Effects of Aloe Vera on Probiotic Properties of *Lacticaseibacillus rhamnosus GG* (LGG) *and Lactobacillus acidophilus LA-5* (LA-5)

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Abstract – Aloe vera is plant which has strong antibacterial, anticancer, antifungal and wound healing. Probiotic bacteria are live microorganisms that confer a health benefit on the host when administered in adequate amounts. Synbiotic is relationship between aloe vera and probiotic bacteria that are found together in the gastrointestinal tract system. Aim of the this study is to investigate effects of aloe vera on probiotic bacteria *Lacticaseibacillus rhamnosus* GG (LGG) and *Lactobacillus acidophilus* LA-5 (LA-5). For this purpose, LGG and LA-5 were grown in different concentrations of aloe vera extract and effects of aloe vera extract on bacterial growth kinetics, bacterial auto-aggregation and resistance of pepsin were investigated. Results showed that aloe vera extracts did not showed inhibitory effect on the microbial growth of LGG and LA-5. Aloe vera increased the auto-aggregation of LA-5 while it decreased the auto-aggregation of LGG. Furthermore, aloe vera increased the pepsin resistance of LGG but it effetc the pepsin resistance of LA negatively. These results indicated that phytic acid may enhance the probiotic property of the LGG and LA-5.

Keywords – Lactic Acid Bacteria, Aloe Vera, Probiotic, Synbiotic

I. Introduction

Aloe vera is medicinal plant that have antibacterial, anticancer, antifungal, antiseptic, antioxidant, anti-inflammatory and wound healing properties. It is one of the most studied and interested plant thanks to its biological properties and health benefits [1][2].

Probiotic bacteria are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" by Food and Agriculture organisation/World Health Organisation (FAO/WHO) [3]. They can be used to prevent and treat some diseases such as diarrhea, inflammatory intestinal diseases, and functional digestive disorders [4]. *L. acidophilus* LA-5 and *L. rhamnosus* GG are the most common and used probiotic bacteria [5].

In the gastrointestinal tract, probiotic bacteria found together with phenolic compounds by forming a synbiotic relationship to exert more beneficial effects on host [6]. The present study aimed to investigate the effects of aloe vera extracts on probiotic bacteria, *Lacticaseibacillus rhamnosus* GG (LGG) and *Lactobacillus acidophilus* LA-5 (LA-5).

II. Materials and Method

A. Growth of probiotic bacteria in the presence of aloe vera extracts

Lacticaseibacillus rhamnosus GG (LGG) and *Lactobacillus acidophilus* LA-5 (LA-5) which were kind gifts of Chr. Hansen, Turkey, were grown in semisynthetic medium for lactic acid bacteria (LABSEM) without shaking at 37°C [7]. The

bacterial cultures were divided into groups, and aloe vera extracts was used in different concentrations (0 – 1000 µg/mL).

B. Effects of phytic acid on probiotic bacteria growth kinetics

LGG and LA-5 were divided into groups and treated with aloe vera extracts, one group as control without aloe vera extracts (LABSEM only). The growth of LGG and LA-5 was observed every four hours by measuring their absorbance at wavelength of 600 nm by adding varying concentrations of aloe vera extracts to LABSEM medium.

C. Auto-aggregation assay for probiotic bacteria

Auto-aggregation is the property that the community formed by microorganisms belonging to the same species by clinging to each other. It is a crucial feature for probiotic bacteria to colonize and attach to the mucosa in the human body [8]. For auto-aggregation assay, bacterial cells were collected in the late logarithmic phase (3200×g, 15 min), washed with phosphate-buffered saline (PBS) and re-suspended in PBS to OD600 0.5 [9]. Auto-aggregation was determined by adding 4 mL of bacterial suspensions to the test tubes after vortex for 10 s (1 h, RT). After incubation, 100 µL from the upper portion of the suspensions was taken, added to the tube containing 900 µL of PBS, and the absorbance was measured at 600 nm. The percentage of auto-aggregation was calculated using the following formula:

$$\%Auto - aggregation = (1 - \frac{A_t}{A_0}) \times 100$$

where At is the absorbance measured after incubation (every hour for 3 h), and A0 is the absorbance measured before incubation [8].

