Original article

Effect of *Aloe vera* juice on growth and activities of Lactobacilli *in-vitro*

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Abstract. In present investigation, different concentrations of *Aloe vera* juice incorporated into the growth media of Lactobacilli were tested to observe the effect on growth and activities of these bacteria. From the results obtained, it was observed that aloe vera juice at a concentration of 5% v/v was effective in promoting the growth of *L. acidophilus, L. plantarum* and *L. casei*, as evident from the fall in pH and increased acidity, as well as from the improved generation time. At 15 to 25% concentration, growth was unaffected as compared to the controls; however, concentration higher than 25%v/v discouraged the growth. Overall, it was concluded that Aloe vera juice or gel at a particular concentration could possibly be used in combination with probiotic *Lactobacillus* strain(s) as a combinational therapy for gastrointestinal disorders and cardiovascular diseases. (www.actabiomedica.it)

Key words: Aloe vera, gastrointestinal diseases, lactobacillus, prebiotics, probiotics, synbiotics

Introduction

Traditional and modern medicine have suggested to use fermented foods as an alternative for the prevention and treatment of malnutrition, chronic diarrhea, gastrointestinal diseases and other conditions, since consumption of these foods provides an entry route for the common biological agents that comprise the beneficial intestinal flora, particularly probiotics (1-4). Probiotics are defined as live microorganisms present in foods which when ingested in sufficient quantities can have specific physiological benefits such as anti-pathogenic, anti-diarrheal, anti-carcinogenic, anti-diabetic, anti-cholesterolemic etc. to individual health (3-14). An important group of probiotics that are used traditionally are lactic acid bacteria i.e. *Lactobacillus, Streptococcus, Lactococcus, Bifidobacterium*.

Aloe vera pulp, due to its composition, is a food plant likely to promote the growth of probiotic agents (15). It has long been used for the treatment and prevention of gastrointestinal diseases in several countries (16). Today, *Aloe vera* has attracted significant interest because of its nutritional and medicinal characteristics, and its potential as a generator of economic activity in arid and semi-arid areas (17). About 320 species of the genus *Aloe* have been reported among which the most commonly known is *Aloe vera (Aloe vera* var *barbariensis*), a species most cultivated in Mexico (18). *Aloe vera* contains most of its carbohydrates in the form of mannose polymers (accemananos), vitamin A, Vitamin B1, B6 and vitamin C (19). Most important properties of *Aloe vera* include its anti-inflammatory, antibiotic, regenerative, anti-diabetic and anti-cholesterolemic (20). In the food industry, *Aloe vera* juice has been used for preparation of soft drinks with healthy nutritional qualities and tonics containing amino acids and minerals.

Traditionally, delivery vehicles used for probiotics are dairy based; however, there is a large variety of fruits and vegetables available locally that could be feasible to be exploited for probiotics (13). Although, both, *Aloe vera* as well as probiotic Lactobacilli, have

individually been well-documented for numerous health benefits; there are scanty of reports available on a combined approach and application. In this milieu, a novel effort was made through present investigation in order to observe the activities of Lactobacilli in *Aloe vera* juice with a prospective approach to blend probiotics and *Aloe vera* that could possibly be exploited as a potential combinational remedy.

Materials and methods

The three Lactobacillus isolates viz. *L. acidophilus NCDC14, L. plantarum NCDC20* and *L. casei NCDC17* were procured from National Collection of Dairy Cultures, National Dairy Research Institute, India, and were maintained in sterile 10% reconstituted skim milk. The *Aloe vera* was collected from home garden (Rajpura, Punjab, India). Freshly harvested, matured *Aloe vera* leaves were cut from the bottom of the plant with a sharp knife, and washed thoroughly. Then, using a sharp knife, the rough edges were removed. The slimy mucilage and transparent gel was scooped out with a spoon from the *Aloe* leaf. It was made sure that the yellow sap exuded from the green part of the leaf was discarded properly. This is called aloin that causes irritation reactions. *Aloe vera* gel was then macerated in a grinder, and was subsequently filtered to separate the fiber and get the juice used in the fermentation. It was preserved in the refrigerator for everyday usage.

