RESEARCH ARTICLE

ISOLATION OF YEASTS, LACTIC ACID BACTERIA AND ACETIC ACID BACTERIA FROM THE NATURAL SOURCES AND THEIR BIOCHEMICAL CHARACTERIZATION FOR BEVERAGE PRODUCTION FROM TOMATO

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Abstract: Yeasts, Lactic acid bacteria and Acetic acid bacteria were isolated from different natural sources like apple, banana, grapes, orange, tomato, jasmine, papaya, guava, milk, curd, fermented meat and honeybees. Totally 10 isolates of yeast, 7 isolates of lactic acid bacteria and 6 isolates of acetic acid bacteria were obtained. Colony and cell morphology of these isolates were studied and biochemical tests like fermentation of sugars, starch hydrolysis, urease test and acid production were carried out and AY isolate of yeast, CL_1 isolate of lactic acid bacteria and BA isolate of acetic acid bacteria were selected for beverage production.

Keywords: Beverage production, Bacteria, Isolation, Tomato

INTRODUCTION

Tomato juice contains moisture (93.1%), carbohydrate (4.89%), vitamins, minerals, and low in protein and fat. Tomato juice is well recognized as one of the popular healthy beverages because it contains vitamin C and lycopene which adds variety of colors and flavours. It helps to reduce risk of several important chronic diseases (Suzuki *et al.*, 2002). Shelf life of tomato is very less as it contains high moisture. Hence it is necessary to process the tomato to enhance its shelf life. Among the tomato processed products, tomato juices are recognized as healthy beverages (Yoon et al., 2006).

The major microorganisms involved in producing a beverage from tomato are yeast, lactic acid bacteria and acetic acid bacteria. Yeasts are defined as unicellular ascomycetous or basidiomycetous fungi, and their vegetative growth results predominantly from budding or fission. They do not form their sexual states within or upon a fruiting body (Kurtzman and Fell, 1998). Acetic acid bacteria are Gram-negative, ellipsoidal to rod-shaped cells that have a required aerobic metabolism with oxygen as the terminal electron acceptor (Gonzalez *et al*., 2004). The lactic acid bacteria (LAB) are a group of Gram-positive bacteria, non-spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates (Axelsson, 1998). Hence these microorganisms can be utilized for the production of beverage with a prolonged shelf life.

MATERIALS AND METHODS

Isolation of yeast and acetic acid bacterial isolates from different natural sources

Different fruits like pineapple, banana, papaya, grapes, pomegranate, orange, apple, tomato and flowers like jasmine, tuberose and honey bees were collected for the isolation of yeast and acetic acid bacteria. Yeast and acetic acid bacteria were isolated from fruit juices by enrichment broth containing cultures and then by standard plate count method using Davis agar medium (Sobia et al., 2007) and Ethanol agar medium (Moryadee and Wasu, 2008) respectively. The pure cultures were observed under the microscope after staining with cotton blue for yeast and Gram staining for acetic acid bacteria. Several morphological and biochemical tests were conducted for identification and characterization of these isolates.

Identification of yeast isolates

The identification of yeast isolates was made by studying their morphological characters by comparing with the reference yeast, *Saccharomyces cervisiae* UCD522.

Colony morphology

Yeasts formed characteristic colonies on Davis agar media, which was a tool for identification. The isolated yeast strains were streaked on petriplates containing Davis agar medium and incubated for two days.

Microscopic observation

Isolated yeast strains were studied for the cell morphology and growth characteristics on the Davis broth. Cotton blue staining was done by using 24 hour old cultures. The stained cells were observed under the microscope.

The budding of yeast isolates was tested by keeping a drop of one hour old cultures on a slide and observed.

Isolation of lactic acid bacterial isolates

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Different milk and curd samples from local dairies were collected for the isolation of lactic acid bacteria. Lactic acid bacteria were isolated from milk, curd and fermented meat by enrichment broth containing cultures and then by standard plate count method using MRS agar medium. The pure cultures were observed under the microscope after staining with Gram staining for lactic acid bacteria. Several morphological and biochemical tests were conducted for identification and characterization of these isolates.

