The Growth of Native Yeasts on Mozzarella Cheese Whey with the Resistance towards High Glucose and Ethanol Contents

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Abstract. The objectives of the research were to determine the growth of native yeasts on mozzarella cheese whey with the resistance towards high sugar and ethanol contents. Native yeasts isolated from 1 ml mozzarella cheese whey that poured into modified Potato Dextrose Agar (Oxoid Ltd.) with the addition of 3% Yeasts Extract/YE (Kraft Foods) and 10 ppm amoxicillin. Yeast-like microorganisms isolated then purified and tested with RapID Yeasts Plus System and analyzed by ERIC (Electronic Code Compendium) http://www.remel.com/eric. The yeasts growth towards high glucose and ethanol was evaluated by growing yeasts in modified Nutrient Broth/NB (Oxoid Ltd.) added with 20 and 30% of glucose monohydrates or 20 and 30% of ethanol concentration and the optical density (OD) measured every 24h until 72h. The results showed that C. tropicalis, Tri.beigelii and Blast.capitatus were native yeasts that could isolated from mozzarella cheese whey. Despite positive growth trend on substrates with high glucose and ethanol concentrations, C. tropicalis and Blast.capitatus showed the best growth.

Keywords: ethanol-resistance, glucose-resistance, mozzarella cheese whey, native yeasts

1. Introduction

The utilization of native yeasts in bioethanol conversions are widely found. Native yeasts has durability and adaptation ability towards the substrate origin and the threat local environment [1]. Nonetheless, native yeast strains also have to show the resistance towards ethanol or even the sugar source on the substrate itself [2].

Mozzarella cheese whey native yeasts likewise need to investigate for its potential in fermenting lactose to ethanol. Lactose is specific sugar from milk-based products which is one of the major numbers of food products that pollute the environment. In producing 1 part of cheese such as mozzarella, 9 part of whey result and it becomes the environmental problem when it disposes directly to the environment [3].

A few type of yeast might be used to ferment ethanol from lactose contained in mozzarella cheese whey [4]. In some occasion, some sugar added to build the ethanol yield, however this actually increases the osmotic stress in the fermentation medium [5]. High sugar and ethanol content in the fermentation medium could affect the growth of yeasts so that high ethanol yield could not be achieved [6].

The research aims to determine the growth of native yeasts isolated from mozzarella cheese whey in high glucose and ethanol contents medium. The ability to grow and adapt high glucose and ethanol medium will be useful in advanced use of native yeasts isolates in fermenting lactose-based substrate into ethanol.
2. Materials and Methods

2.1. Native yeast identification

One ml mozzarella cheese whey poured into modified Potato Dextrose Agar (Oxoid Ltd.) with the addition of 3% Yeasts Extract/YE (Kraft Foods) and 10 ppm amoxicillin, then incubated at room temperature (26°C) for 48h. Three yeast-like isolates purified and one loop from every isolate is added to the RapID Inoculation Fluid until acceptable turbidity based on the inoculation card. After 15 minutes, gently transfer the inoculation fluid into the RapID panel then incubated at 30°C for 4h. After the incubation, add the following reagents to the cavities indicated: 1 drop of RapID Yeast Plus Reagent A to cavities 7 (NAGA) through 14 (PCHO) and 1 drop of RapID Yeast Plus Reagent B to cavities 16 (PRO) through 18 (LGY). After the addition of RapID Yeast Plus Reagent B, allow at least 30 seconds but no more than 1 minute for color development. Read and score the test cavities from left to right using the interpretation guide. The reactivity pattern obtained was used as a basis for identification of yeast to be compared with the pattern of reactivity found in the Electronic RapID Compendium (ERIC™) database [1].

2.2. The growth of native yeasts at high glucose and ethanol medium

Native yeasts isolate taken one loop then grown in modified Nutrient Broth/NB (Oxoid Ltd.) added with 20 and 30% of glucose monohydrates for glucose-resistance test and 20 and 30% of ethanol addition for ethanol-resistance test. Modified NB incubated in room temperature (26°C) then the optical density (OD) measured at λ=600 nm using UV-Vis spectrophotometer for 0h, 24h, 48h, and 72h [7].

3. Results and Discussions

3.1. Native Yeasts Identification

Identification with RapID Yeast Plus System indicate that there are three native yeast species identified as Candida tropicalis, Trichosporon beigelii and Blastoschizomyces capitatus. Yeasts such as Candida spp, and Trichosporon spp. were found as native yeasts from milk-based products also from cheese brines, which is by-product of cheese [8] [9] [10] [11]. Meanwhile, Blastoschizomyces capitatus as Geotrichum capitatum regularly found as contaminant on cheese or dairy products [12].

C. tropicalis is the common species found from whey which is belonging to the Ascomycota of the Hemiascymocetes class [13]. According to [14], C. tropicalis has the ability to use glucose, sucrose, maltose and trehalose. High activities of β-glucoside also shown by C. tropicalis [15].