Different concentrations of *Aloe vera* juice i.e. 5%, 15%, 25%, 50%, 100% were prepared in de Man Rogosa Sharpe (MRS) broth (21). Total volume was kept 50 ml. One flask containing MRS was kept as negative control (uninoculated) and one was kept as positive control (inoculated). Overnight grown cultures of *L. acidophilus, L. plantarum, L. casei* (1%) were inoculated respectively into MRS broth containing different concentrations of *Aloe vera* juice (5%, 15%, 25%, 50% and 100%), and incubated for 48 hr at 37°C. Readings were taken at 0, 24 and 48 hr for pH, acidity and viable counts. The pH of each sample was measured using a pH meter (μ pH Systems 361, Systronics, New Delhi, India) after proper calibration. Total acidity, expressed as percent lactic acid, was determined by titrating with 0.1 N NaOH to pH 8.2, using Phenolphthalein as an end point indicator. The sugar content was estimated in terms of glucose (mg/ml) by the phenol sulfuric acid method, as described by Dubios et al. (22). An aliquot from each treatment was taken at 0 hr and 12 hr and diluted (1:10, v/v) with 0.2% (w/v) EDTA (pH 12.0) and turbidity measured at 640 nm using uninoculated media diluted with EDTA as a blank. Specific growth rate (μ) for each culture was calculated using the equation: $\mu = (\ln D2 - \ln D1)/(t2 - t1)$, where D2 and D1 are the cell densities at times t2 and t1, respectively. Mean generation time was calculated as $Td = \ln 2/\mu$ (23). All the experiments were carried out in triplicates, and the values presented in the manuscript are mean of three replicates.

Results and discussion

Probiotic *Lactobacilli* are gaining enormous attention because of their established health effects such as anti-diarrheal, anti-pathogenic, anti-diabetic, anti-cholesterol and anti-cancer activities etc. (3, 4, 6-14, 24-26). *Aloe vera* juice is also considered extremely healthy because of high content of vitamins, minerals, amino acids (essential and non-essential), trace elements, antimicrobial agents (anthraquinones) and enzymes (27); and hence could serve as a good medium for cultivating probiotics. Therefore, in present investigation, effect of *Aloe vera* juice on growth and activities of probiotic *Lactobacilli* was studied *in-vitro* by incorporating different concentrations of juice in basic media, and incubation for upto 48 hrs. At 0 hr, pH of all the samples containing different concentrations of *Aloe vera* juice was set at 6.7. In case of *L. acidophilus,* it was observed that after 24 hr incubation, pH decreased being highest at 100% (6.2) and lowest at 5% (4.6) (Fig. 1). Most of the lactic acid bacteria are able to reduce the pH of the environment by production of acetic and lactic acid creating an environment adverse to the growth of gastrointestinal pathogens (5, 28). The decrease in pH also indicates the production of other organic acids (24). When compared to pH 4.9 at 0% concentration, pH at 5% (4.6) and 15% (5) was quite same, suggesting that *Aloe vera* had no limiting effect on the growth of *L. acidophilus* and the growth

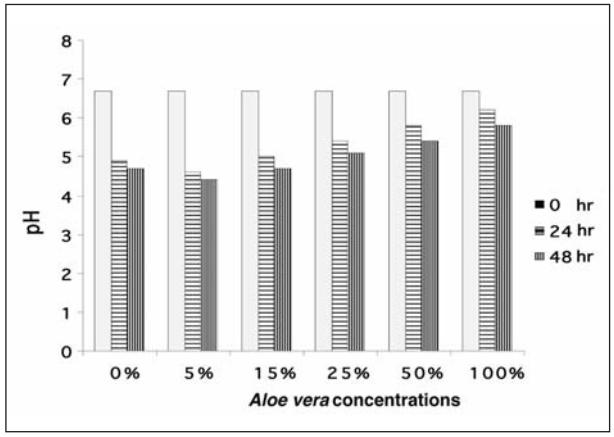

Figure 1. pH of *L. acidophilus* fermented MRS containing different concentrations of *Aloe vera* juice (values are mean of three replicates)