Colony morphology

Lactic acid bacteria formed characteristic colonies on MRS agar media, which was a tool for identification. The isolated lactic acid bacteria strains were streaked on petriplates containing MRS agar medium and incubated for two days.

Microscopic observations

Lactic acid bacterial isolates were studied for the cell shape and Gram's reaction. Gram staining was done using 24 h old cultures

Identification of acetic acid bacterial isolates

Identification of acetic acid bacterial isolates was done by studying their morphological and biochemical tests.

Colony morphology

Acetic acid bacteria formed characteristic colonies on Ethanol agar media, which could be a tool for the primary identification. Each isolate was streaked on ethanol agar medium and incubated for three days.

Microscopic observations

Acetic acid bacterial isolates were studied for the cell shape and Gram's reaction. Gram staining was done using 24 h old cultures

Biochemical characterization of yeast, lactic acid and acetic acid bacterial isolates

Utilization of glucose for acid production

The isolates were inoculated to a test tube containing Ethanol broth with a specific carbohydrate, a pH indicator. Tubes were incubated at 30°C for two days and observed for acid and gas production (De Ley et al.,1984).

Urease activity

The urease activity can be detected by the change of the phenyl red indicator to purple. Inoculate tube of urea with isolated organism and second tube with E. coli (standard test culture). Incubate along with a control tube at 37°C for 24 hrs and old phenyl red to all tubes after incubation. The presence of purple colour is positive for the test.

Hydrolysis of starch

The ability of the isolates to hydrolyze starch was examined. Triplicate of starch agar were inoculated with test cultures and incubated at 30°C for three days. After incubation, the plates were flooded with Lugol's iodine solution, allowed to stand for 15-30 minutes and observed for clear zone around the colony to indicate hydrolysis of starch. The starch agar was prepared by suspending one gram of starch powder in 10 ml of cold distilled water, mixed with 90ml of nutrient agar and autoclaved at 121°C for 20 minutes.

RESULTS

Isolation of yeasts

Yeasts were isolated using Yeast Extract Peptone Dextrose (YEPD) agar medium from Apple, Banana, Grapes, Orange, Tomato, Jasmine, Papaya and Honeybees. All the isolates were subjected to various morphological tests to confirm their identification. (Table 1)

Identification of yeast isolates

Colony and cell morphology of yeast isolates

The isolates formed characteristic smooth, circular, white, creamy, oval, spherical colonies on Yeast Extract Peptone Dextrose agar medium. (Table 2). The cell shape of the isolates is oval, spherical and pear shaped and the size varied from 2.5 to 7.5 µm. (Table 3)

Isolation of lactic acid bacteria

Lactic acid bacteria were isolated using MRS agar medium from Milk, Curd, Fermented meat and Tomato. All the isolates were subjected to various morphological tests to confirm their identification. (Table 4)

Identification of lactic acid bacteria isolates

Colony and cell morphology of lactic acid bacteria isolates

The isolates formed characteristic smooth, circular, white, creamy, oval, rough, spherical colonies on MRS agar medium. (Table 5). The cell shape of the isolates is rods and cocci and the size varied from 0.25 to 2.56 µm. (Table 6)

Isolation of acetic acid bacteria

Acetic acid bacteria are isolated using Glucose Yeast Extract Calcium carbonate (GYC) agar medium from Guava, Banana, Grapes, Papaya, Tomato and Orange. All the isolates were subjected to various morphological tests to confirm their identification. (Table 7)

Identification of acetic acid bacteria isolates

Colony and cell morphology of acetic acid bacteria isolates

The isolates formed characteristic smooth, dull, glistening, circular, irregular, white colonies on GYC agar medium. (Table 8). The cell shape of the isolates are rods and cocci and the size varied from 0.4 to 1.3 µm. (Table 9)

Biochemical characterization of yeasts, lactic acid bacteria and acetic acid bacterial isolates for their characters suitable for beverage production.

