

# WORLD YEAST CONGRESS

May 14-15, 2018 | Montreal, Canada

## Screening and characterization of stress tolerant yeasts in Nipa Sap fermentation from Cagayan and Quezon Provinces, Philippines

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In an effort to establish a holistic and S & T-based village-scale nipa bioethanol industry, an effort to identify super indigenous yeast species to improve ethanol fermentation and to identify microbial community structure residing in and responsible for natural fermentation of nipa sap. A total of 13 yeast isolates were obtained from the natural nipa sap fermentation in two selected sites of the Philippines, Brgy. Cabaggan, Pamplona Cagayan and Brgy. Tapucan, Mauban Quezon. They were purified and categorized into groups based on the combinations of cultural and molecular characteristics. Based on the banding patterns of isolates generated by microsatellite (GTG)<sup>5</sup> fingerprinting, the thirteen isolates were clustered into four groups. Representatives of each group were sequenced using Internal Transcribed Spacer (ITS). After sequencing the ITS PCR genes and phylogenetic analysis using MEGA7, three isolates shared 99% identity of the ITS rDNA genes with *Saccharomyces cerevisiae* and the other one isolate showed 99% match with two *P. kudriavzevii* strains. These four representative yeast isolates were subjected to different stress tolerance tests. Among all yeasts, YC03 strain was

the most highly acid tolerant (tolerated pH 2.0) and high temperature tolerant (tolerated 45°C). These interesting characteristics may find applications in further molecular biology researches such as the use of this strain as 'yeast cell factory'. The four profiled yeasts were evaluated for ethanol production efficiency using different ethanol production media and top ethanol producer was chosen. YMU1 strain gave the highest ethanol produced using different media with comparable ethanol yield (51.70 g L<sup>-1</sup>) against reference strain *HBV3* (51.90 g L<sup>-1</sup>) after 48 hour fermentation using synthetic media (YPD broth). However, using molasses-based medium and simulated nipa sap medium, ethanol produced by yeast strain YMU1 was observed to be significantly different from *HBV3* after 48 hours of fermentation. Ethanol yield coefficient and specific growth rate of YMU1 were computed as 0.51 g g<sup>-1</sup> and 0.0072 h<sup>-1</sup>, respectively. The application of selected isolates as fermenting organism in bioethanol production from various feedstock in addition to nipa sap could be investigated in further studies.

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