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Assessment of increase in probiotic potential of *Lactobacillus* strains fortified with *Aloe vera*

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ABSTRACT

Lactobacilli strains are used in probiotic food items because they are associated with health benefits. *Aloe vera* is fortified with many health benefits, to name a few antitumor, antidiabetic, healing wounds and burns, treatment of ulcers and antimicrobial properties. In this study, four *Lactobacillus* strains *L.casei*, *L.acidophilus*, *L.delbrucekie*, *B.bifidium* and their consortia C1, C2, C3, C4, C5, C6, C7, and C8 were fortified with *Aloe vera*. Tolerance to different temperature 25 ^{0}C, 37^0 C and 65^0 C were assessed in presence and absence of *Aloe vera* and it was observed that survival rate increased in presence of *Aloe vera.* Tolerance to different salt level 2%, 4% and 6.5% were also assessed in presence and absence of *Aloe vera* and it was observed that some bacterial strains survived at 2% salt concentration, all at 4% salt concentration and few at 6.5% salt concentration and in presence of *Aloe vera* tolerance percentage was increased. Tolerance of lactic acid bacteria for different pH levels 4, 6.5, 8 and 9 in presence and absence of *Aloe vera* were assessed, it was observed that survival rate of cultures were good at pH 4, moderate at pH 6.5, less at pH 8 and very less at pH 9 in presence of *Aloe vera* whereas in absence survival rate decreased. This study helps in assessment of increase in number of probiotic bacteria in presence of Aloe *vera*.

Keywords: - Antitumor, Antidiabetic, probiotic.

INTRODUCTION

Lactic acid bacteria are gram-positive, nonmotile and non-sporulating. They produce lactic acid as a major product of fermentative metabolism. In dairy industries lactic acid bacteria play a very important role. Lactobacillus are used in making starter culture for dairy products. Lactobacillus are probiotic bacteria “live microorgani-sms” have some specific characteristics like enhancement of immune function, reduction of cholesterol, reduction of risk of diarrhoea, reduction of risk of eczema (Marteau *et al*., 2001, Calder & Kew, 2002, Wright *et al*., 2002, El- Shenawy *et al*., 2012 and Mishra *et al*., 2008) due to which it is beneficial for number of industrial applications.

De-Vries (2006) reported that in order to be beneficial lactobacillus must be able to survive and grow in in vivo conditions in GI tract i.e. organism must be able to tolerate body temperature, low pH, and salt for survival in gut. *Aloe vera* is one of the important plants that has been traditionally used for its medicinal, herbal and therapeutic properties. *Aloe vera* have numerous biological activities, like antimicrobial and antioxidant activities, the gel is susceptible to oxidation, due to which fermentation occurs. Kim and co–workers (2014) developed preliminary investigation of *Aloe vera* pulp fermentation and found the presence of lactic acid bacteria. On the basis of this finding, it can be said that number of these bacteria can be increased by using *Aloe vera* as a fermenting agent. Chemical and therapeutic characteristics of *Aloe vera* lead to development of Lactic acid bacteria as a starter for fermentation.

The objective of this study was to assess the change in number of probiotic bacteria in presence of *Aloe vera*. The different parameters used for this assessment were temperature, pH and salt concentration.

MATERIAL AND METHODS

Cultures taken - Four strains of Lactobacillus – *Lactobacillus casei, Lactobacillus delbrucekie, Bifidobacterium bifidium, Lactobacillus acidophilus* were obtained from national dairy research institute Karnal, India. Using these four bacterial strains, eight constoria were prepared – C1, C2, C3, C4, C5, C6, C7 and C8.C1(*L.acidophilus + L.casei*), C2(*L.acidophilus* + *L.delbrucekie*), C3(*L.casei + L.delbrucekie*), C4(*B.bifidium* + *L.acidophilus*), C5(*L.aciophilus* + *L.casei* + *L.delbrucekie*), C6(L.acidophilus + L.casei + B.bifidium), C7(*L.casei* + *L.delbrucekie* + *B.bifidium*), C8(*L.acidophilus* + *L.casei* + *L.delbrucekie* + *B.bifidium*).

Media for enumeration - MRS broth was used for revival of lyophilized cultures.

Preparation of Aloe *vera* pulp - *Aloe vera* pulp was extracted from leaf and was macerated with water.

Salt tolerance test - To each test tube with 5ml of MRS broth, 2 drops of bromocresol puple indicator added and 50ul of each culture consortia were added. Each tube was set with different salt concentrations 2%, 4%, and 6.5%. In 12 tubes *Aloe vera* preparation was added and other 12 were taken without *Aloe vera*. Each 24 sets of test tubes were kept for incubation for 7 days.

