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Study on Fermentation of Jackfruit (*Artocarpus heterophyllus* L.) Juice by beneficial Lactic Acid Bacteria.

Dushyantha DK*, Raghavendrakumar M and Suvarna VC

Dept. of Agril. Microbiology, UAS, GKVK, Bangalore -560 065.

ABSTRACT

Jackfruit (Artocarpus heterophyllus L.) is one of the important underutilized fruits which are available in plenty during the season. It is a good source of sugars, proteins and also flavouring compounds. So th e production of fermentable product like beverage from the jackfruit is the most suitable processing method to exploit these characters. Lactic acid bacteria were isolated from Jackfruit Phyllosphere; perianth lobes and juice. They were characterized and compared with standard lactic acid bacterial strain Lactobacillus acidophilus for different characteristics. Among the isolated strains, lactic acid bacterial strain JFL_1 was found superior to other isolates in fermenting the jackfruit juice. Hence, this strain was further screened for beverage making. Casein and soy protein were supplemented as a nitrogen source for enhancing the fermentation efficiency of strains. Beverage produced by supplementation of nitrogen source was found superior to wine produced without supplementation of nitrogen source.

Key words: Lactic acid bacteria, Fermentation, Nitrogen source, beverage.

Corresponding author
Email: dush_hsn@rediffmail.com

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INTRODUCTION

Jackfruit (*Artocarpus heterophyllus*. L) is one of the underutilized fruits belonging to the family Moraceae and is the largest edible fruit in the plant kingdom and occupies the top most rank with respect to quantity of food produced per unit area. The tree is valued for its money earning capacity and there are instances in which a single tree is reported to have generated an income of thousand rupees in one season alone. Hence, its cultivation is gaining popularity in the farming community. Owing to its multipurpose uses right from its roots to fruits, it is much credited tree in the tropical world. Jackfruit is native to India and is mainly found in tropical Asian countries like India, Srilanka, Bangladesh, Indonesia, Malaysia and Philippines. Jackfruit is quite popular in Eastern and Southern India and is cultivated widely in Kerala, Karnataka, Andhra Pradesh, Tamil nadu, West Bengal, Maharashtra, Assam, Andaman and Nicobar islands. The total area under jackfruit in India is approximately 1.02 lakh hectares. It is cultivated in an area of about 0.11 lakh hectares mostly in Southern Plains and Western Ghats of Karnataka producing about 2.6 lakh tones of fruits per annum [5].

MATERIAL AND METHODS

The experiments were conducted in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bangalore. Lactic acid bacteria were isolated from perianth lobes, phyllosphere and juice of jackfruit by standard plate count technique using Mann Rogosa and Sharpe's (MRS) agar medium [1]. The isolated lactic acid bacteria were further purified. Lactobacillus acidophilus was used as reference culture for characterization. These isolates were observed under the microscope for cell shape Gram reaction and production. Biochemical tests like Catalase activity, utilization of glucose, gelatin hydrolysis, fermentation of carbohydrates, Dextran production and growth on neutral red chalk agar medium were conducted for characterization of these isolates. [2].These isolates were screened for the production of wine from jackfruit. A loop full of inoculum of lactic acid bacterial culture was transferred to test tubes containing 20 ml of jackfruit juice. The tubes were kept overnight at 25 ^{0} C for growth. This lactic acid bacterial culture was then added to 300 ml jackfruit juice in a 500 ml flask. This culture was used at 5 per cent (v/v) for fermentation.

The fruits were procured from Regional Research Station, GKVK. Optimum matured jack fruits with golden yellow color bulbs were used for the study. Fruits were cut to get the bulbs with minimum damage. Bulbs with water (2:1) were blended in a mixer to get fine slurry. This was filtered using muslin cloth manually. The clear juice was collected and used for further experiments.

