



FERMENTATION OF AMLA JUICE BY PROBIOTIC LACTIC ACID BACTERIA

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Abstract: Lactobacillus in amla drinks with the aim of formulation of pro biotic drink and to analyse the nutritive value and self-life of the product. Lactobacillus was capable of growing in 15% amla drink at natural pH 30°C. Microbial population pH and organic acid metabolism were measured during the fermentation period and the viability of all strains was also determined during the storage time at 4°C within 4 weeks. The result indicated that Lactobacillus increased the pH at the initial stage. Amla drink could be stored at refrigerated condition for 30 days. Amla juice proved to be a suitable media for production of a fermented pro biotic drink.

Keywords: Lactobacillus, amla, fermentation, probiotic, Microbial activity.

I. INTRODUCTION

Amla extract are used in various food beverage preparations, such as jam, yoghurts and dietary supplements. Amla is highly nutritious and is one of the richest sources of vitamin C and minerals. Amla fruit is widely used as medicine as alone or in combinations with other plants to treat common cold and fever or diuretic, laxative, liver tonic, refrigerant, stomachic, restorative, hair tonic to prevent ulcer and dyspepsia. Modern scientist have studied amla unique nutrient blend proved to have positive influences on everything from metabolic syndrome and blood flow to anti-aging processes. Amla is known to have considerable health promoting properties with antimicrobial, anticancer, antioxidant effects. The fresh juice contains 85% of water and considerable amount of total solvable solids, total sugars and proteins has been reported to be a rich source of antioxidants. These antioxidants are more potent on a molar basis than many other antioxidants including vitamin c. The potential for Amla extract as a food ingredient is increasing substantially, owing to the growing global nutraceuticals and functional food market. Increase in the demand of health drinks has given a role for the development of various value added beverages. Present study was carried to examine the growth of Lactobacillus in Amla drink. According to findings to be a probiotic product it should contain a minimum number of probiotic population ranged.

Food with required population of beneficial microorganisms in human health is known as probiotic food. Probiotic is a relatively new word meaning for life and it's generally used to name bacteria associated with beneficial effect for humans. Probiotic beverage by using whey and amla juice and used Lactobacillus as the probiotic microorganisms. The different combinations were prepared by using amla juice with stable 10% sugar. probiotic are defined as live microbial feed such as Lactobacillus which are supplement by food that supplementation of biotitic to food provides several health benefits such as immune system, lower risk of colon cancer. Probiotics also can fight against pathogen, probiotics are live Bacteria and yeasts that are good especially for your digestive system. Probiotics are increasingly used as food supplement, maintenance of a healthy gut micro flora may provide production against gastro intestinal disorder including infection.

II. MATERIAS AND METHODS

2.1 Sample collection and isolation

The lactic acid bacteria were mainly found in milk, curd, yoghurt, etc., So for the isolation of lactic acid bacteria here the curd was used as the source for isolation. Curd sample was collected from store, sample were transferred into the sterile screw capped bottle. The organism was isolated in MRS agar medium.

2.2 Identification of the organism

Gram staining – Smear was prepared on a clean glass slide and it was flooded with crystal violet for 1 minute. The stain was poured off and slide was flooded with gram's iodine for 1 minute and it was poured. It was decolorized by washing the slide briefly with Gram's decolorize. Slide was washed thoroughly with water to remove the acetone. Slide was then flooded with safranin counterstain for 1 minute and was washed with water and blot dried.

2.3 Biochemical characterization

The presumptive colonies of the selected organisms were then subjected to gram staining and a series of biochemical tests such as Indole, Methyl Red, Voges - Proskauer, Citrate and Carbohydrate fermentation test.

2.4 Fermentation of amla juice

Experiment was carried out as batch Fermentation (in flasks), pasteurized the amla juice for 5 min at 80°C and cooled. A 24-h cultivation of lactic acid bacteria in the MRS broth was carried out at 30°C. For obtaining an initial cell density of 107 c.f.u/ml in the final juice, 15 ml of the cultivated MRS broth was centrifuged at 4,000g for 10 min and the biomass was introduced into the juice (150 ml). The juice was then incubated at 30°C for 72 h and sampling was taken every 24 h for microbiological analysis. Viable cell (c.f.u/ml) were determined by the standard plate count method using MRS medium. After 72 h of fermentation, the fermented samples were stored at 4°C for 4 weeks and the viability of probiotic bacteria was measured during storage time. The microbial population was measured at weekly intervals and expressed as c.f.u/ml.

2.5 Estimation of microbial count of fermented amla juice

The microbial count was determined by the standard plate count method using MRS Medium, after 72 hours of Fermentation the fermented samples were stored at 4°C for 4 weeks and the viability of probiotic bacteria was measured during storage time, the microbial population was measured at weekly intervals.

