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Isolation and identification of *Acetobacter* from Toddy sample for production of Acetic Acid

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ABSTRACT

This study was concerned with the isolation and characterization of *Acetobacter aceti* from toddy for acetic acid production. The samples were inoculated in sterilized GYP standard media and then incubated at 28°C for 48 hours. Successive subculture was performed to screen out the strains. In Gram's staining, the morphology of the isolated bacteria exhibited pink, small rod shaped single, pair and chain in arrangement, in the hanging drops technique, all the isolates revealed motile. Biochemical tests were performed by fermentation of five basic sugars by producing both acid and gas bubbles in Durham tube. Acidity of the media gradually increased from day 0 to day 8 and pH of the media decreased from 6.8 to 5.5 during the period. This protocol was successful for enriching *Acetobacter aceti*, which was essential for Acetic acid production.

Keywords: *Toddy sample Acetic acid , Acetobacter spp.*

1. INTRODUCTION

Manzi is a typical tropical alcoholic beverage, produced by fermentation of sugary coconut sap is trapped from palm tree that grow at the coastal region of maharastra. manzi is a white-shish effervescent,acidic alcoholic brevrage (Swins and De Ley,1977) trapping process of toddy used in this study was as explained by Kadare *et al*. (2004).it is a product of mixed alcoholic lactic and acetic fermentation. AAB (acetic acid bacteria utilizing the glucose and sucrose might be present in an earlier stage of the manzi fermentation (okafar,1975) ***Acetobacter*** is a genus of acetic acid bacteria. Acetic acid bacteria are characterized by the ability to convert ethanol to acetic acid in the presence of oxygen. Of these, the genus *Acetobacter* is distinguished by the ability to oxidize lactate and acetate into carbon dioxide and water. Bacteria of the genus *Acetobacter* have been isolated from industrial vinegar fermentation processes and are frequently used as fermentation starter cultures Acetic acid bacteria (AAB) is a group of gram-negative, aerobic and motile rods that carry out incomplete oxidation of alcohol and sugars, leading to the accumulation of organic acids as end products. These bacteria are heterogeneous assemblage organisms (Sokollek *et al*., 1998; Madigan et al., 2008). Among the most important acetic acid bacteria, the strains of genus Acetobacter are mainly involved in vinegar production. Acetobacter is a gram negative, obligate aerobe coccus or rod-shaped bacterium with the size of 0.6-0.8 X 1.0 - 4.0 µm, nonmotile or motile with peritrichous flagella, catalase positive and oxidase negative biochemically.

2. MATERIAL & METHODS

The coconut wine or toddy were collected 4 sample for Acetic acid production were obtained from Patan coastal region of Maharashtra .The freshly trapped toddy samples were collected in sterile cantainer.

Isolation of *Acetobacter spp.* from toddy sample.

1)Sample collection:

Toddy sample was collected from Patan Tal-Karad Dist.-Satara of coastal region of Maharashtra. Sample was collected in sterile container Daniel *et al*.2021).

Toddy sample was enriched by GYP Broth In 1week incubation period at a 30°Croom temperature. (Daniel *et al*.2021).

3)Isolation method:

Isolation of microorganisms from Toddy .it was along enriched sample and spread on GYP Agar plates Agar plates and incubate at room temperature for 2 to 3 days. (W Scharf *et al*. 2013).

4)Chemical Analysis Determination of Acidity:

The method of SLS: 168:1999 was used. The acidity of the sample was determined as acetic acid (% w/w) by titrating 10 ml of the sample against 0.1 N NaOH using phenolphthalein as an indicator.

5)Production of acetic acid using the submerged fermentation process

The effect of temperature (28, 30 and 32 ˚C), shaking (200 rpm) and inoculum size (5, 10, 20 and 30 ml) on formation of acetic acid in submerged fermentation were studied.

6)Confirmation Test of Acetic Acid.

When the litmus test is done, acetic acid turn blue litmus to red. Acetic acid reacts with basses to from salt of sodium acetate and water. its acid -base neutralization reaction.

7)Estimation of Acetic Acid

The production medium is aerated and samples were taken at 48 hrs interval .Estimation of acetic acid -5 m L of cultured is mix with 20 m L. Distilled water .3-5 drop phenolphthalein indicator is add .the solution is titrated against 05 NaOH the amount of acetic acid produce in 100 ml of medium is calculate using the titration reading x0.03 x20 (Guillaume-François Rouelle *et al*.1754).

Acetic acid (g100ml)=

Volume of 0.5N NaOH(ml)used in titration×0.03×20

3. RESULT

After incubation to check the staining ,biochemical and marphological characterization.and identified as AAB according to the standard guidelines of Bergey's manual of Determinative Bacteriology

Pure cultures of AAB isolates

variable +- positive – negative

Biochemical characterization of AAB

Biochemical Characterization

4 isolates showing significant zones of clearance on GYP agar and which were found to be catalase positive and oxidase negative were preliminarily identified as AAB according to the standard guidelines of Bergey's manual of Determinative Bacteriology

Chemical Analysis Determination of Acidity:

Acidity obtained from the medium having toddy inoculated with A1, A2 and control (without bacterial strain) was significantly increased

Production of acetic acid using the submerged fermentation process

There were no significant differences in the formation of acetic acid (8.74%) between the size of the inoculum, whereas, that of acetic acid formation showed a significant difference during the fermentation period. Inoculum's size, such as 5, 10 and 20 % showed the same acetic acid production A3 (4.5%) at 8th, 5th and 7th day of fermentation respectively

Estimation of Acetic Acid

BACTERIAL STARIN	A1	A2	A3	A4
Gram Stain	V	V	V	+
Catalase	+	+	+	+
Oxidase	-	-	-	-
Growth In Gelatine	+	+	+	+
Growth In pH2.5	-	-	-	-
Growth In pH 4.5	+	+	+	+
Growth In pH 7	+	+	+	+
Motility	+	+	+	+

Sr.no	**Sample**	**Total yield**
1	A1	3.5%
2	A2	4.2%
3	A3	4.5%
4	A4	2.3%

4. CONCLUSION

In this study, acetic acid-producing bacteria were selected by using toddy , based on the acetic acid production, morphological characterization also the formation of gel-like layers in the fermentation medium. Among the three processes of acetic acid production, the submerged process yielded the highest amount of acetic acid A3 (4.5%)at the 8^{th} day of fermentation

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Competing interest

There is high potential Competing interest

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