

## Biotechnological potential of bacterial flora from Cheend juice: Alcoholic beverage from Bastar, India

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**Abstract:** Cheend is an alcoholic beverage of tribal people of Bastar region of Chhattisgarh State in India. It is extracted from *Phoenix dactylefera*. From a sample of Cheend juice seven different bacteria were isolated which were of four genera: four of Bacillus, one each of Paenibacillus, Micrococcus and Streptococcus. All the seven bacteria produced ethanol, PHA and EPS in varying capacity.

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Key words: Bacteria; ethanol; PHA; EPS; Cheend

### Introduction

Cheend juice is one of the alcoholic beverages of tribal people of Chhattisgarh state in India. Cheend juice is extracted from a palm *Phoenix dactylefera*. The juice of this palm is used as an alcoholic beverage by the tribal people of Chhattisgarh since ages. Cheend is extracted from the inflorescence of the palm. The young inflorescence is cut and an earthen pot is tied below the cut to collect the juice. The juice is collected very slowly as it oozes from the cut into the pot. The fresh juice is sweet in taste and as it is kept microbial activity within the juice converts the sugars in the juice into acids and alcohols. Therefore after sometime the taste of the juice turns from sour to bitter. Cheend is of much practical importance to the tribal people of the state of Chhattisgarh as the juice collected from it provides a means of economic benefit to the people owning the palm.

Beer was brewed by Babylonians and also exported to Egypt around 3000 BC (Abegaz, 2007). Boza from Turkey (Hancioglu and Karapinar, 1997), borde and tej from Ethiopia (Abegaz, 2007, Bahiru *et al.* 2001), sobia from Saudi Arabia (Gassem, 2002), Fermented milk product from Fulani (a tribe) of Burkina Faso (Savadogo *et al.* 2004), pulque a traditional Mexican alcoholic beverage (Escalante *et al.* 2004), suusac from Kenya (Lore *et al.* 2005), bhaati jaanr from Eastern India, hamei and marcha from Sikkim and Manipur (Tamang and Thapa, 2006, Tamang *et al.* 2007) are just few fermented food products and beverages. Many others, which are also used, may not have found themselves in the literature. Cheend is one such product.

Almost all the traditional fermented beverages have microbial activity within them which makes these drinks alcoholic. These drinks harbor many types of bacteria and fungi. Many different uses of these microorganisms have been found in the modern day

fields which are found in abundant in these traditional drinks.

The purpose of this study was of to isolate bacteria from juice of Cheend along with their characterization and probable identification along with the testing of biotechnological potential of the isolated bacteria like production of PHA, EPS along with ethanol production.

### Material and Methods

Cheend juice was brought to the laboratory from Garhbengal, Narayanpur, Dist. Narayanpur (C.G), India. The sample was inoculated onto the NAM media plate. Morphologically distinct colonies were pure cultured on NAM slants. The pure cultured colonies were then characterized morphologically, physiologically and biochemically. NAM was used for obtaining colonies from Cheend juice and maintaining pure cultures.

Morphological tests performed were Gram's test and endospore test. Physiological tests performed were growth at different temperatures, pH and NaCl concentrations. Different biochemical tests were performed for probabilistic identification. Ethanol production was tested on various substrates. Production of PHA and exopolysaccharides were also tested in the bacteria isolated from Cheend juice.

### Qualitative estimation of ethanol:

0.5 ml H<sub>2</sub>SO<sub>4</sub> and 1 ml K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> is taken in a test tube. 1 ml-distilled solution is added to the chromate solution. Blue green color indicated the presence of ethanol in the distilled liquid.

### Quantitative estimation of ethanol:

Quantitative estimation of ethanol from the distilled liquid is done the method given by Caputi Jr. *et. al.* (1968).

0.5 ml H<sub>2</sub>SO<sub>4</sub> and 1 ml K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> are taken into a test tube and a series is made with ethanol. Along with 0.5 ml H<sub>2</sub>SO<sub>4</sub> and 1 ml K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 3 ml of distilled liquid is taken for the test sample.

#### Production of PHA and EPS:

For production of PHA the seven bacteria were grown on specialized medium as given by Lee and Choi (1999). PHA production was tested at 37°C. Same sample was used for extraction of exopolysaccharides.

#### Extraction of PHA and EPS:

PHA was extracted from the bacterial cells using sodium hypochlorite method (Law & Slepecky, 1960, Matsuyama *et al* 1999, Garcia *et al* 2001) and EPS by method given as in Welman *et al*, 2003. The following procedure was employed for extraction of PHA and exopolysaccharides from a single sample.

10 ml broth containing bacterial cultured was centrifuged for 20 minutes at 1500 rpm. The bacterial pellet was taken for PHA extraction and the supernatant for extraction of exopolysaccharides. Supernatant was again centrifuged for 20 minutes at 1500 rpm to separate remaining cells. 5 ml supernatant was taken in fresh tube and 3 times

volume ethanol was added to it and was incubated for 1 hour to precipitate the exopolysaccharides. The bacterial pellet was resuspended in 5 ml distilled water and PHA was extracted from bacterial cells using sodium hypochlorite method.

#### Results and discussions:

From the sample of Cheend juice seven different bacteria were isolated on NAM and were named A to G. On the basis of various tests performed on the bacterium four different genera were identified. A, D, E and G were identified as bacteria of *Bacillus* genera, while B is of *Paenibacillus*, C is of *Micrococcus* and F belongs to *Streptococcus*.