Biochemical characterization of yeast isolates

All the isolates of yeast are positive for gram reaction, urease production, acid production, fermentation of sugars expect GY_1, BY_1, BY_2, HY which are tested negative for fermentation of lactose and positive for starch hydrolysis except GY_1, GY_2, BY_2, HY, TY are negative for starch hydrolysis.(Table 10)

Biochemical characterization of lactic acid bacteria isolates

All the isolates are positive for gram reaction, fermentation of sugars, urease production, and acid production and negative for endospore formation. Among the isolates ML_2 and CL_2 are positive for starch hydrolysis and ML_1, CL_1, FL and TL are negative for starch hydrolysis. (Table 11)

Biochemical characterization of acetic acid bacteria isolates

All the isolates are negative for gram reaction, endospore formation, starch hydrolysis and positive for fermentation of sugars, urease production and acid production.(Table 12).

Table 1. Yeasts obtained from different sources

S.NO	Sources	No of isolates obtained	Yeasts Population (Yeast×10^4cfu/ml)	Coding
1.	Apple	1	12.34	AY
2.	Banana	2	9.54	BY_1, BY_2
3.	Grapes	2	14.63	GY_1, GY_2
4.	Orange	1	11.23	OY
5.	Tomato	1	8.65	TY
6.	Jasmine	1	6.48	JY
7.	Honeybee	1	12.22	HY
8.	Papaya	1	5.25	PY
9.	**SE(m)**	-	0.012	-
10.	**CD (P=0.05)**	-	0.036	-
11.	**CV**	-	0.200	-

Table 2. **Colony Morphology of yeast isolates**

S.NO	Isolate	Colour	Shape	Surface	Margin	Colony elevation
1.	GY_1	White	Circular	Smooth	Entire	Flat
2.	GY_2	Thick white	Circular	Smooth	Entire	Convex
3.	JY	White	Circular	Smooth	Entire	Flat
4.	BY_1	White	Circular	Rough	Entire	Convex
5.	BY_2	Thick white	Circular	Smooth	Entire	Raised
6.	HY	White	Oval	Smooth	Entire	Convex
7.	AY	Cream	Circular	Smooth &powdery	Entire	Raised
8.	OY	Milky white	Circular	Smooth	Entire	Convex
9.	TY	Light white	Circular	Smooth	Entire	Convex
10.	PY	Thick white	Oval	Smooth	Entire	Raised

Table 3. Cell Morphology of yeast isolates

Isolate	Shape	Size (μm)	Gram stain
AY	Oval	2.5	+
BY_1	Spherical	3.6	+

BY_2	Oval	4.8	+
JY	Spherical	4.6	+
HY	Spherical	7.5	+
GY_1	Pear	3.9	+
GY_2	Oval	4.5	+
OY	Pear	6.2	+
PY	Oval	2.8	+

Table 4. Lactic acid bacteria obtained from different sources

S.NO	Sources	No of isolates obtained	Lactic acid bacteria population($LAB \times 10^6$cfu/ml)	Codes
1.	Milk	2	16.88	ML_1, ML_2
2.	Curd	2	15.23	CL_1, CL_2
3.	Fermented meat	1	13.39	FL
4.	Tomato	2	10.58	TL_1, TL_2
5.	**SE(m)**	-	0.009	-
6.	**CD (P=0.05)**	-	0.027	-
7.	**CV**	-	0.105	-

Table 5. Colony characteristics of lactic acid bacteria isolates

S.NO	Isolate	Colour	Shape	Surface	Margin	Colony elevation
1.	ML_1	Thick white	Circular	Smooth	Entire	Convex
2.	ML_2	Thick white	Circular	Smooth & Bright	Entire	Raised
3.	CL_1	White	Oval	Smooth & Creamy	Wavy	Convex
4.	CL_2	White	Circular	Rough	Entire	Raised
5.	FL	White	Circular	Smooth	Entire	Convex
6.	TL_1	White	Irregular	Smooth& Powdery	Wavy	Convex
7.	TL_2	Thick white	Oval	Smooth	Entire	Raised

