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# ISOLATION AND CHARACTERIZATION OF *ACETOBACTER ACETI* FROM SUGAR CANE – A REVIEW

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**Abstract:**

*Acetic acid bacteria are airborne and are ubiquitous in nature. They are actively present in environments where ethanol is being formed as a product of the fermentation of sugars. They can be isolated from the nectar of flowers and from damaged fruits. The present review is to investigate the efficiency of acetic acid bacteria to produce good quality vinegar from fruit peels and to isolate the Acetobacter aceti more naturally. Whatever, as we know that acetic acid bacteria play very important role in production of vinegar for acetic acid fermentation. To overcome the disadvantages or side effects from vinegar. In the present review methods for isolation of Acetobacter from sugarcane juice, honey, flowers using GYC media.*

***Keywords:*** *Acetic acid bacteria, Honey, Sugarcane, Yeast extract.*

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## INTRODUTION:

Acetic acid bacteria are a large group of obligate aerobic gram-negative bacteria with the ability to oxidize ethanol to acetic acid **[1] [FIG 1].** They are widely distributed in natural habitats and classified into the family *Acetobacteraceae*. Members of this family are useful in industrial production of vinegar **[2]**. Acetic acid bacteria (AAB) can use substrates such as glucose, ethanol, lactate or glycerol as energy sources. However, most of these compounds are not completely oxidized into $CO_2$, and water and several metabolites, especially acetic acid, are accumulated in the growth medium. AAB are commonly found in nature because of their high resistance to acidity and the variety of substrates that they can use **[3]**. These bacteria have been isolated from alcoholic beverages, vinegar, fruits, flowers, honey, sugarcane, fruit juice, soil and water **[4,5]**. Among the most important acetic acid bacteria, the strains of genus Acetobacter are mainly involved in vinegar **[6,7]**.

The aim of this study is to characterization of the isolated strains from novel food and agricultural resources that could grow at high temperatures and tolerate against high concentrations of ethanol and produce high levels of acetic acid.

## LITERATURE REVIEW:

Tharinee *et.al*. in 2015 referred Isolation of acetic acid bacteria from various kinds of fruits and fermented fruit juices **[8]** . They collected thirty varieties of fruits: apple, black grape, cantaloupe, cherry, Chinese pear, dragon fruit, green grape, guava, lorgan, longkong, lychee, mango, mangosteen, mulberry musckmelon,papaya, peach, persimmon, pineapple, pisang mas, plum, plum mango, rakum plam, rambutan, red grape, rose apple, santol, strawberry, sugarcane and watermelon and 4 fermented juices of kaffir lime, Indian gooseberry, pineapple and star fruit. Ninety-nine isolates of acetic acid bacteria were obtained from 18 varieties of fruits and 4 fermented juices using sterile distilled water supplemented with 4.00% ethanol (v/v) as an enrichment medium. Eighty-nine isolates were identified to be in the genus Acetobacter and 10 isolates were in the genus *Gluconobacter*. Fifty- nine isolates were *Acetobacter aceti* as determined by biochemical tests. Nineteen isolates; P1, P4, P6, P8, P12, K4, K5, K6, K7, S8 and S11 gave the widest yellow zone on bromocresol green ethanol agar. They were selected for acetic acid production and compared with *Acetobacter aceti* TISTR354 in ethanol-yeast extract medium supplemented with 6.00% (v/v) ethanol. It was found that P1, P4, P6, P12 and *Acetobacter aceti* TISTR354 gave the highest yield of acid 4.06%, 3.70%, 3.89%, 4.00% and 4.03% respectively. All the isolates were tested for their tolerance to ethanol and acetic acid. It was suggested that their fruits should be ripe fruits which are appropriate for enrichment technique. It was found that they were able to grow at 4% and 6% ethanol. Moreover, isolates P1, P4, P6, P12, K6, K7, K8, S1, S2, S11 were able to grow at 10.00% ethanol.

The study conducted by J. Kowser *et.al.*,2015 with various samples which are inoculated in sterilized GYC standard media then incubated at 30°C for 48 hours **[9].** 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. All of the isolates were Indole, Voges-Proskauer (VP) and Oxidase negative, Methyl Red (MR) and Catalase positive. The growth rate of isolated strain was optimized by weighing dry cell and turbidity at 600 nm at different concentrations of dextrose (1%, 5% and 10%). Ten (10) % dextrose solution showed rapid growth and higher cell mass than 5% and 1% solution respectively. Acidity of the media gradually increased from 0.102% to 2.18% from day 0 to day 7 and pH of the media decreased from 6.8 to 5.5 during the period. They were successful in doing enrichment of *Acetobacter aceti*, which was essential for vinegar production.

