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The Isolation and Identification of Stress Tolerance Ethanol-fermenting Yeasts from Mozzarella Cheese Whey

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***Abstract*— Bioethanol conversions has long been considered as a possible solution for whey utilization. The existence of wild ethanol-fermenting yeasts that able to hydrolyze lactose become an important thing. Aims of the research was to isolate and identified the potential ethanol-fermenting yeasts from mozzarella cheese whey. Research done experimentally and analyzed descriptively. Yeast candidates isolated from mozarella cheese whey with pour plate method and purified on Potato Dextrose Agar (PDA) with the addition of 3% Yeast Extract and 10 ppm of Amoxycillin. The candidates were tested to stress tolerance towards various sugar and ethanol contents. Ethanol-fermenting yeast candidates tested in converting whey into bioethanol by inoculating 2% isolates into mozzarella cheese whey and incubated in room temperature (26-28°C) for 48 hours, then the ethanol contents tested by Gas Chromatography. The isolate that gave the highest ethanol contents identified using RapID Yeast Plus System. Results showed that there are 5.8 x 10^5 cfu/ml yeasts population in mozzarella cheese whey with four isolates that has different macroscopic character. Four candidate showed endurance towards 30% sugar and 20% ethanol contents and isolate W2 chosen as best isolate that produce the highest ethanol contents of 1,515.973 ppm. RapID Yeast Plus System has identified the isolate W2 has 96.43% similarity with *Candida lambica*.**

***Keywords*— ethanol, identification, isolation, mozzarella cheese whey, yeasts**

I. INTRODUCTION

Bio-conversions of cheese whey into ethanol becoming an interesting alternative for reducing the amount of pollutant disposal into the environment. Whey utilization could reduce disposal of high organic matter into stream which manifested in biochemical oxygen demand (BOD) of 30-50 g/L and chemical oxygen demand (COD) of 60-80 g/L with lactose as the most part that responsible for it [1][2]. The future trend for cheese factories is to move towards zero discharge, i.e., move away from high disposal costs and find more environmentally friendly and profitable applications such as fermenting lactose [3]. Although rare, lactose fermentation to ethanol could be done by yeasts. *Kluyveromyces spp.* and *Candida spp.* was the popular yeasts that could ferment lactose into ethanol [1][5][6]. *Saccharomyces cerevisiae* was a frequently used yeast in ethanol producing process because the good fermentative capacity and ethanol tolerance that allowing to produce up to 20% (v/v) ethanol, however wild *S.cerevisiae* strain are unable to metabolise lactose [9]. The search of other lactose to ethanol fermenting yeasts, especially wild yeasts that has endurance towards high ethanol stress were still done to enhance the results [1].

Diversity of wild yeasts in cheese whey will depend on the cheese production process. One of the most cheese produced in recent years is mozzarella cheese which using chymosin and lactic acid culture in their cheese-making process [10]. Previous research showed several yeasts with specific capability originally isolated from chesees and milk products, has capability in utilizing variety of milk components and its derivatives as growth substrates [11]. Many of yeasts strain with ethanol fermenting capabilities such as *S. cerevisiae, K. Marxianus, K. lactis, C. Kefyr* and *C. Sphaerica* was found in mozzarella cheese which is possible become residues in their whey [13].

Aims of the research was to isolate and identify the potential ethanol-fermenting yeasts from mozzarella cheese whey. Meanwhile, the tolerance of high ethanol and sugar concentrations are important characteristics that should be possessed by yeasts for industrial ethanol production [14]. Therefore, yeasts ability in producing ethanol determined and the resistance towards high ethanol and sugar concentration also tested. Chosen isolate that produced highest ethanol content and has ability to tolerate high ethanol and sugar content were identified with RapID Yeasts Plus System.