intestinal mucus [7],[8]. and human enterocyte-type Caco-2 cell cultures[9]. However, this method was expensive and taking more time. Therefore, a reliable and simple method was used for preliminary screening of potential adherent strains.Thepurpose of our research was to isolate and identify probiotics and determine the hydrophobicity, aggregation, and co-aggregation of selected bacteria.

MATERIAL AND METHODS

Sample collection:

Different probiotic samples Vizylac, Darolac, Sporlac, and Yakult were obtained from the pharmacy and were used for the isolation of the bacteria. Different probiotic samples like Vizylac, Darolac, Sporlac, and Yakult were used for the isolation of microorganisms.

Isolation and screening of bacteria from probiotic samples:

Four probiotic samples each from different pharmacy stores were collected. The isolation of microorganisms was done as follows. 1 gram of probiotic was taken in 9 ml sterile distilled water and 1

ml sample was transferred into sterile MRS broth and incubated at 37 ºC for 24 hours anaerobically. 0.1 ml of inoculated MRS broth samples were plated on sterile De Man, Rogosa, and Sharpe (MRS) agar medium and incubated at 37 ºC for 48 hours anaerobically. A single colony was picked up and streaked on sterile MRS agar plates to get pure culture. Well, isolated colonies were observed for morphological characterization. Seven organisms were selected for further analysis to check adhesion properties and aggregation properties.

Morphological observation of isolates:

The colony characteristics and gram reactions of different isolates were performed using the standard microbiological methods.

Adhesion property: Hydrophobicity

Isolated organisms were inoculated in MRS broth and incubated anaerobically at 37ºC for 24 hours. After overnight incubation, the broth was centrifuged at 10,000 rpm at 4°C for 10 min. Obtained pellet was washed twice with PBS buffer solution and resuspended in the same solution. 1 ml of xylene hydrocarbon was added in 3 ml of cell suspension. Absorbance (OD_{600}) was taken at 0h and after vortexing both phases for 2 min. Absorbance was taken again after 2h incubation [6].

Hydrophobicity % = $\{(A_0 - A_t) / A_0\} \times 100$

Where A_t = Absorbance at time t=2

A_0 = Absorbance at time t=0

Aggregation property:

Auto-aggregation:

Isolated seven organisms were inoculated in MRS broth and incubated anaerobically at 37ºC for 24 hours. After overnight incubation, the broth was centrifuged at 10,000 rpm at 4°C for 10 min. Obtained pellet was washed twice with PBS buffer solution and re-suspended in the same solution, followed by incubation at 37°C for 4 h. An equal amount of aliquot was taken and absorbance was measured at OD_{600} at 0, 1, 2, 3, and 4 h [10].

Auto-aggregation % = $1- (A_t / A_0) \times 100$

Where, A_t =Absorbance after incubation at 1, 2, 3 and 4 h,

A_0 = Absorbance at 0 h.

Co-aggregation:

Select four organisms with the highest auto-aggregation activity for further analysis. Mixtures were made for four isolates with pathogenic bacteria viz. *Bacillus cereus*, *Escherichia coli,* and *Staphylococcus aureus* in 1: 1 ratio. Probiotic bacterial cells and indicator microorganisms were kept as control and incubated at 35°C for 4 h. Absorbance (OD_{600}) was observed for the mixture and each individual strain. The co-aggregation percentage was calculated by Handley's equation [3].

$$\text{Co} - \text{aggregation}(\%) = \left[\frac{\left[A_{(\text{LAB})} + \frac{A_{(\text{Pathogen})}}{2}\right] - A_{(\text{Mixture})}}{\left[A_{(\text{LAB})} + \frac{A_{(\text{Pathogen})}}{2}\right]}\right] \times 100$$

Where A_{LAB} =Absorbance of lactic acid bacterial suspension

$A_{Pathogen}$ = Absorbance of indicator microorganisms

$A_{Mixture}$ = Absorbance of LAB suspension and indicator organisms

RESULTS AND DISCUSSION

Sample collection from the different medical store:

In the present study for the isolation of bacteria, different probiotic samples (Vizylac, Yakult, Darolac, and Sporlac) were collected from different pharmacy stores nearby the region Udhna, Surat, Gujarat.

Isolation and screening of probiotic microorganisms:

By screening on the MRS agar plate, 10 isolates were found. In which 7 isolates were selected by further screening.

