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SELECTION AND UTILIZATION OF LACTIC ACID BACTERIA FROM FRUIT WASTE FOR LACTIC ACID PRODUCTION USING JACKFRUIT RAG AND PINEAPPLE PEEL

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ABSTRACT

The fruit waste of pineapple peel and jackfruit rag contains rich amount of carbohydrates can be tuned as substrates for the cultivation of bacteria and fungi. These wastes were also used as raw material for production of lactic acid. Increasing demand of lactic acid in food, bakery, pharmaceutical and cosmetic industries *etc*., had made it necessary to develop a sustainable and conventional method for lactic acid production by fermentation. The present study aimed for lactic acid production from pineapple peel and jackfruit rag. A total of 15 isolates were isolated from different fruit wastes by streak plate method and were identified as Lactic acid bacteria based on gram staining and biochemical tests. The physicochemical properties of pineapple peel and jackfruit rag were: pH (4.3 and 6.2), TSS in brix (12 and 19.5), moisture content (83.8% and 80%), respectively. Different pre-treatment methods were employed for the extraction of reducing sugars from pineapple peel and jackfruit rag. Among six pre-treatments, the substrate with 1 per cent hydrochloric acid followed by autoclave resulted the highest value of reducing sugars. The lactic acid production was carried out by inoculating the 5% lactic acid bacterial isolates (MM, G1 and A2) in acid hydrolysate of substrates and incubated at 30°C with 120 rpm for three days. Maximum Lactic acid yield was recorded with pineapple peel substrate (3.15%) than jackfruit rag (2.7%) after 3 days of incubation with MM isolate. This study highlights the potential of pineapple peel and jackfruit rag waste as a low cost and economically viable fermentation substrate for the production of lactic acid.

KEY WORDS : Lactic acid bacteria, Fruit wastes, Gram staining, Sugar utilization, Lactic acid.

INTRODUCTION

The food and agricultural industries produce large volumes of wastes annually worldwide, causing a serious disposal problem. This is especially problematic in countries where the economy is largely based on agriculture and where the farming practice is very intensive. Currently, these agro-wastes are either allowed to decay naturally on the fields or are burnt. However, these wastes are rich in sugars due to their organic nature, are easily assimilated by microorganisms and hence, make them potential substrates for exploitation as raw materials in the production of industrially relevant compounds through microbial conversion. In addition, the reutilization of biological wastes is of great interest since, due to legislation and environmental reasons the industry is increasingly being forced to find an alternative use for its residual matter (Rodriguez-Couto, 2008).

"Fruit and vegetable waste" refers to indigestible parts that are thrown away at different stages such

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as collection, handling, shipping and processing. Fruit and vegetable waste can be produced at different steps from farm to consumer, involving both pre- and post-consumer stages of the food supply chain. Fruit based industry produces large volume of wastes, these carbohydrate rich wastes can be tuned as valuable substrates for the commercial production of various valuable products.

There are many ways in which fruit waste can be re-used and re-cycled. As fruit waste is rich in starch, pectin and cellulose can be used for generation of bio-fuels. Fiber and pulp in fruit waste can be used for the production of composite materials, textiles and paper. Fruit waste is a good growth substrate for the cultivation of fungi and bacteria. These organisms produce value-added products during growth such as enzymes. It can also be utilized in composting. It is a raw material for production of organic acids such as lactic acid, citric acid, and acetic acid (Reddy *et al*., 2008).

The pineapple (*Anana comosus*) is one of the most significant fruits in the world and the most prominent edible member of the bromeliaceae family. Sugars and lignocellulosic elements are a bound in pineapple fruit waste. Leaves, roots and stem scraps from the farm typically make up pineapple waste. About 13.48% of the fruit is made up of fruit peel. Because of this, pineapple waste also rises in direct proportion to the increase in pineapple production (Salve and Ray, 2020).

Jackfruits (*Artocapus heterophyllus*) are a common tropical fruit that is usually consumed in the Southeast Asia region. Jackfruits are composed of several berries of yellow pulp and brown seeds encased in a hard shell and are rich in carbohydrates, complex B vitamins and minerals. The waste produced is 65-80% of the total weight of the jackfruit. Jackfruit wastes such as skin, straw and seed have no economic value, which can lead to environmental problems (Zainuddin *et al*., 2014).

Lactic acid is one of the broadly known organic acids. It can be formed by fermentation processes and/or chemical processes. Lactic acid production has received significant attention due to its potential applications in food, cosmetics, pharmaceutical and chemical industries. Lactic acid is classified as GRAS (Generally Regarded As Safe) for use as food additive by the US FDA (Food and Drug Administration). Microbial production of optically pure lactic acid has extensively been studied because chemically synthesized lactic acid is a racemic mixture (Bustamante *et al*., 2020). Therefore, the objective of the present study was to investigate the production of lactic acid from lactic acid from jackfruit rag and pineapple peel by lactic acid bacteria.

