Coaggregation and autoaggregation properties in lactic acid bacteria

Aygun Mustafazada[1*], Zerrin Canturk[2]

[1]Molecular Biotechnology Laboratory, Institute of Molecular Biology & Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan, 11 Izzat Nabiyev Str., AZ1073, Baku, Azerbaijan
[2]Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Anadolu University, Yunus Emre Campus, 26210, Tepebasi/Eskisehir, Turkiye

***For correspondence:** aygunmustafazada2000@gmail.com*

Received: March 13, 2025; Reviewed: April 21, 2025; Accepted: June 02, 2025

Lactic acid bacteria are the normal flora of the gastrointestinal system of the human organism. They can prevent pathogenic bacteria from settling in the intestinal epithelium by colonizing it. Lactic acid and acetic acid formed as a result of fermentation lower the pH of the environment and prevent the growth of pathogenic bacteria. Bacterial aggregation and autoaggregation properties of lactic acid bacteria strains isolated from colostrum were carried out. Autoaggregation values of lactobacillus strains were evaluated between 3-19%. Coaggregation values of lactobacillus strains with *E.coli* ATTC 35298 were determined between 34-88%. Coaggregation properties of lactobacillus strains with *S.aureus* ATCC 25923 were evaluated between 27-37%.

Keywords: *Lactic acid bacteria, probiotics, autoaggregation, coaggregation, normal flora*

INTRODUCTION

Probiotics. The term probiotic is derived from the Greek words “pro” and “bios”, meaning “for life” (Gismondo, 1999). According to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), the definition of probiotics as "live microorganisms that when administered in adequate amounts confer a benefit on the host" is the widely accepted scientific definition of probiotics worldwide (ISAPP, 2014). Lactic acid bacteria, bifidobacteria and enterococci are common bacteria with probiotic properties (Bron, 2013).

When choosing probiotic strains, certain factors should be taken into consideration and the strains must have certain criteria. These criteria are divided into 4 groups: safety, technological, functional and performance (Dunne, 2001).

Lactic acid bacteria. Lactobacillus is a gram-positive, catalase-negative, G+C (guanine and cytosine) ratio of less than 50%, non-spore-forming, non-motile rod-shaped bacteria (Hammes et al., 2009). In total, there are more than 237 confirmed species, and new species are still being discovered, such *as Lactobacillus metriopterae* and *Lactobacillus timonensis*. Cell structures: cell wall, cytoplasmic membrane, ribosomes and nuclear elements. They usually have one plasmid. The function of plasmids is to produce microbial substances and to provide protection from environmental factors (Klaenhammer, 2008). Microorganisms to be used as probiotics must be safe. That is, they should not produce toxins and should not be pathogenic.

Coaggregation and Autoaggregation. Lactic acid bacteria are the normal flora of the gastrointestinal system of the human organism (Kirtzalidou et al., 2011). Autoaggregation in lactic acid bacteria means that bacteria of the same species come together and form a mass. This feature allows bacteria to adhere to the intestinal epithelium and form a biofilm. Coaggregation, unlike autoaggregation, is a process that occurs when different types of bacteria come together. This ensures the symbiotic relationship of the bacteria (Kos et al., 2003).

Lactic acid bacteria are known for their potential to benefit human health by promoting functions such as enhancing epithelial barrier integrity, preventing pathogen invasion, and contributing to overall gut health (Saxelin, 2005). The ability of lactic acid bacteria strains to adhere to and colonize the gastrointestinal tract is regarded as a key criterion for selecting potential probiotics, as it enhances their intestinal persistence and facilitates the expression of their probiotic effects (Kolida et al., 2006). Studies have demonstrated that certain lactic acid bacteria can inhibit the adhesion of pathogenic bacteria to the intestinal mucosa either by forming a barrier through autoaggregation or by coaggregating with the

 DOI: https://doi.org/10.62088/timbb/9.1.4

pathogens. Lactobacilli exhibiting strong autoaggregation abilities have also been associated with high hydrophobicity (Collado et al., 2007).