Preparation of wine

The extracted juice was ameliorated to obtain 18^{0} Brix by adding 200 g cane sugar per litre of juice. The acidity was maintained at 0.5 per cent by adding citric acid. Wild yeasts

present in the juice were suppressed by adding Potassium metabisulphite @ 200 ppm. The juice was inoculated with lactic acid bacterial starter cultures at the rate of 5 per cent (v/v) for fermentation. Fermentation was carried out with occasional mixing of the juice and the fermentation was stopped by fall in T.S.S (^{0}Brix).The wine was filtered using cheese cloth and filled in bottles for completion of slow fermentation. Finally, wines were clarified by adding 0.4 per cent Bentonite clay. Further, clear wine was siphoned into clean pasteurized bottles and tightly corked. These samples were used for further analysis and evaluation.

There were 8 treatments consisting reference strain and lactic acid bacterial isolates along with soy protein and casein and details of the treatment are as follows:

C_1: L. acidophilus
C_2: L. acidophilus + 1 % casein
C_3: L. acidophilus +1 % soy protein
C_4: L. acidophilus + 1 % casein +1 % soy protein
D_1: jack fruit lactic acid bacteria$_1$
D_2: jack fruit lactic acid bacteria$_1$ + 1 % casein
D_3: jack fruit lactic acid bacteria$_1$ + 1 % soy protein
D_4: jack fruit lactic acid bacteria$_1$ + 1 % casein +1 % soy protein

Flow diagram illustrating wine preparation from jack fruit

Jackfruit
↓
Deseeded bulb collection
↓
Homogenization
↓
Blending and mixing with water
↓
Filtration
↓
Brix adjustment (18^0 Brix)
↓
Addition of potassium metabisulphite at 200 ppm
↓

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Addition of starter culture (5% v/v)

↓

Fermentation for 12 days

↓

Filtration

↓

Racking (3- 4 times)

↓

Addition of Bentonite clay (400 mg/l)

↓

Filtration

↓

Pasteurization at 62^0 C for 30 min.

↓

Stored for maturation

Biochemical analysis

To assess the chemical composition of wine, the samples were analyzed for pH, titrable acidity, total sugars, reducing sugars, alcohol and protein content. The total soluble solids of the wine were determined with the help of "ERMA" hand refractometer having a range of 0 to 32^0 Brix at room temperature. The pH of the wine was measured using digital pH meter of analog model (Corin research USA). Titrable acidity (%) was estimated by following the method given by [3]. Nitrogen estimation was carried out by Micro-Kjeldahl method [4]. Reducing sugars were estimated by following the method as given by Shaffer-Somogyi micro method [4]. The data obtained from the investigation was subjected to analysis of variance by completely randomized design using AGREES-C. The treatment differences were separated at 1 per cent significance level using Duncan's multiple range tests using M-STAT.

RESULTS AND DISCUSSION

The colonies that appeared after 48 hrs on Mann, Rogosa and Sharpe's (MRS) agar medium were tiny and pin point shaped. The results in the table 1 revealed that no colonies appeared after 24 hr on sucrose agar medium. So, all the isolates were negative for Dextran production. All the test isolates along with the standard (Lactobacillus acidophilus) were stained using malachite green for spore production. The results in the table 1 revealed all were non

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spore formers. The results of carbohydrate fermentation by the isolates are presented in the table 1. The results showed that standard (Lactobacillus acidophilus), JF_1, JFL_4 and JFL_5 changed the colour from red to yellow due to the production of acids but, JFL_2 and JFL_3 showed no change in colour. Collection of gas in Durham's tube was observed in tubes inoculated with the standard (Lactobacillus acidophilus) and isolates JFL_1 and JFL_3 and there was no gas collection in JFL_2 JFL_4 and JFL_5. these results are similar to the findings of [7]. The experimental results on pH are presented in table 2. There was significant difference between the treatments. The highest pH (3.30) was recorded in control. Among the strains, the highest pH (3.25) was recorded by Lactobacillus acidophilus compared to the other two strains.