MATERIALS AND METHODS

Collection of samples

Different fruit waste samples were collected from the local fruit juice market and used for the production of lactic acid by isolating the Lactic acid bacteria (LAB).

Isolation of Lactic acid bacteria

After 24 hrs of fermentation of fruit waste, 0.1ml of 10^{-6} dilution of the sample was then spread plated on MRS agar and incubate at 37 ^{0}C for 24 to 48 hrs. Colonies are randomly selected based on their morphological appearance and streak plating was then used to purify the isolates. The same procedure was repeated after every 24 hrs for 7 days.The individual bacterial colonies were stored in MRS agar slant at 4 ^{o}C for further analysis.

Identification of Lactic acid bacteria

The pure culture isolates were identified by gram staining followed by biochemical tests (Nyanga *et al*., 2020) where morphological characters are determined.

Sugar utilization test

Prepare basal broth tubes (peptone-7.5g, yeast extract-4.5g, bromo thymol blue-10 ml, distilled water-1 lit, carbohydrates-3%) using different sugars-glucose, lactose, sucrose, xylose, maltose, fructose with inverted Durham tubes. Autoclave the broth tubes with sugars at 121°C for 20 mins. Inoculate the broth tubes with test organism and incubate at 37 °C for 48 hrs. Observe turbidity and gas.

Production of Lactic acid

Substrate preparation for fermentation

The pineapple peel and jackfruit rags are collected and they are powdered after drying in tray dryer and also made in paste by mortar and pestle.

Physico-chemical properties

pH

The pH of pineapple peel and jackfruit rag was measured by using pH meter (AOAC, 2000).

TSS

The refract meter is used to measure the total soluble solids of food products. It is a simple instrument used for measuring concentrations of aqueous solutions such as gases, liquids, and translucent solids. The commonly used scale for refract meter is °Brix scale (AOAC, 2000).

Moisture content

The moisture content of pineapple peel and jackfruit rag was estimated by tray dryer.

$$\text{Moisture content (\%)} = \frac{\text{(Initial Weight – Final Weight)}}{\text{Initial weight}} \times 100$$

Reducing sugars

The reducing sugars in substrate is determined by digital colorimeter

Pre-treatment of substrate

Total pre-treatments carried out for prepared pastes and powders of pineapple peel and jackfruit rag

T1- Boiling (80-90 ºC for 5 mins)
T2- Autoclave (121 ºC for 20 mins)
T3- Boiling + Autoclave
T4- 1% HCl
T5- 1% HCl + Autoclave
T6- Control

Estimation of reducing sugars

The reducing sugars were estimated by di-nitrosalicylic acid (DNSA) method.

DNSA

One gram 5 di-nitrosalicylic acid was dissolved in a little amount of 2N NaOH and 30 g of sodium potassium tartarate was added and volume was made upto 100 ml with 2N NaOH.

Preparation of stock solution of glucose

The standard stock solution was prepared @1mgml^1 by dissolving 100 mg of D-glucose in distilled water and the final volume was made upto 100 ml with distilled water.

Procedure

There presentative samples of 0.1 ml from each treatment and replications was taken into thin walled boiling test tubes and 0.9 ml of distilled water was added. A reagent blank containing one ml of distilled water was also prepared. Similarly, standards were also included ranging from 100 µg to 1000 µg concentration of glucose.

DNSA reagent @ 0.5 ml was added to each sample, mixed well and kept in boiling water bath for 5 minutes. The samples were cooled and final volume was making up to 25 ml using a volumetric flask. Absorbance in terms of optical density of the standard and the samples were read at 540 nm using colorimeter.

Fermentation media preparation

After pre-treatment of substrate, fermentation media was prepared. Add 100 ml of filtered sterilized peel hydrolysate to 10 ml of sterile MRS medium in separate 250 ml conical flasks. These conical flasks were inoculated with 5% of LAB isolated cultures and incubated at 37pc in a shaker incubator (120 rpm) for 3 days. The substrate product (Lactic acid) was estimated (Umesh and Preethi, 2014).

Estimation of Lactic acid concentration

The production of lactic acid was detected by estimating the titrable acidity of the fermentation medium against 1N NaOH. Two millimeters of culture broth was taken from the fermented broth in a flask and 8 ml of distilled water was added to make up 10 ml and then boiled (80 ºC) for 1 min to remove air. A drop of phenolphthalein indicator was added.

