



Endogenous ethanol- and thermo-tolerant strain from Malaysia pineapple peel: Screening and molecular characterisation

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ABSTRACT

The current study aims to identify an endogenous acetic acid bacterial strain isolated from a Malaysian pineapple capable of tolerating high ethanol and temperatures. A total of 23 strains of presumed acetic acid bacteria were isolated from the pineapple peel waste. Subsequently, three isolates with the highest acid yield were subjected to 16S rDNA sequencing; *Acetobacter tropicalis* (*A. tropicalis*), *Staphylococcus haemolyticus*, and *Staphylococcus ureilyticus*. Tolerance evaluations against pH, temperature, ethanol, sugar, and acetic acid demonstrated that all three strains tolerated significant temperatures, ethanol, and sugar levels. Based on the results, *A. tropicalis* possesses the potential to reduce cooling system load during acetic acid manufacture in hot climate regions with a notable acetic acid yield.

1. Introduction

Generally, acetic acid is employed in the food industry as a vinegar, flavouring agent, and health beverage (Selvanathan & Masngut, 2023). The substance offers numerous advantageous effects, including anti-obesity, antimicrobial, antihypertensive, antioxidative, and anti-diabetic. The health benefits arise from the diverse bioactive constituents of acetic acid, including polyphenols and micronutrients (Es-sbata et al., 2020).

Acetic acid bacteria (AAB) form substantial microorganism clusters and are widely utilised in acetic acid production due to their abilities to efficiently or partially oxidise ethanol into acetic acid (Gerard et al., 2020; Selvanathan & Masngut, 2023). Moreover, the efficacy of AAB encompasses various industrial and pharmaceutical applications, including nitrogen fixation, pigment production, exopolysaccharides and bacterial cellulose (BC) synthesis (Yang et al., 2022).

Typically, AAB is found on flower and fruit surfaces and in sweet substances and alcoholic beverages. The aerobic microorganism is

catalase-positive and could exhibit Gram-negative or variable Gram properties (El-Askri et al., 2022). AAB belongs to the Acetobacteraceae family, which includes 19 genera, such as *Acetobacter*, *Acidomonas*, *Ameyamaea*, *Asaia*, *Bombella*, *Commensalibacter*, *Endobacter*, *Gluconacetobacter*, *Gluconobacter*, *Granulibacter*, *Komagataebacter*, *Kozakia*, *Neo-asaia*, *Neokomagataea*, *Nguyenibacter*, *Saccharibacter*, *Swaminathania*, *Swingsia*, and *Tanticharoenia* (Gomes et al., 2018).

Conventionally, microbial identification primarily relied on physiological and chemotaxonomic properties assessments. Selective media, such as Carr, Mannitol, or glucose-yeast-calcium carbonate (GYC), have been utilised as initial screening tools to distinguish between acid and non-acid-producing bacteria. On the other hand, AAB attributes have been validated via Gram staining (Mathew et al., 2019), oxidase, and catalase evaluations. Nevertheless, the procedures are limited and do not permit genus- and species-level identifications.

Current molecular biology development offers various genotypic assessments. Novel technological advancements, such as partial 16S ribosomal gene sequencing and bioinformatic tool and sequence databases, have facilitated AAB nomenclature updates and classifications,

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