Morphological characteristics of the isolated microorganisms on MRS agar plate:

Total seven numbers isolates were preceded for their morphological characterization. The colony characteristics of the obtained isolates from the different probiotic samples were studied on an MRS agar plate which gave circular, white, small colonies after 48 hours of anaerobic incubation. All 7 colonies were Gram-positive as examined by Gram's staining method under an oil-immersion microscope. Among them, 5 colonies were of long rods, 1 colony of small rods, and 1 colony of cocci (Table:-1).

Table 1:- Morphological characteristics of isolated organisms

Colony characteristics	ND1	ND2	ND3	ND4	ND5	ND6	ND7
Size	Small	Pin point	Small	Large	Small	Small	Small
Shape	Round	Round	Irregular	Circular	Circular	Round	Round
Opacity	Translucent	Transparent	Opaque	Opaque	Opaque	Opaque	Translucent
Margin	Entire	Entire	Rough	Entire	Entire	Entire	Entire
Elevation	Bumpy	Convex	Convex	Convex	Convex	convex	Bumpy
Consistency	Smooth	Smooth	Smooth	Smooth	Smooth	smooth	Smooth
Pigmentation	Non	Non	Non	Non	Non	Non	Non
Gram reaction	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive
Morphology	Long rods in chain	Short rods	Long rods	Long rods	Single cocci	Long rods in chains	Long rods

Adhesion property: Hydrophobicity

Cell surface hydrophobicity is the nonspecific interaction between host and bacterial cells. The cell surface properties of lactic acid bacteria are key components of adhesion. Initially, this interaction is weak, but it is enhanced by the adhesion process mediated by cell surface proteins and lipoteichoic acid [11],[12].Bacterial adhesion to xylene was tested to study the Lewis acid-base characteristics of the bacterial cell surfaces. Xylene is a non-polar solvent. Determination of bacterial adhesion to xylene is a valid qualitative phenomenological approach [13]. The result of this study showed that the probiotic strains exhibited strong hydrophobicity towards non-polar solvent xylene. ND7 exhibited the highest adhesion (91.19%) and ND2 exhibited the lowest adhesion (29.90%) towards xylene (Table:-2). Higher hydrophobicity is required for the colonization and adhesion of bacteria to the epithelial cells of the gut [14].It is believed that the presence of S-layer proteins in the cell wall of Lactobacillus with a high isoelectric point shows a strong affinity for non-proteins. It has been proposed that the properties of the cell surface play a key role in self-aggregation and hydrophobicity [15].Adherence to epithelia helps in evaluating the surface hydrophobicity of the non-polar solvent. A good probiotic must possess high auto-aggregation and strong hydrophobicity.

Table 2:- Adhesion of isolated organisms to xylene hydrocarbon

Isolate culture no.	OD_{600}		% Hydrophobicity of xylene
	0 h	**2 h**	
ND1	1.565	1.089	**30.41**
ND2	0.755	0.529	**29.90**
ND3	2.385	1.028	**56.89**
ND4	0.294	0.168	**42.85**
ND5	1.273	0.728	**42.81**
ND6	0.767	0.088	**88.52**
ND7	0.636	0.056	**91.19**

Aggregation property:

Auto-aggregation:

Interaction of the bacterial strain with itself (clumping of the cell) determines the autoaggregation capability. Probiotic bacteria should adhere to the enterocytes cellular lines of oral cavity and GIT in order to exhibit their beneficial effects [16]. Bacterial aggregation depends on the amount of biofilm production which helps in adhesion of the cell [17]. The exact mechanism is not known of autoaggregation. Checked the self-aggregation based on the deposition rate. The sedimentation rate was observed during the 4 hours of incubation. The ability of strains to auto-aggregate increased with the increase of the incubation period. ND6 showed 68.97% aggregation after 4 h incubation (Figure:-1). The observed auto-aggregation could be due to cell surface component as they were not lost after washing and suspending of the cells in phosphate saline buffer [18].