Tahir Zahoor *et al.,* in 2006 made a study conducted by to isolate vinegar culture *(Acetobacter aceti)* from sugar cane juice, rotten apples, flowers, wine, canal water and vinegar as a primary source for *Acetobacter* by continuous sub-culturing on standard medium glucose, yeast extract and calcium carbonate (GYC) **[10]**. GYC Agar described by Swings in 1992 detects the presence of acid-producing microorganisms and is regarded as "standard growth medium" .The culture was identified on the basis of colony characteristics and morphology. It was finally confirmed by different biochemical/enzymatic tests and further specified by nutritional and temperature requirements for the growth. The isolated strain was later used for the production of vinegar through fermentation. Among canal water, crushed apples, sugar cane juice, alcohol, vinegar and flowers, the alcohol and vinegar were found to be the most suitable sources for isolation of *Acetobacter spp*. The colonies of purified culture were found to be pale to off-white, circular, raised, convex, smooth and not 3 mm in diameter with morphology of Gram -ve, ellipsoidal, rods, squat bacilli, roundish, single, in pairs and in chains. The isolated and identified spp. Gave excellent results for the production of vinegar and this vinegar was more acceptable rather than commercially available fermented vinegar. At industrial level good quality vinegar can be

produced by using pure culture of *Acetobacter aceti* for acetic acid fermentation.

Potential acetic acid bacteria were investigated from different readily available sources. Seven different samples (sugarcane bagasse, sugarcane juice, sugarcane juice processing water, soil, rotten apples, rotten red grapes and rotten white grapes) were collected from local market. After processing and enrichment, samples were inoculated on Glucose Yeast Calcium carbonate (GYC) agar plates and incubated at 30°C for four days. Nineteen different bacterial colonies were selected and isolated on the basis of clear zone formation on GYC medium. The bacterial isolates were identified on the basis of their morphological, biochemical and physiological characterization. Among nineteen isolates, one was identified as *Acetobacter aceti*, one as *Acetobacter Pasteurian us*, one as *Acetobacter Orleans* is, two were identified as *Acetobacter fibrinogenesis*, and the remaining fourteen isolates were identified as *Gluconobacter spp*. As potential acetic acid producers, only the *Acetobacter* isolates were further assessed for their acid production capability under different temperature and pH using 'Potency Index' as a potency determining parameter. Temperature 30°C and pH 5.5 were found to be the optimum temperature and pH respectively for maximum acetic acid production by most of the species. *Acetobacter Pasteurian us* with the highest P.I. value of 3.78 was the most potent acetic acid producer among these isolates **[11]**.

**FUTURE PROSPECTIVES:**

Acetic Acid Bacteria are most commonly known for their role in vinegar production. It is necessary to develop pure vinegar cultures for vinegar production so that the use of synthetic vinegar may be avoided as it is prohibited in most countries. Keeping in view all of these points. The present work is conducting to isolate a pure culture of *Acetobacter aceti* and the maintenance of this culture. This strain could be a potential strain for production of vinegar type with a new and desirable taste.

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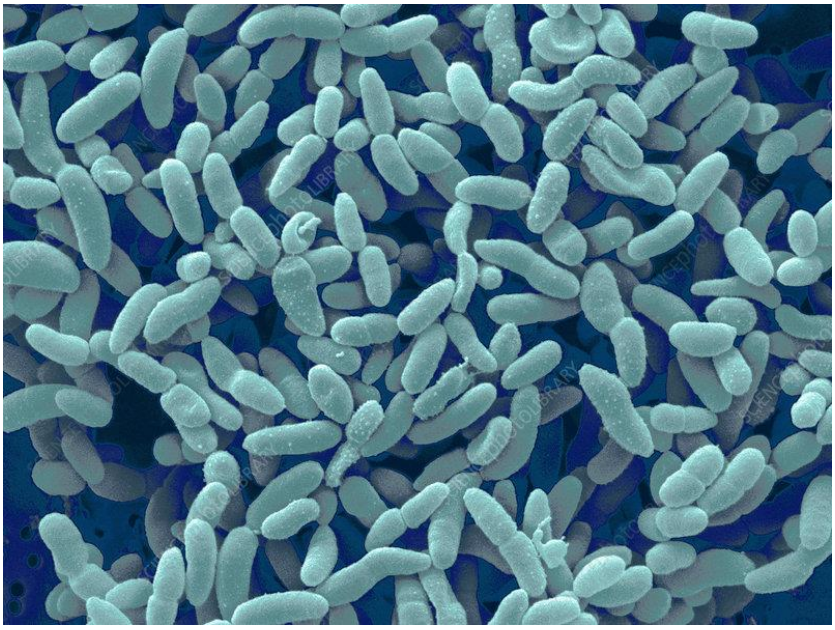

**FIG 1 :** *Acetobacter aceti* microscopic view