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Isolation and characterization of lactic acid bacteria from banana pseudostem

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Abstract
Banana pseudostem comprises several polymers such as cellulose, hemicellulose, pectin and lignin that constitute fibbers with good mechanical properties. These sugars can be used for production of various organic acids and alcohol. With the availability of such huge biomass as substrate, a wide range of microorganisms like bacteria and fungi grow on it. Lactic acid bacteria can grow on such sugars and can be isolated from banana pseudostem. In present study, six lactic acid bacteria (LAB) were isolated from banana pseudostem core using MRS agar. Characterized as LAB based on their morphological and biochemical character. Further, milk coagulation study showed that some isolates of LAB coagulated milk at 12 h of incubation.

Keywords: *lactobacillus* bacteria, (lab), banana pseudostem

Introduction
Banana is one of the important fruit crops grown almost in every state of India (7.1 lakh ha). Apart from fruit, it generates huge quantity of biomass as waste in the form of pseudostem, leaves, suckers etc., of these, on an average about 60 to 80 t/ha is pseudostem alone. Presently, the banana pseudostem is absolute waste in most of the states of India. In order to develop value added products exclusively from banana pseudostem on large scale, a project entitled, "A Value Chain on Utilization of Banana Pseudostem for Fibre and Other Value Added Products" was sanctioned during June 2008 under World Bank funded-NAIP (Component II), ICAR, New Delhi in consortium mode with Navsari Agricultural University, Navsari (Gujarat) as lead centre and Central Institute for Research on Cotton Technology (ICAR), Mumbai (Maharashtra), Manmade Textile Research Association, Surat (Gujarat) and J. K. Paper Mills Ltd., Songadh (Gujarat) as partners.
The banana is an important global commodity. The stem of each plant is of concentric sheaths with tender core at the centre. Banana Pseudo-stem (BPS) constitute a major part of plant biomass, which are usually left in the plantation or incinerated and wasted. BPS appears to be rich in fibre, total carbohydrate and cellulose. Banana plantations face the problem of disposing the pseudo-stem which goes as a waste after harvesting of the trees. In hectare, on an average about 60 to 80 tonnes is of pseudo-stem alone. The stem of banana, commonly known as pseudo-stem is an aggregation of leaf stalk bases in cylindrical form. Pseudo-stem of Banana normally goes as waste though it could be used in pulp and paper industries due to its cellulosic content. It is also consumed as juice in fresh form. The banana central core finds use in south Indian cuisine. Banana stem is a rich source of fibre and helps to control obesity. It also aids to detoxify the body. In southern India, it is consumed as fresh juice to prevent kidney stones (Dawn *et al*., 2016) [4].
Lactobacilli (LAB) are Gram-positive, non-spore forming, catalase-negative rods belonging to the group of lactic acid bacteria. Lactobacillus acidophilus is one of the major species of this genus found in human and animal intestines. They are able to create equilibrium between beneficial and harmful microbiota of the guts if present in sufficient numbers, as probiotics. There are many reports of isolation of lactic acid bacteria from various fruits, vegetables and their wastes. Mayer and Hillebrandt (1997) [17] reported characterization of six isolates done from Lactobacillus genera *viz., Lactobacillus brevis, L. casei, L. delbrueckii, L. helveticus, L. lactis* and *L. plantarum* with a population of 107-109 cells/g wet pulp of potato. The study concluded that potato pulp was one of the agricultural waste products obtained in high quantities during starch production containing starch, cellulose, hemicelluloses, pectin, proteins,

free amino acids and salts. Kim *et al*. (1998) [10] isolated lactic acid bacterial strains from kimchi, *viz*., *Lactobacillus acidophilus, L. plantarum, Leuconostoc mesenteroides*, with or without Saccharomyces cerevisiae and were used as inoculants in fruit-vegetable juice fermentation.

Fermented vegetable and fruit-based beverages have even been claimed to be the ideal vehicle for functional microorganisms and prebiotic ingredients. Thus, to carry out successful controlled vegetable and fruit fermentations, the selection of LAB to be used as starter cultures should be based mainly on pro-technological, sensory, and/or nutritional criteria. Furthermore, LAB belonging to specific niches could present distinctive metabolic traits as a result of environment adaptations.