D. Resistance of probiotic bacteria to pepsin

Probiotic bacteria taken into the body are exposed some digestive enzymes such as pepsin in the gastrointestinal tract (GIT). Therefore, resistance of probiotic to pepsin is important feature for survival of probiotic bacteria to exert beneficial effects on host. For the analysis, control and treatment groups were collected after growth by centrifugation, washed with PBS, and incubated for 3 hours at 37 ℃ in PBS containing 3 mg/mL pepsin. The cultures were then grown on MRS agar and colony counts were performed after 48 hours [5].

III. RESULTS

A. Growth of probiotic bacteria in the presence of aloe vera extracts

Figure 1 indicates microbial growth kinetics of *Lactobacillus rhamnosus* GG treated with 0 – 1000 µg/mL aloe vera extract and Figure 2 shows microbial growth kinetics of *Lactobacillus acidophilus* LA-5 treated with 0 – 1000 µg/mL aloe vera extract.

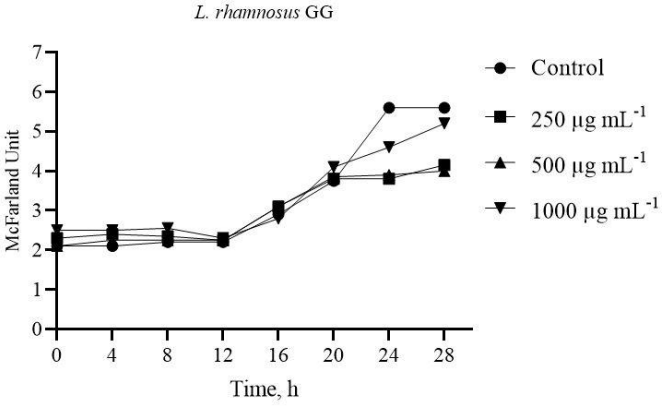

Fig. 1 Growth kinetics of *Lactobacillus rhamnosus* GG grown in the presence of 0 – 1000 µg/mL aloe vera extract

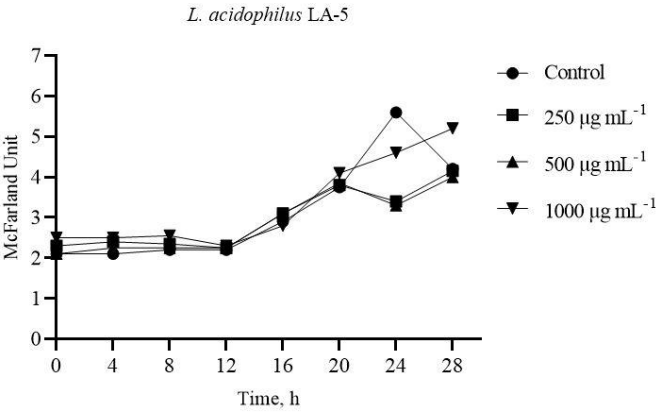

Fig. 2 Growth kinetics of *Lactobacillus acidophilus* LA-5 grown in the presence of 0 – 1000 µg/mL aloe vera extract

B. Effects of aloe vera extracts on auto-aggregation property of Lactobacillus acidophilus LA-5 and Lactocaseibacillus rhamnosus GG

The auto-aggregation feature of *Lactobacillus acidophilus* LA-5 and *Lactocaseibacillus rhamnosus* GG grown in the presence of 0 – 1000 µg/mL aloe vera extract was examined for 5 hours and the results are shown in Figure 3 and Figure 4.

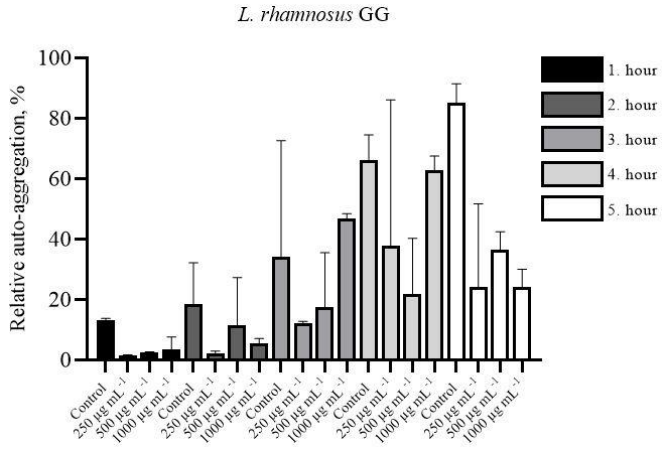

Fig. 3 Effects of aloe vera extract on auto-aggregation of *Lactobacillus rhamnosus* GG