Table 6. Cell Morphology of lactic acid bacterial isolates

Isolate	Shape	Size(µm)	Gram -reaction	Endospore formation
ML_1	Rods	1.02	+	-
ML_2	Rods	1.39	+	-
CL_1	long rods	2.56	+	-
CL_2	Cocci	0.79	+	-
FL	Rods	1.82	+	-
TL_1	Cocci	0.25	+	-
TL_2	Rods	1.25	+	-

Table 7. Acetic acid bacteria obtained from different sources

S.NO	Sources	No of isolates obtained	Acetic acid bacteria population (AAB×10^6cfu/ml)	Codes
1.	Grapes	1	11.75	GA
2.	Guava	1	12.32	GuA
3.	Tomato	1	13.25	TA
4.	Papaya	1	9.34	PA
5.	Banana	1	12.65	BA
6.	Orange	1	10.25	OA
7.	**SE(m)**	-	0.007	-
8.	**CD (P=0.05)**	-	0.023	-
9.	**CV**	-	0.106	-

Table 8. Colony characteristics of acetic acid bacterial isolates

S.NO	Isolate	Colour	Shape	Surface	Margin	Colony elevation
1.	PA	Light white	Irregular	Smooth & Dull	Undulating	Flat
2.	BA	Thick white	Circular	Smooth	Entire	Convex
3.	TA	White	Circular	Smooth	Entire	Convex
4.	GA	Transparent	Irregular	Smooth & Glistening	Entire	Raised
5.	GuA	White	Circular	Smooth	Entire	Convex
6.	OA	White	Circular	Smooth	Entire	Raised

Table 9. Cell Morphology of acetic acid bacterial isolates

Isolate	Shape	Size(µm)	Gram reaction	Endospore formation
GA	Cocci	0.5	-	-
GuA	Rods	0.8	-	-
TA	Cocci	1.1	-	-
PA	Rods	0.4	-	-
BA	Rods	1.3	-	-
OA	Rods	0.2	-	-

Table 10. Biochemical characterization of yeast isolates

S.NO	Isolate	Fermentation of Sugars				Starch hydrolysis	Urease	Acid production
		Fructose	Sucrose	Lactose	Glucose			
1.	GY_1	+	+	-	+	-	+	+
2.	GY_2	+	+	+	+	-	+	+
3.	JY	+	+	+	+	+	+	+
4.	BY_1	+	+	-	+	+	+	+
5.	BY_2	+	+	-	+	-	+	+
6.	HY	+	+	-	+	-	+	+
7.	AY	+	+	+	+	+	+	+
8.	OY	+	+	+	+	+	+	+
9.	TY	+	+	+	+	-	+	+
10.	PY	+	+	+	+	+	+	+

Table 11. Biochemical characterization of lactic acid bacterial isolates

S.NO	Isolate	Fermentation of Sugars				Starch hydrolysis	Urease	Acid production
		Fructose	Sucrose	Lactose	Glucose			
1.	ML_1	+	+	+	+	-	+	+
2.	ML_2	+	+	+	+	+	+	+
3.	CL_1	+	+	+	+	-	+	+
4.	CL_2	+	+	+	+	+	+	+
5.	FL	+	+	+	+	-	+	+
6.	TL_1	+	+	+	+	-	+	+
7.	TL_2	+	+	+	+	-	+	+

Table 12. Biochemical characterization of acetic acid bacterial isolates

S.NO	Isolate	Fermentation of Sugars				Starch hydrolysis	Urease	Acid production
		Fructose	Sucrose	Lactose	Glucose			
1.	PA	+	+	+	+	-	+	+
2.	BA	+	+	+	+	-	+	+
3.	TA	+	+	+	+	-	+	+
4.	GA	+	+	+	+	-	+	+

5.	GuA	+	+	+	+	-	+	+
6.	OA	+	+	+	+	-	+	+

+ Positive, - Negative

CONCLUSION

Yeasts, lactic acid bacteria and acetic acid bacteria were isolated from different natural sources in which AY isolate of yeast, CL_1 isolate of lactic acid bacteria and BA isolate of acetic acid bacteria are found to be efficient for the production of beverage from tomato based on their biochemical characterization.

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