Material and Methods

The present study entitled "Isolation and characterization of lactic acid bacteria from banana pseudostem" was carried out at College of Horticulture, Dr. Y.S.R Horticultural University, Anantharajupeta, and College of dairy technology Tirupati during year of 2020-21. The details of the material and methods used for the study are presented in this chapter.

Location of the site where the laboratory work was done at postharvest technology laboratory, central laboratory, pathology laboratory, college of horticulture, Anantharajupeta and lactic acid bacteria Laboratory college of dairy technology, S.V.V.U, Tirupati.

Isolation of Lactic acid bacteria isolation from banana pseudostem from different villages in RLY Kodur Mandal.

Different lactic acid bacteria were isolated from different sources of banana pseudo stem core samples collected from two varieties (Grand Naine and Amruthapani) of banana crop.

1. Isolation of Lactic acid bacteria from banana pseudostem collected from different villages of Rail way Kodur Mandal

Sample collection and isolation

- Different LABs were isolated from different sources of banana pseudostem core samples.
- Serial dilution method
- Gram stain of LAB and morphological characteristics were determined after 24 h of incubation on MRS (de Man, Rugose, Sharpe) agar.
- Sub culture for pure LAB isolation

2. Identification and characterization of Lactic acid bacteria

Observations to be recorded

Morphological characterization

- Colony colour
- Colony shape
- Gram's reaction
- Cell shape

Biochemical characterization

- Carbohydrate fermentation
- Catalase activity
- Acid and gas production
- Different lactic acid bacteria were isolated from different sources of banana pseudo stem core samples. The populations of lactic acid bacteria were enumerated in samples by standard plate count method using de Mann, Rogosa and Sharpe's medium (MRS) medium.

Mann, Rogosa and Sharpe's agar (De Mann *et al*., 1960) [5], Oxoid peptone: 10.00 g, Meat extract: 10.00 g, Yeast extract: 5.00 g, K2HPO4: 2.00 g, Diammonium citrate: 2.00 g, Glucose: 20.00 g, MgSO4: 0.58 g, MnSO4: 0.25 g, Sodium acetate: 5.00 g, Agar: 18 g, Distilled water: 1000 ml, pH: 6.2-6.6.

All chemicals and dyes used in this study were of analytical grade, purchased from Merck, India. The bacteriological media were obtained from HiMedia Laboratories Pvt. Ltd., India. Banana pseudostem core for isolation of Lactobacillus was collected from different villages in RLY Kodur mandal and kept in sterile plastic container. Immediately after collection the sample was stored aseptically in low temperature (4 °C) for the isolation of Lactobacillus.

3.1.1 Isolation and Identification of *Lactobacillus* (lactic acid bacteria)

Lactobacillus was isolated from locally available banana pseudostem by using de Mann Rogosa Sharpe (MRS) agar media (De Man *et al*., 1960) [5]. 10 gm of pseudostem core sample was mixed with 90 ml of sterile saline (Nacl 8g/1000 ml distill water), homogenized gently, serially diluted 10 fold in saline and pour plated aseptically on MRS agar media (MRS broth 55.5 g, agar 15 g per 1000 ml distil water). Plates were incubated at 37 ^{o}C for 48 hrs in anaerobic condition. Colonies differ in morphology, pigmentation; shape and size were sub cultured in MRS broth. Initially all of the isolates were examined for Gram staining and catalase production. Only the Gram-positive, catalase-negative and rod shape isolates were then purified by streak plating using the same medium. After several subcultures, finally the single colony of Lactobacillus was isolated by observing their colony morphology and some biochemical tests (Gram staining, catalase, endospore and motility test) and the culture was maintained at 4 ^{o}C in MRS broth pH 5.5

Final identification was done using classic microbiology tests including Gram-staining for detecting morphology, catalase and carbohydrates fermentation (galactose, lactose and sucrose)

Morphological identification

Lactic acid bacteria, on de Mann, Rogosa and Sharpe's media, formed characteristic colonies which were used as a tool for the preliminary identification. Each isolate was streaked on MRS medium and incubated for two days.