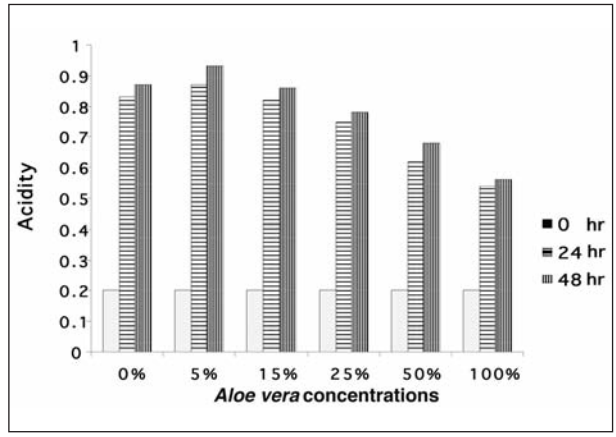

Figure 2. Acidity (%lactic acid) of *L. acidophilus* fermented MRS containing different concentrations of *Aloe vera* juice (values are mean of three replicates)

rate was normal at 5% and 15% concentrations. *Aloe vera* pulp because of its nutritional composition, mainly carbohydrates and vitamins, is a food plant that is expected to promote the growth of probiotic microorganisms (15). This could be the reason for the survival of probiotic bacteria at different concentrations of *Aloe vera* in present study. In case of 25%, 50% and 100% juice concentration, the pH was higher, i.e. 5.4, 5.8 and 6.2 respectively, as compared to pH 4.9 at 0% concentration indicating slow growth of *L. acidophilus*. From the above data, it could be analyzed that the growth of *L. acidophilus* was highest at 5% and lowest at 100%. The pH further decreased after 48 hrs incubation, being highest at 100% (5.8) followed by 50% (5.4), 25% (5.1), 15% (4.7) and lowest at 5% (4.4), when compared with 0% (4.7) concentration. Hypothesis thrown in previous studies (15, 18) suggest that the acemanane and use of glucomannan from *Aloe vera* could increase the growth and production of some antimicrobial metabolites (short-chain organic acids) that could inhibit the bacterial.

Acidity of the culture decreases as the pH increases. In present study, the results of acidity coincided with that of pH. At 0 hr, the acidity of *L. acidophilus* was observed to be 0.2 at all concentrations (0%, 5%, 15%, 25%, 50% and 100%) of *Aloe vera*. After 24 hrs, acidity was highest at 5% (0.87) followed by 15% (0.82), 25% (0.75), 50% (0.62) and lowest at 100% (0.54) (Fig. 2). When compared to 0% (0.83), the acidity at 5 and 15% was quite same. It could be due to the presence of saccharides like cellulose, glucose, mannose, L-rhamniose, aldopentose present in the *Aloe vera* juice (27). From this, it was again observed that the rate of growth of *L. acidophilus* was highest at 5% followed by 15% and slower at 25%, 50% and 100%. The acidity further increased after 48 hrs incubation being highest at 5% (0.93) followed by 15% (0.86), 25% (0.78), 50% (0.68) and lowest at 100% (0.56), when compared with 0% (0.87) concentration.

In case of *L. plantarum,* it was analyzed that after 24 hrs incubation, pH decreased from 6.7 being highest at 100% (5.7) followed by 50% (5.5), 25% (5.2), 15% (5) and lowest at 5% (4.6) (Fig. 3). When compared with 0% (4.9), the growth was found to be nearly equal at 5% and 15% concentrations but slower at 25%, 50% and 100% concentrations. The pH further decreased after 48 hrs incubation, being highest at 100% (5.5) followed by 50% (5.4), 25% (4.8), 15% (4.5) and lowest at 5% (4.3). When compared with 0% (4.6), the growth was found to be nearly same at 5% and 15% concentrations and slower at 25, 50 and 100% concentrations. The results for acidity also followed the same trend as that for pH. At 0 hr, the acidity of *L. plantarum* was 0.24 at all concentrations (0%, 5%, 15%, 25%, 50% and 100%) of *Aloe vera*. After 24 hrs incubation, acidity was highest at 5% (0.87) followed by 15% (0.82), 25% (0.70), 50% (0.68) and lowest at 100% (0.50) (Fig. 4). From this it was analyzed