Temperature tolerance test - To each test tube with 5ml of MRS broth, 2 drops of bromocresol purple indicator and 50ul of each culture and consortia were added. In 12 tubes 500ul of *Aloe vera* preparation was added and other 12 were taken without *Aloe vera*. Each 24 sets of test tubes were kept for incubation for 7 days in different temperature conditions as 25^0C, 37^0C and 65^0C.

pH tolerance test - To each test tube with 5ml of MRS broth, 2 drops of bromocresol purple indicator and 50ul of each culture and consortia were added. Different pH range 4, 6.5, 8 and 9 was set for each tube. In 12 tubes 500ul *Aloe vera* preparation were added and other 12 were taken without *Aloe vera*. Each 24 sets of test tubes were kept for incubation for 7 days.

RESULT AND DISCUSSION

In varying salt concentration

In present study the lactic acid bacterial cultures were able to tolerate following NaCl concentration – 2%, 4% and 6.5%. as shown in Table 1. NaCl is present in GI tract and act as an inhibitory substance, it can inhibit growth of certain types of bacteria. If the lactic acid bacteria were sensitive to NaCl then it would not be able to show its activity in presence of NaCl so it was essential to test the NaCl tolerance of lactic acid bacterial culture. In given table result shows that toleration towards NaCl increases in presence of *Aloe vera*. The present experimental result was similar to the work done by Adebayo-tayo and Onilude (2008). After 7 days incubation appearance of yellow color shows positive result means growth of lactic acid bacteria.

In varying temperature conditions

In present study, Lactic acid bacteria and their consortia were able to survive at temperature 25^0C and 37^0C but not at 65^0C. In presence of *Aloe vera*, survival rate of bacteria get increased. The bacterial growth was drastically affected by temperature. The reason for selecting this parameter was to detect whether the cultures were able to grow within range of normal body temperature or not. As if the cultures were not able to survive within the selected

Table 1 : In varying salt concentration

S.no	Name of Bacteria	2% NaCl		4% NaCl		6.5 % NaCl	
		Aloe +nt)	(Aloe –nt)	Aloe +nt)	(Aloe –nt)	Aloe +nt)	(Aloe –nt)
1.	*L.acidophilus*	+	+	+	+	+	-
2.	*L.casei*	+	+	+	+	+	-
3.	*L.delbrucekie*	+	+	+	-	+	-
4.	*B.bifidium*	+	+	+	-	-	-
5.	C1	+	+	-	-	-	-
6.	C2	+	+	-	-	-	-
7.	C3	+	+	+	-	-	-
8.	C4	+	+	+	-	+	-
9.	C5	+	+	+	-	+	-
10.	C6	+	+	+	-	-	-
11.	C7	+	+	+	-	+	-
12.	C8	+	+	+	-	-	-

+ means growth or survival, - means absence or no growth

Table 2 : In varying temperature conditions

S.no	Name of Bacteria	Temperature = 25^0C		Temperature = 37^0C		Temperature = 65^0C	
		Aloe +nt)	(Aloe –nt)	Aloe +nt)	(Aloe –nt)	Aloe +nt)	(Aloe –nt)
1.	*L.acidophilus*	+	+	+	+	+	-
2.	*L.casei*	+	-	+	+	-	-
3.	*L.delbrucekie*	+	+	+	+	+	-
4.	*B.bifidium*	-	-	+	+	-	-
5.	C1	+	-	+	+	-	-
6.	C2	-	-	+	+	-	-
7.	C3	+	-	+	+	-	-
8.	C4	+	-	+	+	-	-
9.	C5	-	-	+	+	+	-
10.	C6	-	-	+	+	-	-
11.	C7	+	-	+	+	-	-
12.	C8	+	-	+	+	-	-

+ means growth or survival, - means absence or no growth

Table 3 : In varying pH conditions

S.no	Name of Bacteria	pH=4		pH = 6.5		pH= 8		pH = 9	
		Aloe +nt)	(Aloe –nt)	Aloe +nt)	(Aloe –nt)	Aloe +nt)	(Aloe –nt)	Aloe +nt)	(Aloe –nt)
1.	*L.acidophilus*	+	+	+	+	+	+	+	+
2.	*L.casei*	+	+	+	+	+	+	-	-
3.	*L.delbrucekie*	+	+	+	+	+	-	+	-
4.	*B.bifidium*	+	+	+	+	+	-	-	-
5.	C1	+	+	+	-	+	-	-	-
6.	C2	+	+	+	-	-	+	-	-
7.	C3	+	+	+	-	-	-	-	-
8.	C4	+	+	+	-	+	-	-	-
9.	C5	+	+	+	-	+	-	+	-
10.	C6	+	+	+	+	+	+	-	-
11.	C7	+	+	+	-	+	-	-	-
12.	C8	+	+	+	+	+	-	-	-