3.1.1 Gram staining

Lactic acid bacterial isolates were studied for their cell morphology and Gram reaction. Gram staining was done using 24 hr old cultures. A thin smear of bacterial culture was made on a clean slide. Smear was air-dried and heat fixed. Smear was covered with crystal violet dye for 1 minute and washed with distilled water. Then the smear was covered with Gram's iodine solution for 1 minute. Iodine solution was washed off with 95 per cent ethyl alcohol. Ethyl alcohol was added drop by drop, until no more colour flows from the smear. Slides were washed with distilled water and drained. Safranin was applied to smear for 1 minute as counter stain, washed with distilled water and blot dried with absorbent paper. Slides were examined microscopically using oil immersion objective.

Characterization of lactic acid bacteria

- Gram staining
- Carbohydrate fermentation

- Catalase activity

Biochemical characterization

3.2.1 Catalase activity

A loop full of 24 hr old culture suspension was placed on a clear glass slide to which a drop of freshly prepared hydrogen peroxide (3%) was mixed and observed for the occurrence of effervescence or bubbles.

3.2.2 Carbohydrate fermentation

Prepare broth media (phenol red) by mixing 16.9 grams/1lit of distilled water and heating gently to dissolve it, add 0.5% to 1% the desired carbohydrates (glucose, lactose and sucrose), insert inverted durham tubes in to all tubes (13 X 1000 mm), the durham tubes should be fully filled with broth, sterilize in an autoclave at 120 ºC centigrade for 15 minutes, transfer the sugars in to screw-capped tubes or fermentation tubes and label properly. Added the 200 µl of MRS broth culture in to fermentation tubes and incubated at 37 ºC for 24h. When utilization of different carbohydrates (lactose, sucrose and glucose) and then turn yellow was taken as positive for acid production.

3.2.3 Acid and gas production

The bacterial isolates were tested for acid and gas production by inoculating to ten ml presterilized glucose broth in test tubes containing Durham's tube and phenol red broth (16.1g/l 0.04 per cent solution) as pH indicator. The tubes were incubated for 1 to 2 days at above 35 °C. The accumulation of gas in the Durham's tube was taken as positive for gas production and change in color of medium to yellow was taken as positive for acid production.

Milk fermentation test

Sterilized skim milk (10% reconstituted skim milk powder) was inoculated with 1% active culture and incubated at 37 °C. Observation for coagulation of milk was recorded at 12 and 24 h intervals and the change in pH was recorded at 24 h of incubation.

Result and Discussion

The experimental results of isolation and characterization of lactic acid bacterial strains is as follows.

Isolation of lactic acid bacteria from banana pseudostem collected from from different villages in Rail way Kodur mandal

Isolation of Lactic acid bacteria isolation from banana pseudostem collected from different villages are Turupalli, lakshmipuram in Rail way Koduru mandal and HRS (Horticulture Research Station) at Anantharajupeta (Table 1).

Isolation and identification of lactic acid bacteria

4.1.1 Isolation

Lactic acid bacteria were isolated using de Mann, Rogosa and Sharpe's (MRS) medium. The results pertaining to isolates from banana pseudostem from different villages. All the lactic acid bacterial isolates were subjected to morphological and biochemical tests to confirm their identity.

4.1.2 Isolation and characterization of lactic acid bacteria

A total of 3 samples comprising of banana pseudostem collected from various places were plated on MRS agar and after 48 h of incubation, typical colonies showing different morphological characteristics were picked up (plate 1). Total 15 isolated colonies were inoculated in MRS broth and after 24 h of incubation; the cell morphology was studied by Gram staining. The isolates Catalase reaction. From among 15 isolates, only 6 were found to be Gram positive rods or cocci (plate 2) and Catalase negative. Hence, these 6 isolates were further evaluated for different physiological and biochemical characterization

