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# Isolation and identification of indigenous acetic acid bacteria from Sri Lankan coconut toddy for vinegar production

W. M. S. H. Ranasinghe* and M. M. Gunawardane

Department of Microbiology, University of Kelaniya, Sri Lanka,

*sandunihans92@gmail.com

The aim of this study was to isolate and identify indigenous acetic acid bacterial (AAB) isolates in Sri Lanka, from local coconut toddy samples, which are capable of producing the highest acetic acid concentration that can be further used for vinegar production. Coconut toddy samples were collected from 03 districts in Sri Lanka and AAB isolates were isolated using CARR agar medium. Six bacterial isolates produced yellow color around the colonies after 3 days of incubation, indicating the production of acetic acid. These isolates were primarily identified as AAB. Further confirmation of the bacterial isolates were carried out using colony morphology, motility, endospore staining and biochemical tests. The colonies that produced yellow color around the colonies after extended incubation for 10 days (30 °C) in CARR medium, were identified as *Gluconobacter* isolates, while colonies that produced yellow color initially but returned to its original color after extended incubation of 10 days were identified as *Acetobacter* isolates. Based on the results of morphological and biochemical tests, the isolates were identified as *Acetobacter aceti, Acetobacter xylinum, Gluconobacter hansenii* and *Gluconobacter liquefaciens.* Three isolates were identified as *A. aceti* strains. To select the bacterial isolate with the highest acetic acid production, isolates were allowed to grow on ethanol-yeast extract broth for 14 days at 30°C. Five milliliters from the broth was taken out at every 48 hrs and titrated with NaOH in the presence of phenolphthalein to find out the acetic acid concentration of each broth. Isolates were also tested for ethanol tolerance, temperature tolerance and acetic acid tolerance in order to check the ability of being used as an industrial acetic acid producer. One of the isolates (*Acetobacter aceti)* was identified as the best isolate that can withstand above mentioned conditions. It was able to produce acetic acid concentration of 5.62% within 14 days of shaking incubation. Also it was capable of growing at temperatures of 30°C, 37°C, and 40ºC. Moreover, it was able to tolerate ethanol concentrations of 4-10% and remained viable at acetic acid concentrations of 2-4%. This indigenous *Acetobacter aceti* bacterial isolate produced vinegar with 5.62% acetic acid concentration under very mild conditions, which can be developed in to a mass scale vinegar producer.

**Keywords:** Acetic acid bacteria, CARR agar medium Toddy, Vinegar