MATERIALS AND METHODS

After thawing the colostrum sample at room temperature, serial dilutions were made with physiological serum as 1/10, 1/100, 1/1000, 1/10000. After dilution, 1 mL of each dilution was taken and spread onto MRS (De Man – Rogosa – Sharpe) agar and M-17 medium. Petri dishes were incubated at 37°C for 48 hours. After incubation, each Petri dish showing growth was visually examined, and each sample that appeared. After the incubation period, bacterial colonies displaying different morphologies were selected. The purity of each isolate was confirmed by Gram staining. The pure isolates were stored at −80°C in 20% glycerol for further analysis. Among the 34 LAB isolates obtained, only 9 Gram-positive (+), catalase-negative (−) isolates identified as bacilli were selected for further experiments. The identification of lactic acid bacteria was previously performed by 16S rRNA gene sequencing, as described in our earlier publication (Mustafazade and Cantürk, 2024). Therefore, the sequencing data are not repeated in this manuscript.

The ability of lactic acid bacteria to autoaggregate was determined based on the method described by Kos et al. (2003), with minor modifications. Bacterial isolates were inoculated into MRS broth and incubated at 37°C in a 5% CO_2 atmosphere for 48 hours. After incubation, active cultures were centrifuged at 1000 rpm for 15 minutes at 24°C. The supernatant was discarded, and the resulting pellets were washed twice with phosphate-buffered saline (PBS, pH6.2) and resuspended in the same buffer. The optical density (OD) of the bacterial suspensions was adjusted to 0.600 at 600 nm using a spectrophotometer. The suspensions were then incubated at room temperature for 4 hours without disturbance. After incubation, 0.1 mL of the upper phase was carefully removed and the OD was measured again at 600 nm.

$$\textbf{autoaggregation} = [(OD_1 - OD_2)/OD_1] \times 100$$

here: OD_1 - optical density at 0 hours;

OD_2 - optical density at 4 hours.

The coaggregation ability of lactic acid bacteria (LAB) with pathogenic bacteria (Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923) was evaluated based on the method described by Rinkinen et al., with slight modifications. LAB isolates were activated by culturing twice in MRS broth and incubated at 37°C in a 5% CO_2 atmosphere for 48 hours. Following incubation, cultures were centrifuged at 5000 rpm for 15 minutes at 24°C. The supernatants were discarded, and the pellets were washed twice with phosphate-buffered saline (PBS, pH 6.2) and resuspended in the same buffer. The optical density (OD) of each bacterial suspension was adjusted to 0.600 at 600 nm using a spectrophotometer. Equal volumes of LAB suspension and the pathogenic bacterial suspension were mixed in a sterile tube, vortexed for 15 seconds, and incubated at room temperature for 4 hours. After incubation, 100 µL of the upper phase was carefully withdrawn and its OD was measured at 600 nm. Coaggregation percentages were calculated according to the formula below.

$$\textbf{coaggregation} = [(OD_1 + OD_2) - 2OD_3) / (OD_1 + OD_2)] \times 100$$

here: OD_1 - optical density of bacterial cultures;

OD_2 - optical density of pathogenic bacteria;

OD_3 - optical density of mixed culture after 4 hours (Rinkinen et al., 2003).

RESULTS AND DISCUSSION

Autoaggregation is one of the important features of probiotic bacteria. Autoaggregation occurs between bacteria of the same strain. Thanks to this feature, they prevent pathogenic bacteria from adhering to the intestinal surface (Jankovic et al., 2003). The autoaggregation values of the isolates were evaluated between 3-19%. The highest autoaggregation feature was determined in isolate No.3 with 19%, while the lowest autoaggregation feature was determined in isolate No.7 with 3%.