4.2.1 Catalase activity

Results related to catalase activity by the lactic acid bacterial isolates were presented in the Tab. The data revealed that all the isolates showed negative for catalase activity indicating that isolates showed similar characteristics as that of Lactobacillus spp. (Table 3)

4.2.2 Biochemical characteristics

The colonies that appeared after 48 hrs on Mann, Rogosa and Sharpe's (MRS) medium were cream, smooth, oval submerged colonies. The isolates and reference strain of lactic acid bacteria underwent several biochemical tests for their identification. The results in Table 3 revealed that all the lactic acid bacterial isolates showed negative for spore and dextran production. All the lactic acid bacterial isolates were tested for their confirmation of the acid production on phenol red lactose fermentation. The turn yellow indicated the acid production by the isolates indicated the characteristics as that of Lactobacillus spp (plate 3)

Lactic acid is the major metabolic end product of carbohydrate fermentation by lactic acid bacteria, responsible for the sour taste and improved the microbiological stability and safety of the food. Almost all the selected isolates were able to utilize hexose sugars like Glucose (G), Lactose (L) and Sucrose (S) at different rate. None of the isolates gas indicating that all were homofermentative (Table 3)

4.2.3 Milk fermentation result

The milk coagulation test showed all the isolates have the ability to coagulate milk at 24 h of incubation at a temperature of 37 ºC. There was significant difference observed in the milk coagulation capacity of different isolates. Two of the 6 isolates LAB-1and LAB-4 took 12 h to coagulate the milk of the 6 isolates, LAB-2, LAB-3, LAB-5 and LAB-6 took the long time 24 h to coagulate the milk that indicating their slow growth. (plate 4)

In the present study, we observed reduction of pH from 3.8 to 5.4. A lower pH of 3.8 and 3.94 were recorded for LAB-1 and LAB-4 isolates, respectively that coagulated milk at 12 h of incubation. Among the 6 Lactobacillus isolates, the LAB -1 isolated from milk with a pH of 3.80 was found to be efficient in milk fermentation.

The pH is an important factor which indicates the fermentation of milk and producing lactic acid (Hoque *et al*., 2010) [9].

Table 1: The experimental results of isolation and characterization of lactic acid bacterial strains

SI. No.	Lactic acid bacteria	Source
1	BPSLAB 1	Thurupali
2	BPSLAB 2	Lakshmipuram
3	BPSLAB 3	HRS (Horticulture Research Station), Anantharajupeta.
4	BPSLAB 4	Thurupali
5	BPSLAB 5	Lakshmipuram
6	BPSLAB 6	HRS (Horticulture Research Station), Anantharajupeta.

Table 2: All the isolates showed negative for catalase activity indicating that isolates showed similar characteristics as that of *Lactobacillus* spp.

SI. No	Isolate	Colony Colour	Colony shape	Cell shape
1	BPSLAB 1	Creamy white	Round	Rods
2	BPSLAB 2	Creamy white	Round	Rods
3	BPSLAB 3	Creamy white	Round	Cocci
4	BPSLAB 4	Creamy white	Round	Cocci
5	BPSLAB 5	Creamy white	Round	Rods
6	BPSLAB 6	Creamy white	Round	Rods

Table 3: All the lactic acid bacterial isolates showed negative for spore and dextran production

SI. No.	Isolate	Gram's reaction	Catalase activity	Glucose Utilization AG	Lactose Utilization AG	Sucrose Utilization AG
1	BPSLAB 1	+	-	+ -	+ -	+ -
2	BPSLAB 2	+	-	+ -	+ -	+ -
3	BPSLAB 3	+	-	+ -	+ -	+ -
4	BPSLAB 4	+	-	+ -	+ -	+ -
5	BPSLAB 5	+	-	+ -	+ -	+ -
6	BPSLAB 6	+	-	+ -	+ -	+ -