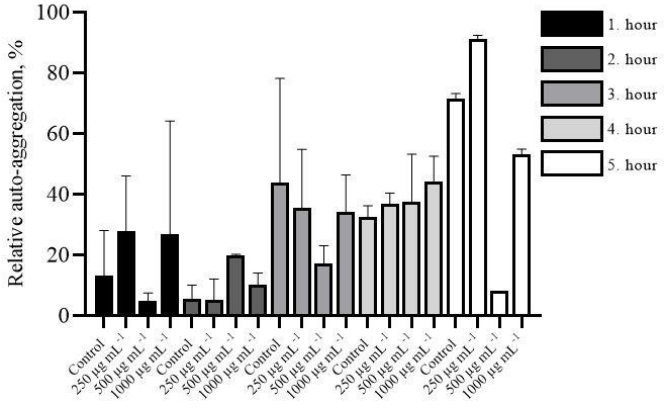

Fig. 4 Effects of aloe vera extract on auto-aggregation of *Lactobacillus acidophilus* LA-5

C. *Resistance of probiotic bacteria to pepsin*

Figure 5 shows resistance of *Lactobacillus acidophilus* LA-5 and *Lactocaseibacillus rhamnosus* GG grown in the presence of aloe vera extract to pepsin.

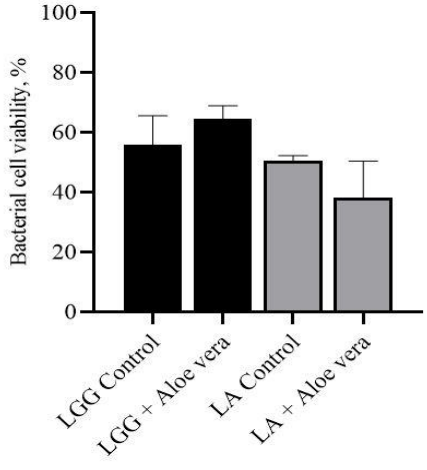

Fig. 5 Pepsin resistance of *L. rhamnosus* GG *and L. acidophilus* LA – 5

IV. DISCUSSION

Aloe vera is plant that have antibacterial property against Gram-negative and Gram-positive bacteria [10]. Figure 1 and Figure 2 showed growth kinetics of LGG and LA-5 bacteria treated with different concentrations of aloe vera extract (0 – 1000 µg/mL). The results showed that all of the aloe vera extract concentrations did not showed inhibitory effects on the microbial growth of LGG and LA-5 which are Gram-positive bacteria.

Auto-aggregation is necessary property for probiotic bacteria to adhere to mucosal layer and form colony to exert beneficial effect on host [11]. Figure 3 shows auto-aggregation of LGG treated with different concentrations of aloe vera extracts. 1000 µg/ml of aloe vera extract increased the auto-aggregation ability of LGG at third hour, while all of the concentrations of aloe vera extracts affect negatively auto-aggregation of LGG. Figure 4 shows auto-aggregation of LA treated with different concentrations of aloe vera extracts. All of the aloe vera concentrations increased the auto-aggregation of LA at every hour except third hour.

Probiotic bacteria taken orally can survive and colonize in gastrointestinal tract (GIT) to exert their probiotic properties. Therefore, probiotic bacteria can resist to pepsin and the pH of the stomach [12]. While aloe vera affect positively pepsin resistance of LGG, it decreased the pepsin resistance of LA.

V. CONCLUSION

In the present study, the effects of aloe vera extract on microbial growth, auto-aggregation and resistance to pepsin of *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* LA-5. Symbiotic relationship is interaction between phenolic compounds and probiotic bacteria which are found together in gastrointestinal tract (GIT). There is no more information about symbiotic relationship between phenolic and probiotic bacteria. Aloe vera has antibacterial property against Gram-positive and Gram-negative bacteria. We showed that aloe vera did not affect the microbial growth of LGG and LA-5, which are Gram-positive bacteria.