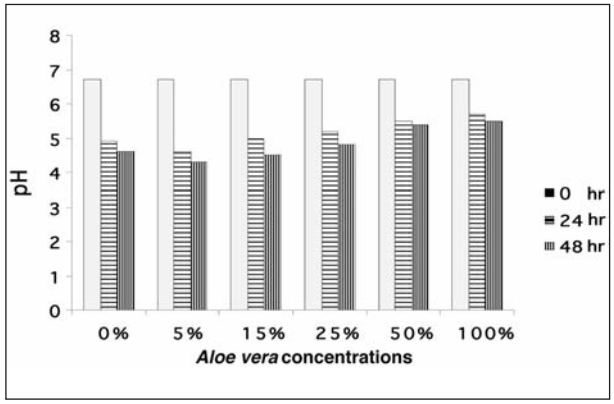

Figure 3. pH of *L. plantarum* fermented MRS containing different concentrations of *Aloe vera* juice (values are mean of three replicates)

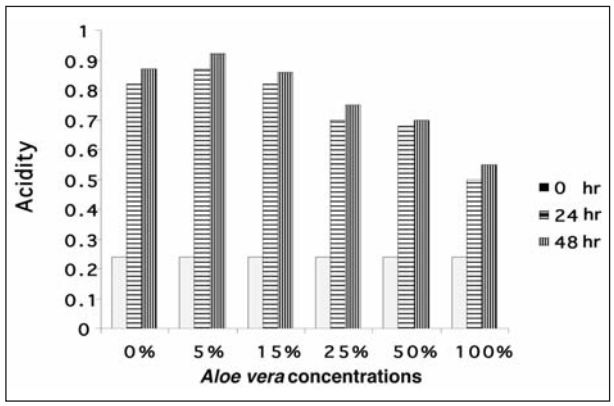

Figure 4. Acidity (%lactic acid) of *L. plantarum* fermented MRS containing different concentrations of *Aloe vera* juice (values are mean of three replicates)

that the rate of growth of *L. plantarum* promoted was normal at 5% and 15%; but slower at 25%, 50% and 100%. In case of *L. casei* also, a same trend of pH and acidity was observed as that observed in case of *L. acidophilus* and *L. plantarum* (data not shown here).

During the growth kinetics for 12 hrs for *L. acidophilus, L. casei* and *L. plantarum* using medium with different concentrations of *Aloe vera* juice, it was observed that in case of *L. acidophilus* at different concentrations of *Aloe vera* (0%, 5%, 15%, 25%, 50% and 100%) , the generation time (in minutes) was 53 (0%), 48 (5%), 60 (15%), 92 (25%), 208 (50%), 217 (100%) supporting the results obtained for pH and acidity (Table 1). In case of *L. plantarum,* it was found to be 62 (0%), 59 (5%), 71 (15%), 103 (25%), 203 (50%), 213 (100%); and for *L. casei,* it was found to be 58 (0%), 61 (5%), 81 (15%), 132 (25%), 196 (50%), 210 (100%). The results again supported the observation that the viability of *L. acidophilus, L. plantarum* and *L. casei* was promoted at 5% *Aloe vera* concentration but started reducing with increased concentration. In case of *L. casei,* growth rate was highest at 0% but a slight reduction was observed at 5%. The generation time was observed to be reduced further with increased concentration.