III. RESULT AND DISCUSSION

3.1 Isolation of curd sample

Lactobacillus was isolated from curd sample were plating and the growth of Lactobacillus was confirmed by the nutrient agar plate method (white color colonies).

3.2 Biochemical characterization

Biochemical characteristics of the bacterial strain were determined by using various biochemical tests like catalase , carbohydrate test, MR-VP test, indole test, urease test, TSI test etc., their results were recorded in the following table.



Figure 1: Picture showing the IMViC test result

S.No	Biochemical test	results
1	Indole test	Negative
2	Methyl red test	positive
3	Voges-proskauer test	Negative
4	Citrate test	Negative

Table 1: IMViC results shown in table

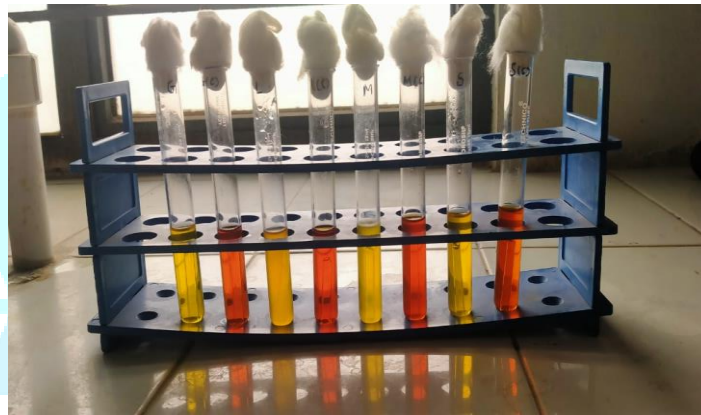


Figure 2: Picture showing results of carbohydrate fermentation test

S. No.	Carbohydrate test	result
1	Glucose	Positive
2	Lactose	Positive
3	Sucrose	Positive
4	Mannitol	Positive

Table 2: Table showing results of carbohydrate fermentation test

3.3 Fermentation of amla juice:

Viable cell if organisms during fermentation process indicates capability of growth and survival of organism in the juice result shows the organism were capable of growing in amla juice in natural pH level.

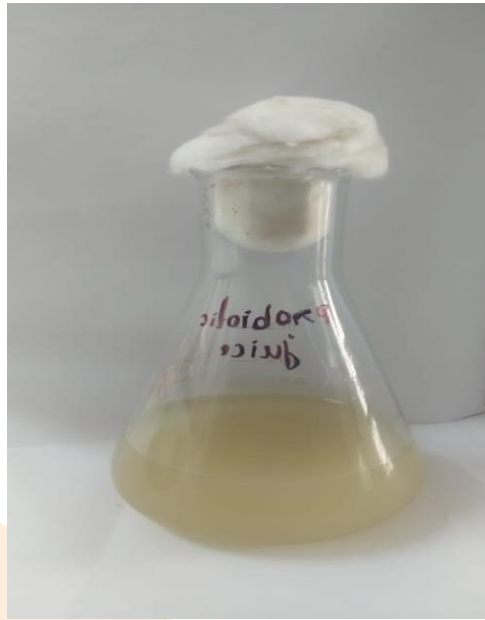


Figure 3: Picture showing result of fermentation of probiotic juice

3.4 Estimation of microbial count of fermented amla juice

Fermentation and the population was increased at 72 hours of Fermentation. In 1st day it was noticed there was less growth; 2nd day there was an increased growth and 3rd day the growth was too numerous to count. The weekly analysis was constant and stable in growth which was too numerous to count.