Aloe vera extracts decreased the auto-aggregation of LGG except 1000 µg/ml of aloe vera at third hour while aloe vera extracts increased the auto-aggregation of LA-5. Furthermore, while aloe vera increased pepsin resistance of LGG, it decreased the pepsin resistance of LA. Results indicate aloe vera

may effect the probiotic properties of LGG and LA-5.

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REFERENCES

[1] S. Rahman, P. Carter, and N. Bhattarai, "Aloe Vera for Tissue Engineering Applications," *J. Funct. Biomater.*, vol. 8, no. 1, p. 6, 2017, doi: 10.3390/jfb8010006.

[2] D. I. Sánchez-Machado, J. López-Cervantes, R. Sendón, and A. Sanches-Silva, "Aloe vera: Ancient knowledge with new frontiers," *Trends Food Sci. Technol.*, vol. 61, pp. 94–102, 2017, doi: 10.1016/j.tifs.2016.12.005.

[3] D. Pradhan, R. H. Mallappa, and S. Grover, "Comprehensive approaches for assessing the safety of probiotic bacteria," *Food Control*, vol. 108, no. July 2019, p. 106872, 2020, doi: 10.1016/j.foodcont.2019.106872.

[4] V. Liévin-Le Moal and A. L. Servin, "Anti-infective activities of Lactobacillus strains in the human intestinal microbiota: From probiotics to gastrointestinal anti- infectious biotherapeutic agents," *Clin. Microbiol. Rev.*, vol. 27, no. 2, pp. 167–199, 2014, doi: 10.1128/CMR.00080-13.

[5] D. Alp, H. Kuleaşan, and A. Korkut Altıntaş, "The importance of the S-layer on the adhesion and aggregation ability of Lactic acid bacteria," *Mol. Biol. Rep.*, vol. 47, no. 5, pp. 3449–3457, 2020, doi: 10.1007/s11033-020-05430-6.

[6] J. S. Michael de Vrese, "Probiotics, prebiotics and synbiotics," *Funct. Foods Sources, Heal. Eff. Futur. Perspect.*, no. May, pp. 143–207, 2008, doi: 10.1201/b15561-2.

[7] H. U. Celebioglu, M. Delsoglio, S. Brix, E. Pessione, and B. Svensson, "Plant Polyphenols Stimulate Adhesion to Intestinal Mucosa and Induce Proteome Changes in the Probiotic Lactobacillus acidophilus NCFM," *Mol. Nutr. Food Res.*, vol. 62, no. 4, pp. 1–11, 2018, doi: 10.1002/mnfr.201700638.

[8] B. Kos, J. Šušković, S. Vuković, M. Sĭmpraga, J. Frece, and S. Matošić, "Adhesion and aggregation ability of probiotic strain Lactobacillus acidophilus M92," *J. Appl. Microbiol.*, vol. 94, no. 6, pp. 981–987, 2003, doi: 10.1046/j.1365-2672.2003.01915.x.

[9] H. U. Celebioglu *et al.*, "Differential proteome and cellular adhesion analyses of the probiotic bacterium Lactobacillus acidophilus NCFM grown on raffinose - an emerging prebiotic," *Proteomics*, vol. 16, no. 9, 2016, doi: 10.1002/pmic.201500212.

[10] P. Danish, Q. Ali, H. Mm, and A. Malik, "ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF ALOE VERAPLANT EXTRACT," pp. 1–8, 2020.

[11] Y. Sui *et al.*, "Food Bioscience In vitro probiotic characterization of Lactobacillus strains from fermented tangerine vinegar and their cholesterol degradation activity," *Food Biosci.*, vol. 39, no. December 2020, p. 100843, 2021, doi: 10.1016/j.fbio.2020.100843.

[12] O. Osmanagaoglu, F. Kiran, and H. Ataoglu, "Evaluation of in vitro Probiotic Potential of Pediococcus pentosaceus OZF Isolated from Human Breast Milk," *Probiotics Antimicrob. Proteins*, vol. 2, no. 3, pp. 162–174, 2010, doi: 10.1007/s12602-010-9050-7.