In food industry also, *Aloe vera* juice has been used for the preparation of soft drinks with nutritional qualities and tonics containing amino acids and minerals. *Aloe* is typically known for easing sunburns, treating dry skin conditions, and healing wounds. However, *Aloe vera* is now being explored as a treatment for gastrointestinal disorders. *Aloe vera* can possibly be used as a prebiotic (29) because of its nutritional composition, particularly acemanane, glucomannan, mannose polymers (accemannose), vitamin A, vitamin B1, B6 and vitamin C etc. Additionally, *Aloe vera* has laxative effects that are caused by aloin, which is found in the sap of the *Aloe* vera plant. Aloin stimulates colon contractions and decreases water absorption in the intestines, which induces and softens stools, respectively, helping in alleviating constipation. *Aloe* also has anti-inflammatory and analgesic effects,

Table 1. Generation time of *L. acidophilus, L. plantarum* and *L. casei* grown at different concentrations of *Aloe vera* juice (values are mean of triplicates)

Isolates	Generation time (min.) *Aloe vera* concentration (%v/v)					
	0	5	15	25	50	100
L. acidophilus	53	48	60	92	208	217
L. plantarum	62	59	71	103	203	213
L. casei	58	61	81	132	196	210

which may help lessen other digestive symptoms. Thus, combining *Aloe vera* and probiotics could form a potential synbiotic (29). It has been hypothesized that synbiotics may have additive or synergistic effects on digestive health, meaning there would be more benefits from using both components together than from using either component alone. Therefore, using *Aloe vera* with probiotics may prove to be better for the digestive tract than using either by themselves.

Conclusion

The present investigation was executed with an innovative concept of merging both probiotics and *Aloe vera* for a prospective combinational therapy for anti-cholesterol and anti-diabetic effects. From the results obtained, it could be clearly advocated that *Aloe vera* could promote the growth of probiotic *Lactobacilli* at particular concentrations, and hence, could be used as a prebiotic for preparation of synbiotic therapeutic products. However, more *in-vitro* as well as *in-vivo* studies such as strain specific effect, effect of purified components etc. followed by animal trials are pre-requisites before authenticating and endorsing this kind of combinational therapy and their health claims.