Thereafter, 1N NaOH solution was added with continuous shaking till the formation of pink color was observed. The quantity of NaOH utilized in the titration was measured and the amount of lactic acid was determined by using the formula.

$$\text{Percentage of lactic acid} = \frac{\text{Ml of NaOH} \times \text{Conc. of NaOH} \times 0.09}{\text{Weight of the sample}} \times 100$$

RESULTS AND DISCUSSION

Isolation of lactic acid bacteria

In this present study, fifteen samples of Lactic acid bacteria were isolated from thirty-two fruit waste samples. All the isolates were cultured on MRS agar by streak plate method and initially subjected to morphological and biochemical tests. Yadav *et al.*, 2020 reported that they isolated species of lactic acid bacteria namely *Lactobacillus plantarum*, *Lactobacillu spentosus* from raw and fermented fruits and vegetables.

Identification of Lactic acid bacteria

Based on the morphological characteristics of the bacteria, among 32 samples fifteen isolates were found to be Gram-positive and rod shaped as shown in Table 2. The microscopic view of the identified isolates was violet and rod shaped. Nyanga *et al*., (2007) reported that lactic acid bacteria were preliminary identified and the predominant strains found were *Lactobacillus agili s*and *Lactobacillus plantarum* from *masau* fruits.

Sugar utilization test

Different isolates showed different types of sugar utilization patterns as shown in Table 3. Where, all the isolates showed gas production for glucose.

Physico-chemical properties

pH

The pH in the pineapple waste was recorded as 4.3 and jackfruit rag waste was recorded as 6.2 (Table 4).

Table 1. Details of the isolates of lactic acid bacteria obtained from different fruit wastes

S. No	Sample	No. of samples tested	No. of Lactic acid bacteria isolated	Code of pure Lactic acid bacteria isolates
1	Apple waste	4	2	A1and A2
2	Banana waste	3	2	B1and B2
3	Grape waste	5	2	G1and G2
4	Muskmelon waste	3	1	MM
5	Mixed fruit waste	5	2	MFW1and MFW2
6	Orange waste	3	2	O1and O2
7	Pineapple waste	4	2	P1and P2
8	Sapota waste	5	2	S1and S2

Table 2. Morphological characteristics of lactic acid bacterial isolates

S.No	Isolate code	Colony colour	Colonynature	Elevation	Margin	Gramreaction
1	A1	White	Smooth	Convex	Entire	+
2	A2	White	Smooth	Convex	Entire	+
3	B1	White	Smooth	Convex	Entire	+
4	B2	White	Smooth	Convex	Entire	+
5	G1	White	Smooth	Convex	Entire	+
6	G2	White	Smooth	Convex	Entire	+
7	MM	White	Smooth	Convex	Entire	+
8	MFW1	Creamy white	Smooth	Flat	Entire	+
9	MFW2	Creamy white	Smooth	Convex	Entire	+
10	O1	White	Smooth	Flat	Entire	+
11	O2	White	Smooth	Convex	Entire	+
12	P1	White	Smooth	Convex	Entire	+
13	P2	White	Smooth	Flat	Entire	+
14	S1	White	Smooth	Convex	Entire	+
15	S2	White	Smooth	Convex	Entire	+

Table 3. Utilization of sugars by the isolates of lactic acid bacteria

Sample	Glucose	Lactose	Sucrose	Xylose	Maltose	Fructose
A1	+	+	+	+	-	-
A2	+	+	-	+	-	+
G1	+	+	-	+	-	+
MM	+	+	+	+	-	+
O1	+	+	-	+	-	-
P1	+	+	+	-	-	-
P2	+	-	-	+	-	+
S1	+	-	+	+	+	+

Note: ˆ+ˆ indicates positive test and ˆ-ˆ indicates negative test.

Hajar *et al*., (2012) reported that the pH of pineapple peel extract variety N36 was 3. 85.

Moisture content

The moisture content of pineapple waste was recorded as 83. 8% and jackfruit rag waste was recorded as 80.0% (Table 4).

Total soluble solids (TSS)

The total soluble solids recorded in pineapple waste and jackfruit waste were12˄ Brix and 19.5˄Brix respectively (Table 4). Hajar *et al*., (2012) reported that the TSS of pineapple peel extract variety N36 recorded as 2.5˄Brix.

Table 4. Physico-chemical properties of pineapple and jackfruit rag waste

S. No.	Parameters	Pineapple waste	Jackfruit waste
1.	Moisture content (%)	83.8	80.0
2.	pH	4.3	6.2
3.	TSS (brix)	12	19.5
4.	Reducing sugars (mg/ml)	1.13	1.01

Reducing sugars

The reducing sugars recorded in pineapple waste was 1.13 mg/ml and in jackfruit waste was1.01mg/ml (Table 4).