Table 1: Morphological and biochemical characterization of lactic acid Bacterial isolates

Sl.No	Isolates	Shape	Gram's reaction	Catalase activity	Glucose utilization		Gelatin hydrolysis	Spore production	Dextran production
					A	G			
1	RLAB	Rods	+	-	+	+	-	-	-
2	JFL_1	Rods	+	-	+	+	-	-	-
3	JFL_2	Rods	+	-	-	-	-	-	-
4	JFL_3	Rods	+	-	-	+	-	-	-
5	JFL_4	Rods	+	-	+	-	-	-	-
6	JFL_5	Cocci	+	-	+	-	-	-	-

Note: RLAB: Reference lactic acid bacteria (*Lactobacillus acidophilus)*
JFL_1: Jackfruit lactic acid bacteria$_1$ JFL_2: Jackfruit lactic acid bacteria$_2$
JFL_3: Jackfruit lactic acid bacteria$_3$ JFL_4: Jackfruit lactic acid bacteria$_4$
JFL_5: Jackfruit lactic acid bacteria$_5$
A: Acid production
G: Gas production

With respect to TSS, significant differences were noticed between the strains. There were highly significant differences between the treatments with casein + soy protein having a TSS (8.80^0Brix) and was on par with the other treatments. The highest titrable acidity (0.90%) was noticed in casein + soy protein treatment. Among the strains Lactobacillus acidophilus recorded the highest titrable acidity (0.93%). The maximum residual sugar (2.18 %) was noticed in wine fermented with Lactobacillus acidophilus. With respect to the treatments maximum residual sugar (2.19 %) was recorded for casein + soy protein treatment compared to others. These results are similar to the reports [6]. With respect to nitrogen content of jackfruit wine (Table 4), there were significant differences between treatments. The highest nitrogen (0.49 %) was recorded in casein + soybean treatment. There was a significant difference between the strains. The highest nitrogen (0.46 %) was recorded in JFL_1. Significantly high amount of protein (2.89 %) was recorded in wine prepared by supplementation of casein + soy protein. Irrespective of the treatments, there were significant differences among the strains. The highest protein (3.13 %) was recorded in wine fermented with Lactobacillus acidophilus.

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Table 2: Effect of nutrient amendments and isolates on pH and TSS (%) content of Jackfruit wine.

Treatments	pH			TSS		
	LAB strains			LAB strains		
	RL	JFL_1	Mean	RL	JFL_1	Mean
Control	3.25	3.34	3.30[a]	8.20	8.60	8.40[a]
Casein	3.28	3.18	3.23[b]	8.60	8.70	8.65[a]
Soy protein	3.31	3.17	3.24[b]	8.70	8.40	8.55[a]
Casein + SP	3.19	3.12	3.16[b]	8.70	8.90	8.80[a]
Mean	3.25[b]	3.20[b]		8.55[b]	8.65[b]	
Source	Sem ±	CD at 1%		Sem ±	CD at 1%	
Strains (S)	0.20	0.06		0.15	0.09	
Treatments (T)	0.80	0.06		0.70	0.09	
Interaction (TxS)	0.10	0.12		0.27	0.19	

Note: RLAB: Reference lactic acid bacteria (*Lactobacillus acidophilus)*
JFL_1: Jackfruit lactic acid bacteria$_1$ JFL_2: Jackfruit lactic acid bacteria$_2$
JFL_3: Jackfruit lactic acid bacteria$_3$ JFL_4: Jackfruit lactic acid bacteria$_4$
JFL_5: Jackfruit lactic acid bacteria$_5$

Table 3: Effect of nutrient amendments and isolates on the titrable acidity (%) and residual sugar (%) of jackfruit wine