Blastoschizomyces capitatus which is also commonly called Geotrichum capitatum or Trichosporon capitatus were included into the genus Geotrichum sp., have the ability to use sucrose, hydrolysis of one type of aryl-substituted glucoside, and hydrolysis of two types of arylamide [16]. According to [17], Geotrichum sp. has high arylamidase activity, but low activity of α and β-glucosidase. [18] states that B. capitatus cannot assimilate trehalose.

T. beigelii is one species of the genus Trichosporon which is a yeast-like organism and classified as basidiomycetes, although since 1994, the nomenclature has actually been reclassified into 6 new species however the naming of T. beigelii is still used [19] [20]. T. beigelii isolates showed hydrolysis activity against some aryl-substituted glucoside, namely ρ-Nitrophenyl-α, D-glucoside, ρ-Nitrophenyl-β, D-glucoside, ρ-Nitrophenyl-α, D-galactoside, and ρ-Nitrophenyl-β, D-fucoside. The two isolates also showed urease activity and hydrolysis of all types of arylamides, namely Proline-β-naphthylamide, Histidine β-naphthylamide, and Leucyl-glycine β-naphthylamide. Some types of Trichosporon sp. can secrete enzymes α and β-glucosidase, fucosidase, galactosidase, urease and arylamidase activity such as T. asahii which have extracellular β-glucosidase activity and T. cutaneum with α and β-glucosidase activity, and also α and β-galactosidase, α-fukosidase and arylamidase [21] [22]. Meanwhile, urease activity has been shown to be positive for all types of Trichosporon sp. [23].
GLU (Glucose), MAL (Maltose), SUC (Sucrose), TRE (Trehalose), RAF (Raffinose), LIP (Fatty Acid Ester), NAGA (p-Nitrophenyl-N-acetyl-β,D-galactosaminide), αGLU (p-Nitrophenyl-α,D-glucoside), βGLU (p-Nitrophenyl-β,D-glucoside), ONPG (p-Nitrophenyl-phosphate), αGAL (p-Nitrophenyl-α,D-galactoside), βFUC (p-Nitrophenyl-β,D-fucoside), PCHO (p-Nitrophenyl phosphorocholine), URE (Urea), PRO (Proline-β-naphtylamide), HIST (Histidine-β-naphtylamide), LGY (Leusil-glisin-β-naphtylamide)

Figure 1. Native yeasts identification results by RapID Yeast Plus System

3.2. Native Yeasts Growth

3.2.1. The growth of native yeasts on high glucose medium

Figure 2 showed that three native yeasts have the glucose-resistance ability with the increasing growth trend from 0-72h at the medium with 20-30% glucose contents. Candida tropicalis and B.capitatus shown the highest OD which represent the growth at high contents of glucose. The increase of glucose until 30% shown the highest growth decrease toward Tri.beigelii. Candida tropicalis and B.capitatus shown longer logarithmic phase at 30% of glucose contents until 72h, meanwhile 20% glucose contents given faster growth rate so that the stationer phase achieved at 48-72h.

Figure 2. The Growth of Native Yeasts on the Medium with 20% (A) and 30% (B) Glucose Addition
Glucose contents up to 30% can repress the growth of yeast which shown by the decrease of turbidity which also shown on the OD value [24] [25]. High contents of glucose gave high osmotic pressure that causing slow rate of yeast growth [26]. However, some yeasts can use glycerol to cope with the osmotic pressure resulted from high sugar contents [27]. Mixed of glucose and glycerol as carbon source give slow growth rate at the beginning of fermentation [1]. Besides that, Non-Saccharomyces yeasts high glucose-resistance ability shown by the capacity to absorb succinic and acidic acid that resulted by osmotic pressure condition [28].

3.2.2. The growth of native yeasts on high ethanol medium

The results on Figure 3 showed that three native yeasts have ethanol-resistance ability for 48. The growth of three native yeasts tend to increase for 24-48h then decrease at 72h. The increase of ethanol content gave lower growth towards Tri.beigeli, meanwhile C.tropicalis and B.capitatus are constantly grew. Tri.beigeli was the best ethanol-resistance yeast at 20% ethanol, while B.capitatus showed the highest OD value at 30% ethanol contents medium.

![Figure 3. The Growth of Native Yeasts on the Medium with 20% (A) and 30% (B) Ethanol Addition](image)

Yeasts are threaten by the contents of ethanol because it can obstruct the growth by creating disturbance in substrate utilization [29]. Besides that, the yeasts intracellular construction can be damaged by ethanol then the growth, fermentation and cell generation will be disturbed [30].

Ethanol-resistance ability in yeasts has found at the ethanol addition up to 20% [31]. The ability to resist the ethanol threat identified at yeast cell walls that shown controlled fatty acid synthesis [32]. Besides that, yeasts ability in ethanol synthesis was the fundamental factor that affects the ethanol-resistance ability [33] [34].

4. Conclusion

*C. tropicalis*, *Tri.beigeli* and *Blast.capitatus* has been identified as native yeasts isolated from mozzarella cheese whey. Three native yeasts shown glucose-resistance and ethanol-resistance ability with positive trend of growing at high glucose and ethanol contents medium. *C. tropicalis* and Blast.capitatus showed the highest OD and constant growth at all of the treatments, so it is potential for advance use as ethanol-fermenting yeasts for lactose-based substrates.

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