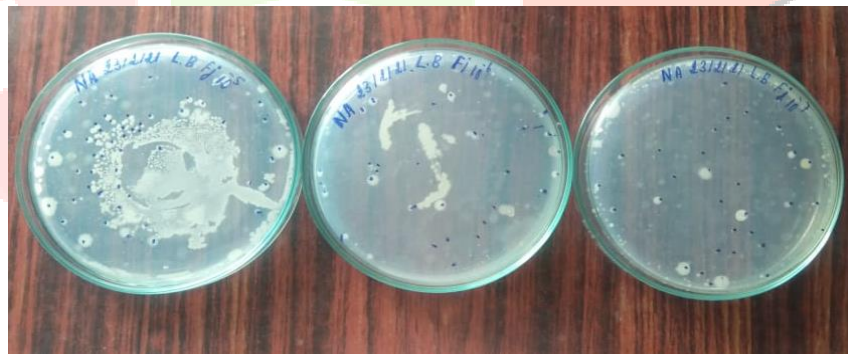


Figure 4: 1st day colony growth



Figure 5: 2nd day colony growth

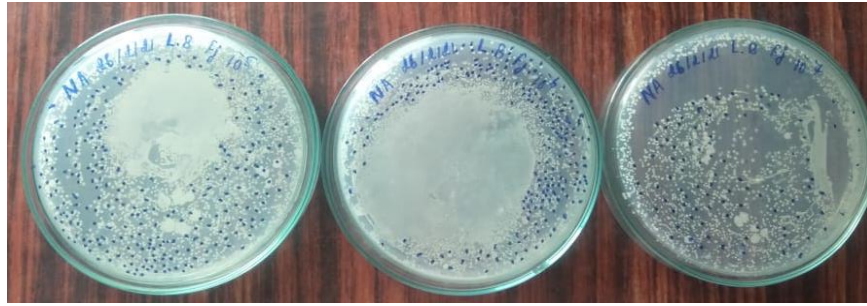


Figure 6: 3rd day colony growth



Figure 7: 1st week microbial analysis

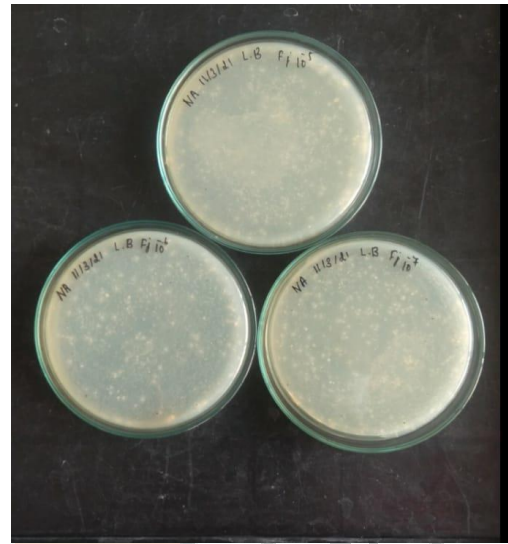


Figure 8: 2nd week microbial analysis

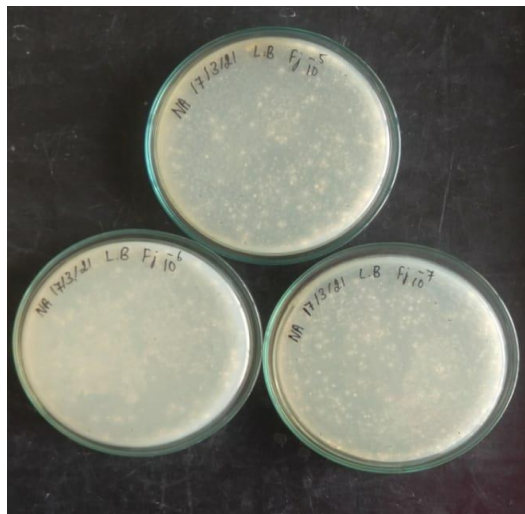


Figure 9: 3rd day microbial analysis

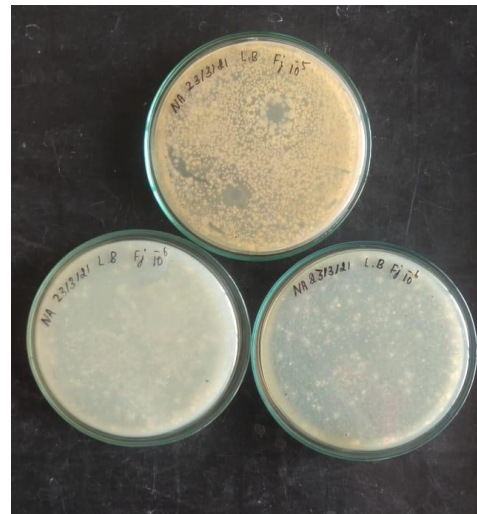


Figure 10: 4th day microbial analysis

IV. SUMMARY AND CONCLUSION

In this probiotic acid bacteria were studied to investigate suitability of using Amla juice as a probiotic drink. The specific health effects of selected probiotic strains are becoming increasingly accepted thanks to an expanding volume of documentation from clinical studies. During 72 hours fermentation and viability of selected strains has been also studied. In particular cultures, shortening of diarrhea and treatment of allergies are now well established. The different antioxidant properties of the different juices were compared like vitamin C concentration antioxidant activity, Tannin content with their content was found maximum in case of the Pat Anjali Amla juice but if we compare the different extraction methods the mechanical extracted juice had the more antioxidant activity, vitamin C. The Amla juices have showed antimicrobial activity as the concentration of the juices increase the percentage activity also increase. The activity of the juices was checked against the *Lactobacillus*. All the strains gave the positive results. It may be concluded that the Amla juice can be taken in place of the antibiotics as it is safe for the health and having not any side effects. There after growth rate, substrate metabolism and viability to selected strains has been also studied. Citric acid and sugar metabolism by Lactic acid bacteria during this proved that *Lactobacillus* were more capable to consume higher amount of substrate. Bacteria consumed glucose and fructose as the carbon energy source where glucose was the preference.

V. ACKNOWLEDGEMENT

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