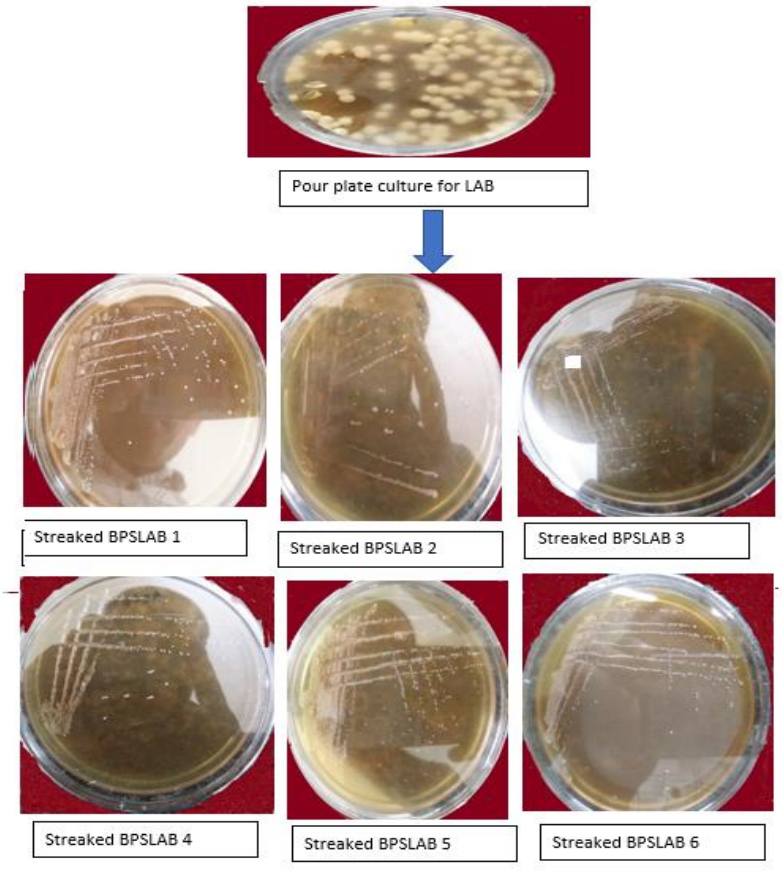


Plate 1: Isolated of lactic acid bacteria (LAB) on MRS agar medium

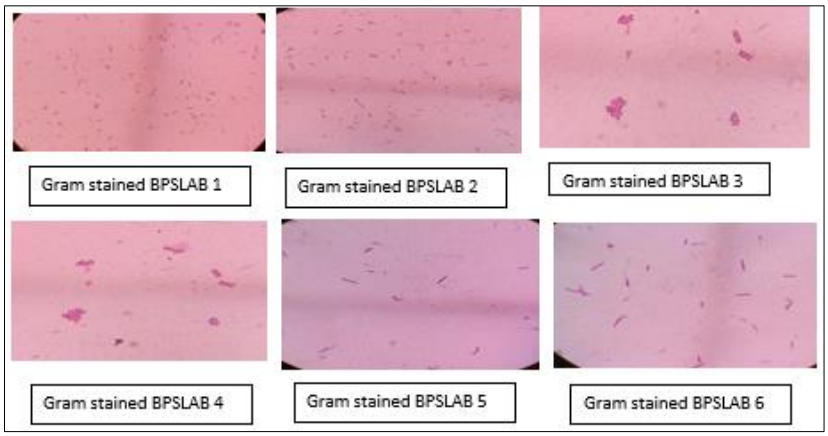


Plate 2: Gram Staining Result

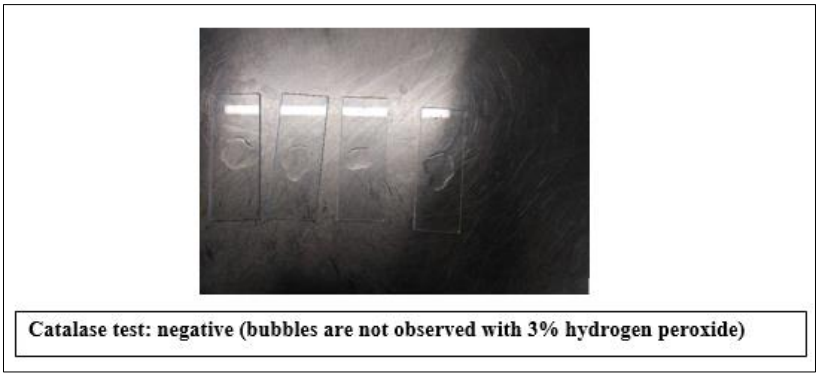
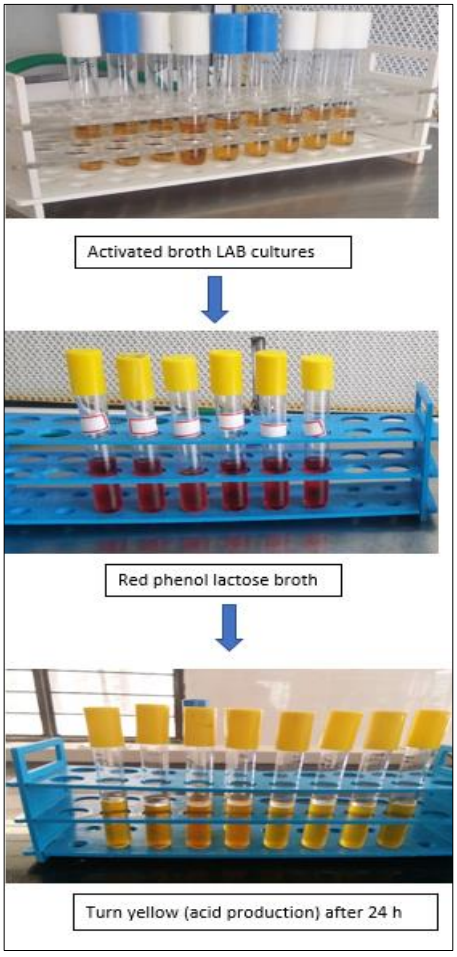


Plate 3: Catalase test result



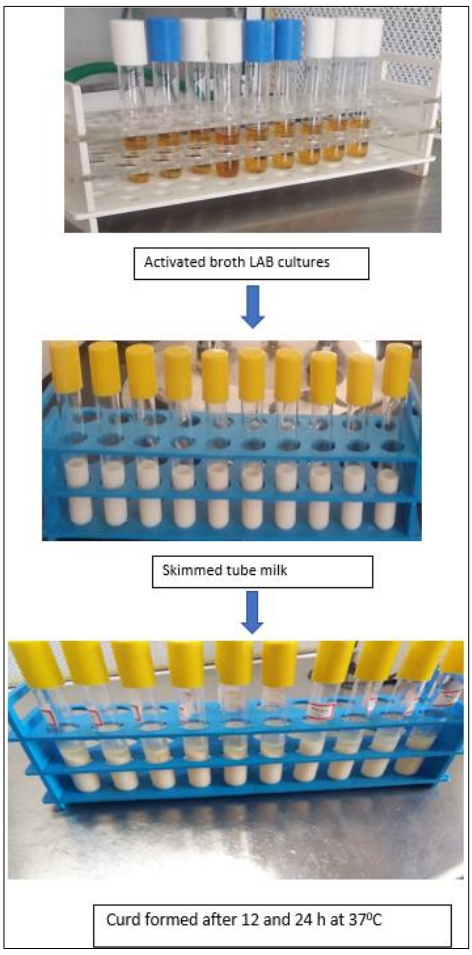


Plate 4: Carbohydrate Fermentation Result **Plate 5:** Milk Fermentation Result

Conclusion

Fifteen bacterial strains were isolated from banana pseudostem. Among them, 6 isolates could produce high amount of lactic acid. All the lactic acid bacterial isolates formed characteristic cream, smooth, round, oval, submerged/raised colonies on de Mann, Rogosa and Sharpe's Medium. The lactic acid bacterial isolates were Gram positive and rod, coci shaped cells. They were catalase negative. They were tested for gas and acid production from lactose, sucrose, glucose and observations showed that isolates were homo-fermentative; they produced only lactic acid and did not produce any gas during growth. Among the 6 *Lactobacillus* isolates, the LAB -1 isolated from milk with a pH of 3.80 was found to be efficient in milk fermentation.

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