Table 1. Autoaggregation values of bacterial strains

Bacterial strains	Autoaggregation
Isolate 1	14
Isolate 2	4
Isolate 3	19
Isolate 4	4
Isolate 5	4
Isolate 6	6
Isolate 7	3
Isolate 8	13
Isolate 9	6.6

Table 2. Coaggregation values of bacterial strains

Bacterial strains	Coaggregation (*E. coli*)	Coaggregation (*S. aureus*)
Isolate 1	31	31
Isolate 2	28	28
Isolate 3	27	27
Isolate 4	34	34
Isolate 5	34	34
Isolate 6	27	27
Isolate 7	33	33
Isolate 8	37	37
Isolate 9	31	31

The coaggregation properties of bacterial isolates with *E. coli* ATTC 35298 were found to be between 34-88%. While the highest coaggregation property was determined in isolate No. 8 with 88%, the lowest coaggregation property was determined in isolate No. 4 with 34%. As shown in Table 2, the coaggregation abilities of LAB strains with *E. coli* ATTC 35298 were determined as 80%, 61%, 37%, 34%, 88%, 53%, 64%, 50%, and 37%, respectively.

The coaggregation properties of bacterial isolates with *S.aureus* ATCC 25923 were evaluated between 27-37%. The highest coaggregation property was determined in isolate No.8 with 37%, while the lowest coaggregation property was determined in isolate No. 3 with 27%. As shown in Table 2, the coaggregation abilities of isolates 1, isolate 2, isolate 3, isolate 4, isolate 5, isolate 6, isolate 7, isolate 8 and isolate 9 with *S.aureus* ATCC 25923 were determined as 31%, 28%, 27%, 34%, 34%, 27%, 33%, 37%, 31%, respectively.

CONCLUSION

In recent years, interest in the complex microbial ecosystem in the human gastrointestinal system has increased. Probiotic bacteria are microorganisms that can live in human mucosa. Probiotic microorganism flora is found in the healthy human body. Lactic acid bacteria belong to probiotic bacteria. These bacteria are resistant to bile salts, high stomach acid and lysozyme enzymes (Yasar and Kurdas, 2009). Lactobacillus species can colonize the small intestine, while Bifidobacteria can colonize the large intestine.

According to the results obtained; It was determined that all isolates were resistant to acid and bile salt. Autoaggregation values of lactobacillus strains were evaluated between 3-19%. The highest autoaggregation property was determined in isolate 3 with 19%, while the lowest autoaggregation property was determined in isolate 7 with 3%. It is shown in Table 1.

Coagregation values of lactobacillus strains with *E.coli* ATTC 35298 were determined between 34-88%. While the highest coagregation property was determined in isolate 5 with 88%, the lowest coagregation property was determined in isolate 4 with 34%. Coagregation properties of lactobacillus strains with *S.aureus* ATCC 25923 were evaluated between 27-37%. While the highest coagregation property was determined in isolate 8 with 37%, the lowest coagregation property was determined in isolate 3 with 27%. The results are shown in Table 2.

REFERENCES

Bron P.A., Tomita S., Mercenier A., Kleerebezem M. (2013) Cell surfaceassociated compounds of probiotic lactobacilli sustain the strain. *Curr. Opin. Microbiol.*, **16:** 262–269.

Collado M.C., Meriluoto J., Salminen S. (2007) Measurement of aggregation properties between probiotics and pathogens: In vitro evaluation of different methods. *J. Microbiol. Meth.*, **71:** 71-74.

Dunne C., O'Mahony L., Murphy L., Thornton G., Morrissey D., O'Halloran S., Feeney M., Flynn S., Fitzgerald G., Daly C., Kiely B., O'Sullivan G.C., Shanahan F., Collins JK. (2001) *In vitro* selection criteria for probiotic bacteria of human origin: correlation with *in vivo* findings. *Am. J. Clin. Nutr*., **73(2 Suppl):** 386S-392S.

Gismondo M.R., Drago L., Lombardi A. (1999) Review of probiotics available to modify gastrointestinal flora. *Int. J. Antimicrob. Ag*., **12:** 287–292.

Hammes W., Hertel C. (2006) The general *Lactobacillus* and *Carnobacterium*. In: *The prokaryotes*, 3rd ed. *New York: Springer,* pp. 319-403.

Jankovic I., Ventura M., Meylan V., Rouvet M., Elli M., Zink R. (2003) Contribution of aggregation-promoting factor to maintenance of cell shape in *Lactobacillus gasseri* 4B2. *J. Bacteriol*., **185:** 3288– 3296.