Results of various testes performed on the bacteria isolated from Cheend juice are given in different tables. Results of morphological testes are presented in Table 1, physiological tests in Table 2, ethanol production in Table 3 and PHA and EPS production in Table 4.

All the bacteria produced highest ethanol in either apple juice, radish juice of mango pulp (Table 3). Species of *Micrococcus* i.e. C gave highest PHA production while one of the *Bacillus* species gave highest EPS production (Table 4).

It can be said that Cheend juice has diversity of bacteria and as it has been shown all the bacteria isolated were able to produce ethanol, PHA and EPS which also is an indication that bacteria present in Cheend juice would have biotechnological potential and can be utilized as such.

**Table 1: Results of Morphological tests performed on the bacterial strains**

So.No	TEST	A	B	C	D	E	F	G
1	Colour	Creamy	creamy	Brown Yellow	Off white	creamy	creamy	Creamy
2	Margin	Entire	Wavy	Entire	Entire	Entire	Entire	Wavy
3	Elevation	Elevated	Elevated	Elevated	None	None	Elevated	None
4	Pigment	None	None	Present	None	None	None	None
5	Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Transperent	Opaque
6	Surface	Smooth	Rough	Smooth	mucelaginous	mucelaginous	Smooth	Rough
<b>Cell Morphology</b>								
1	Gram Stain	Positive	Positive	Positive	Positive	Positive	Positive	Positive
2	Cell Shape	Rods	Rods	Coccus	Rods	Rods	Coccus	Rods
3	Arrangement	Single	Single arrange in L shape	Arrange in chain	Single arrange in L & V shape	Single group and chain	Bunch	Single
4	Endospores	Present	Present	Absent	Present	Present	Absent	Present
5	Motility	Non motile	Motile.	Non motile	Non motile	Motile.	Non Motile	Non motile

**Table 2: Results of Physiological tests performed on the bacterial strains**

S.No	TEST	A	B	C	D	E	F	G
<b>A</b>	Growth in different temperature							
1	37°C	+	+	+	+	+	+	+
2	40°C	+	+	+	+	+	+	+
3	45°C	+	+	+	+	+	+	+
4	50°C	+	+	+	+	+	+	+
5	55°C	+	+	+	+	+	+	+
6	60°C	+	+	+	+	+	+	+
7	65°C	+	+	+	+	-	-	+
8	70°C	+	-	-	-	-	-	-
<b>B</b>	Growth in different pH (O.D)							
1	2	0.01	0	0	0.02	0.01	0	0.02
2	3	0.02	0.01	0.01	0.04	0.01	0.02	0.02
3	4	0.03	0.03	0.03	0.04	0.02	0.03	0.03
4	5	0.06	0.05	0.04	0.09	0.05	0.04	0.06
5	6	0.04	0.05	0.07	0.1	0.07	0.05	0.07
6	7	0.05	0.06	0.08	0.08	0.06	0.05	0.07
7	8	0.07	0.03	0.05	0.07	0.05	0.06	0.05
8	9	0.04	0.01	0.03	0.06	0.05	0.02	0.03
9	10	0.01	0	0.01	0.05	0.02	0.01	0.01
<b>C</b>	Growth in different NaCl concentration							
1	2%	+	+	+	+	+	+	+
2	4%	+	+	+	+	+	+	+
3	6%	+	+	+	+	+	+	+
4	8%	-	-	-	-	-	-	-

Table 3. Ethanol production by bacteria

S.No	Sources	Percentage of ethanol production (%)						
		A	B	C	D	E	F	G
1	Plum	6.16	5.33	3	5.16	5.5	3.16	3.33
2	Pomegranate	3	5	3.83	3.33	3.83	4.33	4
3	Fig	4	4.5	4.5	4.33	4.5	4.83	4.5
4	Apple	8.33	5.33	7.83	8.33	7.16	7.66	7.16
5	Banana	4.66	4.33	6	5.33	6.66	6	6.33
6	Pearl millet	3.83	3.83	4.33	4	4.83	4.16	4.5
7	Beet	5.83	6	6.83	6.16	7.33	6.16	6.33
8	Carrot	7.5	7.66	7.66	7.83	7.66	7.83	6.83
9	Corn	4.16	4.66	5.66	4.16	4.83	4.83	4
10	Date	6.33	5.66	6.5	6.5	6	6.33	6.66
11	Radish	5.33	5.33	5.66	3.33	6	5.66	5
12	Gorgon Nut	7.16	6.83	5.83	5.66	5.83	6.83	7.83
13	Mango	6.33	7.16	7.83	6.83	7	7.16	6.66
14	Pear	6.16	7	6	6.83	5.83	6	7.33
15	Pine apple	4.66	5	6.33	6	6	6.33	6.33
16	Potato	7.33	7.16	5.16	5.33	5.33	7.66	7.33
17	Rice	6	6	7	6.33	5.33	6.5	6
18	Water chest nut	3.66	4.5	4.66	3.5	3.33	5.5	5
19	Sugar	5.33	5.83	5	5.16	5.33	5.66	5.5
20	Sweet lemon	3.83	4.33	4.83	4.66	2.83	4.83	3.83
21	Wheat	4.5	4.5	4.33	5.16	5	4.16	5.16

Table 4: PHA and EPS production

S.No.	Bacteria	PHA production (g/l)	EPS production (g/l)
1	A	0.03	0.005
2	B	0.01	0.01
3	C	0.1	0.01
4	D	0.02	0.02
5	E	0.02	0.06
6	F	0.01	0.02
7	G	0.012	0.01

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