

Evaluation of Microbial Quality of Unfermented Coconut Sap Collected Under Two Different Systems by Molecular Approaches

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Abstract

Unfermented coconut sap is one of the natural drinks, being traditionally tapped from unopened inflorescence of coconut palm. Unfermented coconut sap undergoes rapid fermentation after contacting with the microorganisms present in the atmosphere; hence the quality of the sap is deteriorated. In the present study, two different sap collection systems were evaluated, namely traditional clay pot system and a poly-bag collection method treated with *Vateria copallifera*. The microbial quality of collected sap was evaluated through molecular methods. DNA was extracted by the modified Cetyl Trimethyl Ammonium Bromide (CTAB) method from the microbial colonies isolated from the collected coconut sap by the two collection systems. ITS1 forward (5' TCCG TAG GTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3') reverse primers were used for yeast species and 27 forward (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 reverse (5'-CGGTTACC TTGTTACGACTT-3') primers were used for the bacterial species for Polymerase Chain Reaction (PCR) amplification. PCR products were analyzed using 1.5 % agarose gel and amplified specific bands were purified with wizard PCR clean-up system. Purified PCR products were subjected to DNA sequencing. Three types (A, B, C) of distinct microbial colonies were isolated from the sap samples collected by two methods. DNA homology data analysis by BLAST concluded that, A, B and C isolates are *Serratia marcescens*, *Achromobacter xylosoxidans* and *Saccharomyces cerevisiae* respectively. *Saccharomyces cerevisiae* is the responsive microorganism for fermentation. *Serratia marcescens* and *Achromobacter xylosoxidans* are environmental abundant opportunistic pathogen and it restricts the direct consumption of unfermented coconut sap. Therefore, hygienic practices need to be applied to increase the quality of coconut sap.

Keywords: Microbial quality, Unfermented coconut sap, Universal primer

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