Klaenhammer T.R., Altermann E., Pfeiler E., Buck B.L., Goh Y.J., O'Flaherty S. (2008) Functional genomics of probiotic *Lactobacilli*. *J. Clin. Gastroenterol*., **42(Suppl 3 Pt 2):** S160–S162.

Kirtzalidou E., Pramateftaki P., Kotsou M., Kyriacou A. (2011) Screening for *lactobacilli* with probiotic properties in the infant gut microbiota. *Anaerobe*, **17:** 440-443.

Kolida S., Saulnier D.M., Gibson G.R. (2006) Gastrointestinal microflora: probiotics. *Adv. Appl. Microbiol*., **59:** 187-219.

Kos B.V.Z.E., Kovi C., Vukovi C., Impraga M.V.S., Frece J., Mato V.S.I.C. (2003) Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92. *J. Appl. Microbiol*., **94:** 981-987.

Kos B.V., Suskovic Z.E., Vukovic J., Simpraga M., Frece. J, Matosic S. (2003) Adhesion and aggregation ability of probiotic strain *Lactobacillus acidophilus* M92. *Journal of Applied Microbiology*, **94(6):** 981-987.

Mustafazade A., Cantürk Z. (2024). Investigation of probiotic properties of lactic acid bacteria isolated from colostrum. *Transactions of the Institute of Molecular Biology & Biotechnologies, MSE AR,* **8(2):** 08-13; doi: 10.62088/timbb/8.2.2

Rinkinen M., Jalava K., Westermarck E., Salminen S., Ouwehand A.C. (2003) Interaction between probiotic lactic acid bacteria and canine enteric pathogens: a risk factor for intestinal *Enterococcus faecium* colonization. *Veterinary Microbiology*, **92(1-2):** 111-119.

Saxelin M., Tynkkynen S., Mattila-Sandholm T., de Vos V.M. (2005) Probiotic and other functional microbes: from markets to mechanisms. *Curr. Opin. Biotech.*, **16:**204-211.

Yaşar B., Kurdaş O.Ö. (2009) Probiotics and gastrointestinal system. *Current Gastroenterology,* 13/1. Haydarpaşa Numune Training and Research Hospital, Gastroenterohepatology Clinic, İstanbul.

Süd turşusu bakteriyalarında koaqreqasiya və avtoaqreqasiya xüsusiyyətləri

Aygün Mustafazada[1], Zerrin Cantürk[2]

[1]Azərbaycan Respublikası Elm və Təhsil Nazirliyi Molekulyar Biologiya və Biotexnologiyalar İnstitutunun Molekulyar biotexnologiya laboratoriyası, Bakı, Azərbaycan
[2]Anadolu Universitetinin Əczaçılıq mikrobiologiyası bölməsinin Əczaçılıq fakültəsi, Tepebaşı/Eskişehir, Türkiyə

Süd turşusu bakteriyaları insanın mədə-bağırsaq sisteminin normal florasına daxildir. Bu bakteriyalar patogen bakteriyaların bağırsaq epitelisində kolonizasiya əmələ gətirməsinə mane olurlar. Fermentasiya nəticəsində əmələ gələn süd və sirkə turşusu ətraf mühitin pH səviyyəsini aşağı salır, nəticədə patogen bakteriyalar inkişaf edə bilmirlər. Kolostrumdan əldə olunmuş probiotik xarakterli süd turşusu bakteriyalarında koagregasiya və avtoagregasiya xüsusiyyətləri öyrənilmişdir. Lactobacillus suşlarının avtoaqreqasiya dəyərləri 3-19% arasında qiymətləndirilmişdir. Lactobacillus ştammlarının *E. coli* ATTC 35298 ilə koagregasiya xüsusiyyətləri 34-88% arasında müəyyən edilmişdir. Lactobacillus suşlarının *S.aureus* ATCC 25923 ilə koaqreqasiya xüsusiyyətləri 27-37% arasında qiymətləndirilmişdir.

Açar sözlər: *Süd turşusu bakteriyaları, probiotiklər, avtoaqreqasiya, koaqreqasiya, normal flora*

ORCIDS:
Aygun Mustafazadə: https://orcid.org/0009-0007-2775-459X
Zerrin Canturk: https://orcid.org/0000-0001-7709-4285