Pre-treatment of substrates

The substrates pineapple peel waste-fresh peel, dried powder and jackfruit waste –fresh rag, dried powder were pre-treated in order to estimate the reducing sugar content. The reducing sugar content for each pre-treatment was represented in Table 5. In fresh pineapple peel waste, the pre-treatment with 1% HCl followed by autoclave (3.80 mg/ml) recorded significantly highest educing sugar content as compared to 1% HCl (2.58 mg/ml), boiling followed by autoclave (2.03 mg/ml). In dried pineapple peel waste, the pre-treatment with 1% HCl followed by autoclave (8.01 mg/ml) recorded significantly highest reducing sugar content followed by 1% HCl (6.08 mg/ml), boiling along with autoclave (5.13 mg/ml). In fresh jackfruit rag waste, the pre-treatment with 1% HCl followed by autoclave (3.03 mg/ml) recorded significantly highest reducing sugar content as compared with 1% HCl (2.51 mg/ml), boiling along with autoclave (1.98 mg/ml). Similarly in the dried jackfruit rag waste, the pre-treatment with 1% HCl followed by autoclave (6.59 mg/ml) recorded significantly highest content of reducing sugars as compared to1% HCl (6.02mg/ml), boiling followed by autoclave (4.95 mg/ml).

Based on the content of reducing sugars, the pre-treatment of 1% HCl followed by autoclave which showed highest values for all the substrates was selected for further studies.

Fermentation for lactic acid production

The pre-treatment (1% HCl followed by autoclave), which showed the highest values in reducing sugar content was considered for fermentation. The isolates (MM, G1 and A2) which showed the presence of lactic acid bacteria were collected inoculated into substrate media. On first day in pineapple peel waste, MM isolate (1.353%) recorded highest lactic acid production followed by G1 isolate (1.257%) and A2 isolate (0.803%). In jackfruit rag waste, MM isolate (0.903%) recorded highest lactic acid production followed by G1 isolate (0.853%) and A2 isolate (0.443%). On second day in the pineapple peel waste, MM isolate (2.247%) recorded highest lactic acid production followed by G1 isolate

Table 5. Effect of pre-treatment on the extraction of reducing sugar content from substrates-pineapple waste and jackfruit rag waste

Pre-treatments	Pineapple peel waste		Jackfruit waste	
	Fresh peel	Dried powder	Fresh rag	Dried powder
Boiling	1.34	3.77	1.08	2.84
Autoclave	1.80	3.99	1.36	3.54
Boiling+Autoclave	2.03	5.13	1.98	4.95
1%HCl	2.58	6.08	2.51	6.02
1%HCl+Autoclave	3.80	8.01	3.03	6.59
Control	1.13	2.60	1.01	2.48
Mean	2.11	4.93	1.83	4.40
S.E(m) ±	0.025	0.087	0.036	0.111
CD at 1%	0.111	0.378	0.157	0.479

Table 6. Production of Lactic acid from the pre-treated substrates

Isolates	Fermentation period (days)	Lactic acid (%) Pineapple peel	Lactic acid (%) Jackfruit rag
MM	1	1.353	0.903
G1		1.257	0.853
A2		0.803	0.443
MM	2	2.247	1.797
G1		1.803	1.243
A2		1.343	0.890
MM	3	3.153	2.703
G1		2.697	1.807
A2		2.257	1.347
S.E(m) ±		0.008	0.007
CD at 1%		0.025	0.024

(1.803%) and A2 isolate (1.343%). In jackfruit rag waste, MM isolate (1.797%) recorded highest lactic acid production followed by G1 isolate (1.243%) and A2 isolate (0.890%). On the third day in the pineapple peel waste, MM isolate (3.153%) recorded highest lactic acid production followed by G1 isolate (2.697%) and A2 isolate (2.257%). In jackfruit rag waste, MM isolate (2.703%) recorded highest lactic acid production followed by G1 isolate (1.807%) and A2 isolate (1.347%). The lactic acid production increased significantly from first day to third day and maximum production was noticed in the pineapple peel (3.153%) and jackfruit rag (2.703%) for the MM isolate on the third day of fermentation. Umesh and Preethi, (2014) reported that the maximum concentration of lactic acid (10.08 g/l) was obtained when mango peel was used as substrate whereas other substrates like orange, pineapple and banana peels recorded 5.74 g/l, 4.68 g/l and 4.68 g/l respectively. Jawad *et al*. (2013) reported the highest lactic acid production (17.484 g/l) at 35 °C with incubation period of 6 days.

CONCLUSION

The substrates of pineapple peel and jackfruit rag with pre-treatment of 1% HCl followed by autoclave were fermented by inoculating the identified isolates MM, G1 and A2. On fermentation, the pineapple peel waste substrate produced more Lactic acid when compared with the jackfruit rag waste. The isolate MM produced more Lactic acid than the other two isolates G1 and A2 in the pre-treated substrates. Thus, it can be concluded that the waste of pineapple peel and jackfruit rag can be successfully used for Lactic acid production.

Conflict of Interest – None

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