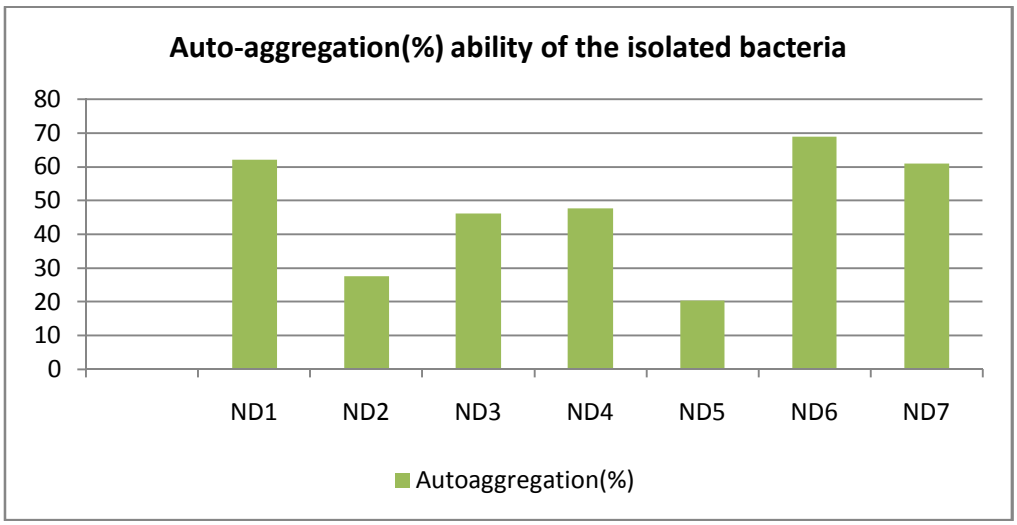

Figure1: Auto-aggregation ability of isolated bacteria.

Co-aggregation:

Auto-aggregation is the important property for adhesion to the fucoid preventing the colonization of pathogenic microorganisms [19]. Probiotics can co-aggregate with pathogenic microorganisms, inhibit their growth and finally kill them by secreting antimicrobial compounds that directly attack the cells of pathogenic bacteria [20]. ND7 exhibited higher co-aggregation ability with *Staphylococcus aureus* (77.00%) and co-aggregation potential of ND4 with *Bacillus cereus* was lower (14.00%) (Table:- 3).The effective co-aggregation potential of probiotics and gram-positive bacteria depends on the same cell wall morphology. The organism has a thick peptidoglycan layer, and its binding is enhanced due to its hydrophobicity [21]. Lactic acid bacteria having co-aggregation potential have a significant role in the human gut as they inhibit the growth of pathogenic strains by co-aggregating with them in the gastrointestinal tract. [22]. Here research shows that *Lactobacillus* spp. exhibited higher co-aggregation ability with *Staphylococcus aureus* [3].

Table 3:- Evaluation of Co-aggregation ability of isolated organisms with test indicators

Indicator	OD_{600} nm					Co-aggregation (%)				
	ND1	**ND4**	**ND6**	**ND7**	**Mean**	**ND1**	**ND4**	**ND6**	**ND7**	**Mean**
Escherichia coli	0.130	0.022	0.032	0.019	**0.650**	69.00	58.00	61.00	71.00	**64.75**
Bacillus cereus	0.130	0.017	0.031	0.021	**0.100**	54.37	14.00	54.00	45.00	**41.84**
Staphylococcus aureus	0.180	0.008	0.020	0.008	**0.166**	66.80	63.00	69.00	77.00	**68.95**

CONCLUSION

In conclusion, seven selected bacterial strains represented desirable auto-aggregation, co-aggregation, and hydrophobicity abilities. ND7:S*Lactobacillus sporogenes* gave high hydrophobicity (91.19%), high co-aggregation (77.00%) with *Staphylococcus aureus*, whereas; ND6:*Bacillus mesentericus*gave high auto-aggregation (68.97%). Auto-aggregation and co-aggregation are important in the formation of biofilm to protect the host from pathogens. Bacterial aggregation and hydrophobicity can be used for preliminary screening to assess their adhesion properties. Therefore, these isolates can be suitable probiotic candidates for use in functional foods including dairy products. Further studies are needed to identify, characterize and evaluate the other important probiotic properties such as their functional properties (antimicrobial activity, survival of gastrointestinal tract conditions), technological properties (viability during storage), resistance to antibiotics, etc.

REFERENCES

1. Okochi, M., Sugita, T., Asai, Y., Tanaka, M., & Honda, H. (2017). Screening of peptides associated with adhesion and aggregation of Lactobacillus rhamnosus GG in vitro. Biochemical Engineering Journal, 128, 178–185.
2. Riaz, M. S., Shaheen, T., Batool, N., Saleem, S., & Hayat, F. (2015). Lactic acid bacteria as probiotic candidate and their application. J. Biol. Today's World, 4, 209-216.
3. Collado, M. C., Meriluoto, J., &Salminen, S. (2007). Adhesion and aggregation properties of probiotic and pathogen strains. European Food Research and Technology, 226(5), 1065–1073.