References

1. Holzapfel W. Appropriate starter culture technologies for small-scale fermentation in developing countries. *Int J Food Microbiol* 2002; 75: 197-213.
2. Fitzgerald RJ, Murray BA. Bioactive peptides and lactic fermentation. *Int J Dairy Tech* 2006; 59: 118-25.
3. Nagpal R, Yadav H, Puniya AK, Singh K, Jain S, Marotta F. Potential of probiotics and prebiotics for synbiotic functional dairy foods. *International Journal of Probiotics and Prebiotics* 2007; 2: 75-84.
4. Nagpal R, Behare PV, Kumar M, et al. Milk, milk products and disease free health: an updated overview. *Critical Reviews in Food Science & Nutrition* 2012a; 52: 321-33.
5. Shah N. Functional foods from probiotics to prebiotics. *Food Tech*(2001; 55: 46-52.
6. Kumar M, Mohania D, Poddar D, et al. A probiotic fermented milk prepared by mixed culture combination reduces pathogen shedding and alleviates disease signs in rats challenged with pathogens. *International Journal of Probiotics and Prebiotics* 2009; 4(3): 211-8.
7. Kumar M, Kumar A, Nagpal R, et al. Cancer-preventing attributes of probiotics: an update. *International Journal of Food Science & Nutrition* 2010; 61 (5): 473-96.
8. Kumar M, Verma V, Nagpal R, et al. Effect of Probiotic Fermented Milk and Chlorophyllin on Gene Expressions and Genotoxicity during AFB1-induced Hepatocellular Carcinoma. *Gene* 2011; 490: 54-9.
9. Kumar M, Nagpal R, Kumar R, et al. Cholesterol lowering Probiotics as potential biotherapeutics for metabolic diseases. *Experimental Diabetes Research* 2012a; (doi: 10.1155/2012/902917) [Epub ahead of print].
10. Kumar M, Nagpal R, Verma V, et al. The Probiotic metabolites as epigenetic target to control colon cancer. *Nutrition Reviews* 2012c; (in-press).
11. Kumar M, Verma V, Nagpal R, et al. Anticarcinogenic effect of probiotic fermented milk and Chlorophyllin on aflatoxin-B1 induced liver carcinogenesis in rats. *British Journal of Nutrition* 2012b; 107: 1006-16.
12. Nagpal R, Kumar A, Arora S. *In-vitro* probiotic potential of lactobacilli from indigenous milk products. *International Journal of Probiotics and Prebiotics* 2010; 5(2): 103-10.
13. Nagpal R, Kumar A, Kumar M. Fortification and fermentation of fruit juices by probiotic lactobacilli. *Annals of Microbiology* 2012b; (doi: 10.1007/s13213-011-0412-5) [Epub ahead of print].
14. Nagpal R, Kumar A, Kumar M, Behare PV, Jain S, Yadav H. Probiotics, their health benefits and applications for developing healthier foods: a review. FEMS Microbiology Letters 2012c; (doi: 10.1111j.15746968.2012.02593.x) [Epub ahead of print].
15. Contreras-Pinzon M, Dominguez-Espinosa R, Gonzalez-Burgos A. Biotransformation process lactic *Aloe vera* juice. *Sci Mag Tech Ed* 2007; 22: 35-42.
16. Arguelles M, Borovoy J, Toussaint G, Garcia-Aranda J. Use of probiotics in children. *Newslett. Med. Hosp. infantile de Mexico* 2000; 57: 454-63.
17. Paez A, Gebre G, Gonzalez M, Tschaplinski T. Growth, soluble carbohydrates ana aloin concentration of *Aloe vera* plants exposed to three irradiance levels. *Env Exp Bot* 2000; 44: 133-9.
18. Contreras-Pinzon M, Dominguez Espinosa R, Fred D. Production development and process design for the production of functional *Aloe vera* drink. Conference proceedings of the 2nd International Exhibition on bsp. Mexico: Achem. America, 2005.
19. Garcia-Hernandez J, Valdez-Cepeda R, Murillo-Arnador B, et al. Preliminary compositional nutrient diagnosis norms in *Aloe vera* grown on calcareous soil in an arid environment. *Env Exp Bot* 2006; *58*: 244-52.
20. Repinski K. Natural remedies that really work. US: Redbook magazine, Hearst communications 1999; 31: 193-7.
21. de Man JD, Rogosa M, Sharpe ME. A medium for the cultivation of *Lactobacilli*. *J Appl Bact* 1960; 23: 130-5.
22. Dubios M, Gilles KA, Hamilton JK, Roberts DA, Smith F. Colorimetric methods for determination of sugars and related substances. *Anal Chem* 1956; 28: 350-6.
23. Shin HS, Lee JH, Pestka JJ, Ustunol Z. Growth and viabil-

ity of commercial *Bifidobacterium* spp. in skim milk containing oligasaccharides and inulin. *Journal of Food Science* 2000; 65: 884-7.
24. Roberfroid B. Prebiotics and Probiotics: are they functional foods? *Am J Clin Nutr* 2000; 71: 1682-7.
25. Ouwehand AC, Isolauri E, Salminen S. The role of the intestinal microflora for the development of the immune system in early childhood. *Eur J Nutr* 2002; 41(1): 132-7.
26. Salminen S, Benno Y, de Vos W. Intestinal colonisation, microbiota and future probiotics? *Asia Pac J Clin Nutr* 2006; 15(4): 558-62.
27. Vogler BK, Ernst E. *Aloe vera*: a systematic view of its clinical effectiveness. *Br J Gen Pract* 1999; 49(447): 823-8.
28. Stanton C, Ross R, Fitzgerald G, Sinderen D. Fermented functional foods based on probiotics and their biogenic metabolites. *Curr Opinion Biotechnol* 2005; 16: 198-203.
29. Nagpal R, Kaur A. Synbiotic effect of various prebiotics on *in-vitro* activities of probiotic lactobacilli. *Ecology of Food & Nutrition* 2011; 50: 63-68.

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