Treatments	Titrable acidity (%)			Residual sugar (%)		
	LAB strains			LAB strains		
	RL	JFL_1	Mean	RL	JFL_1	Mean
Control	0.94	0.82	0.87[b]	2.18	2.14	2.16[c]
Casein	0.89	0.84	0.87[b]	2.20	2.17	2.18[ab]
Soy protein	0.92	0.88	0.89[b]	2.19	2.16	2.18[bc]
Casein + SP	0.97	0.93	0.95[a]	2.17	2.11	2.14[c]
Mean	0.93[a]	0.87[b]		2.18[a]	2.14[b]	
Source	Sem ±	CD at 1%		Sem ±	CD at 1%	
Strains (S)	0.06	0.01		0.08	0.02	
Treatments (T)	0.10	0.01		0.08	0.02	
Interaction (TxS)	0.01	0.03		0.08	0.04	

Note: RLAB: Reference lactic acid bacteria (*Lactobacillus acidophilus)*
JFL_1: Jackfruit lactic acid bacteria$_1$ JFL_2: Jackfruit lactic acid bacteria$_2$
JFL_3: Jackfruit lactic acid bacteria$_3$ JFL_4: Jackfruit lactic acid bacteria$_4$
JFL_5: Jackfruit lactic acid bacteria$_5$

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Table 4: Effect of nutrient amendments and isolates on crude protein (%) content of Jackfruit wine

Treatments	Nitrogen (%)			Protein (%)		
	LAB strains			LAB strains		
	RL	JFL_1	Mean	RL	JFL_1	Mean
Control	0.39	0.38	0.38^{c}	2.44	2.41	2.43^{d}
Casein	0.46	0.45	0.46^{b}	2.90	2.86	2.88^{c}
Soy protein	0.49	0.48	0.48^{b}	3.19	3.06	3.11^{b}
Casein + SP	0.48	0.51	0.49^{a}	3.06	3.20	3.13^{a}
Mean	0.45^{a}	0.46^{a}		2.89^{a}	2.88^{a}	
Source	Sem ±	CD at 1%		Sem ±	CD at 1%	
Strains (S)	0.19	0.01		1.21	0.06	
Treatments (T)	0.22	0.01		1.44	0.06	
Interaction (TxS)	0.05	0.03		0.35	0.12	

Note: RLAB: Reference lactic acid bacteria (*Lactobacillus acidophilus)*
JFL_1: Jackfruit lactic acid $bacteria_1$ JFL_2: Jackfruit lactic acid $bacteria_2$
JFL_3: Jackfruit lactic acid $bacteria_3$ JFL_4: Jackfruit lactic acid $bacteria_4$
JFL_5: Jackfruit lactic acid $bacteria_5$

Lactobacillus acidophilus was used as a reference strain. The isolates grown on Mann Rogosa and Sharpe (MRS) medium showed characteristic submerged colonies. The lactic acid bacteria (LAB) cell shape was determined by staining techniques. They were Gram positive, rods and cocci shaped cells, catalase negative and non spore formers and none of the isolates produced Dextran on sucrose agar medium. The lactic acid bacteria could assimilate glucose as carbon source. All the isolates in this study showed assimilation of glucose in modified MRS broth. In the present study the pH of jackfruit juice was 3.95 and TSS was 18 ^{0}Brix. Titrable acidity of jackfruit juice was 0.52 %. The total sugar includes both reducing and non-reducing sugars and the total sugar content of jackfruit juice was 18.45 per cent. The protein content of jackfruit juice was 3.13 percent. Protein is one of the nutrient components essential to cell growth. The Amount of protein content of wine depends on total amount of nitrogen content of fruits and supplemented nitrogen sources. The Results revealed in this study that the wine treated with casein + soy protein recorded maximum amount of protein. Metabolism of these amino acids in proteins varies from yeast strain to strain as described by [8].

CONCLUSION

Jackfruit is a poor man's fruit which is available in plenty during the season (April-August) and marketed on road side stall and by push cart vendors in most unhygienic conditions. to find out hygienic and scientific methods to process the bulbs for making fermented fruit beverages using beneficial lactic acid bacteria for long time storage and consumption. This endeavor helps not only to utilize the excess produce of the jackfruit during the season but, also ensures the development of a sustained jack fruit processing cottage industry in rural areas.

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