4. Datta, A., Stapleton, F., &Willcox, M. D. (2017). Bacterial coaggregation among the most commonly isolated bacteria from contact lens cases. Investigative ophthalmology & visual science, 58(1), 50-58.
5. Vlková, E., Rada, V., Šmehilová, M., & Killer, J. (2008). Auto-aggregation and co-aggregation ability in bifidobacteria and clostridia. Folia microbiologica, 53(3), 263-269.
6. Li, Q., Liu, X., Dong, M., Zhou, J., & Wang, Y. (2015). Aggregation and adhesion abilities of 18 lactic acid bacteria strains isolated from traditional fermented food. Int J Agric Policy Res, 3(2), 84-92.
7. Kirjavainen, P. V., Ouwehand, A. C., Isolauri, E., &Salminen, S. J. (1998). The ability of probiotic bacteria to bind to human intestinal mucus. FEMS microbiology letters, 167(2), 185-189.
8. Ouwehand, A. C., Niemi, P., &Salminen, S. J. (1999). The normal faecal microflora does not affect the adhesion of probiotic bacteria in vitro. FEMS microbiology letters, 177(1), 35-38.
9. Fajdiga, S., Koninkx, J. F. J. G., Tooten, P. C. J., & Marinšek-Logar, R. (2006). Interference of *Salmonella enteritidis* and Lactobacillus spp. with IL-8 levels and transepithelial electrical resistance of enterocyte-like Caco-2 cells. Folia microbiologica, 51(4), 268-272.
10. García-Cayuela, T., Korany, A. M., Bustos, I., P. Gómez de Cadiñanos, L., Requena, T., Peláez, C., &Martínez-Cuesta, M. C. (2014). Adhesion abilities of dairy Lactobacillus plantarum strains showing an aggregation phenotype. Food Research International, 57, 44–50.
11. Rojas, M., Ascencio, F., & Conway, P. L. (2002). Purification and characterization of a surface protein from Lactobacillus fermentum 104R that binds to porcine small intestinal mucus and gastric mucin. Applied and Environmental Microbiology, 68(5), 2330-2336.
12. Roos, S., &Jonsson, H. (2002). A high-molecular-mass cell-surface protein from Lactobacillus reuteri 1063 adheres to mucus components The GenBank accession number for the sequence reported in this paper is AF120104. Microbiology, 148(2), 433-442.
13. Kiely, L. J., & Olson, N. F. (2000). The physicochemical surface characteristics of Lactobacillus casei. Food Microbiology, 17(3), 277-291.
14. He, T., Priebe, M. G., Zhong, Y., Huang, C., Harmsen, H. J. M., Raangs, G. C., ... &Vonk, R. J. (2008). Effects of yogurt and bifidobacteria supplementation on the colonic microbiota in lactose-intolerant subjects. Journal of Applied Microbiology, 104(2), 595-604.
15. Van der Mei, H. ., van de Belt-Gritter, B., Pouwels, P. ., Martinez, B., &Busscher, H. . (2003). Cell surface hydrophobicity is conveyed by S-layer proteins—a study in recombinant lactobacilli. Colloids and Surfaces B: Biointerfaces, 28(2-3), 127–134
16. Nikolic, M., Jovcic, B., Kojic, M., &Topisirovic, L. (2010). Surface properties of Lactobacillus and Leuconostoc isolates from homemade cheeses showing auto-aggregation ability. European Food Research and Technology, 231(6), 925-931.
17. Rickard, A. H., Gilbert, P., High, N. J., Kolenbrander, P. E., & Handley, P. S. (2003). Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends in microbiology, 11(2), 94-100.
18. 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.
19. Boris, S., Suárez, J. E., Vázquez, F., &Barbés, C. (1998). Adherence of human vaginal lactobacilli to vaginal epithelial cells and interaction with uropathogens. Infection and immunity, 66(5), 1985-1989.
20. Bao, Y., Zhang, Y., Zhang, Y., Liu, Y., Wang, S., Dong, X., ...& Zhang, H. (2010). Screening of potential probiotic properties of Lactobacillus fermentum isolated from traditional dairy products. Food Control, 21(5), 695-701.
21. Arief, I. I., Jenie, B. S. L., Astawan, M., Fujiyama, K., &Witarto, A. B. (2015). Identification and probiotic characteristics of lactic acid bacteria isolated from Indonesian local beef. Asian J. Anim. Sci, 9(1), 25-36.
22. Botes, M., van Reenen, C. A., & Dicks, L. M. (2008). Evaluation of Enterococcus mundtii ST4SA and Lactobacillus plantarum 423 as probiotics by using a gastro-intestinal model with infant milk formulations as substrate. International journal of food microbiology, 128(2), 362-370.

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