+ means growth or survival, - means absence or no growth

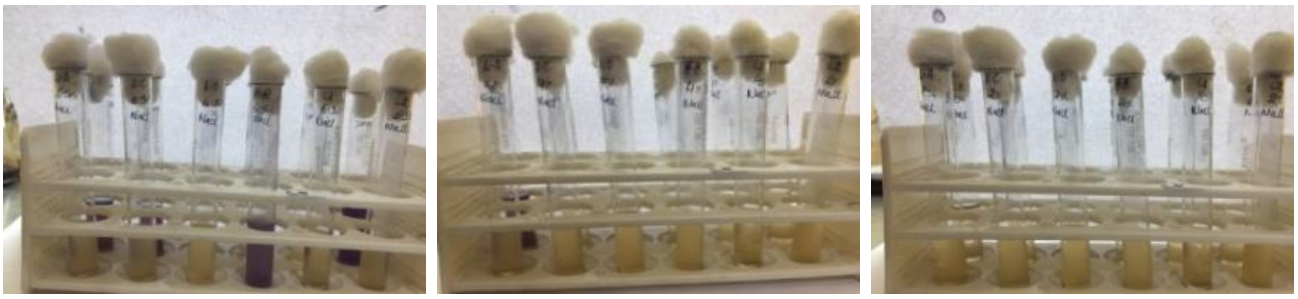

Fig 1:- Salt tolerance (Yellow color shows positive result , purple color shows negative result)

Fig 2:- Temperature tolerance (Yellow color shows positive result , purple color shows negative result)

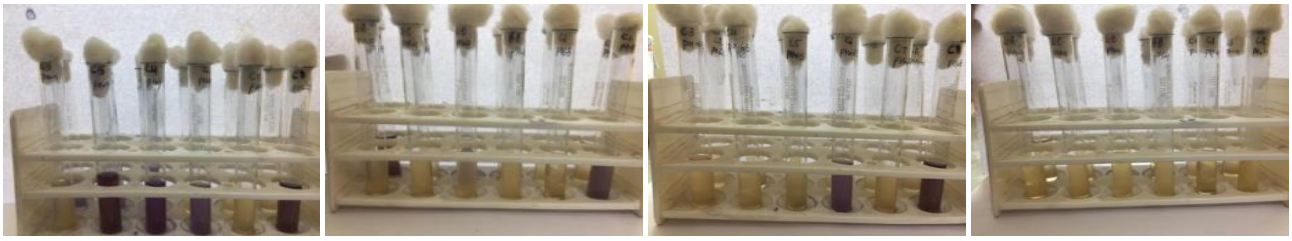

Fig 3:- pH tolerance (Yellow color shows positive result , purple color shows negative result)

temperature range then they would not have been able to survive in the human gut, which is an essential factor of probiotics to show their effectiveness. The results obtained were shown that in presence of *Aloe vera* all cultures show growth at 25^{0}C, 37^{0}C and 65^{0}C temperature range but in absence of *Aloe vera* , growth was obtained only at 25^{0}C and 37^{0}C. After 7 days incubation appearance of yellow color shows positive result means growth of lactic acid bacteria.

In varying pH conditions

The first host factor that may affect commercial probiotics are the high acidity in the proximal intestine (Bakari, D *et al*., 2011). Therefore, being tolerant to acidic condition is an important criterion to be considered during the selection of potential probiotic to assure their viability and functionality. In the present study *Lactobacillus* culture tolerate pH range from 4 to 9, good growth observed at pH 4, moderate at pH 6.5 and get decreased at pH 8 and 9. But in presence of *Aloe vera*, toleration towards different pH range increased. After 7 days incubation appearance of yellow color shows positive result means growth of lactic acid bacteria.

CONCLUSION

By present study we can say that combination of prebiotics and probiotic microorganisms improves quality in the formulation of food. In present study, *Aloe vera* was used as a substrate for lactic acid bacteria fermentation, Aloe *vera* was used as a prebiotic source, its addition effects the growth of probiotic bacteria in positive manner. Increased number of probiotic bacteria in digestive tract provide large health benefits. More research needed in order to confirm whether a synbiotic product using *Aloe vera* as a substrate for lactic acid bacteria fermentation might have a greater effect on consumer's health than individual prebiotic and probiotic products.

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