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Screening of Thermotolerant Yeast from Thai Traditional Starters for Ethanol Production

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Abstract

The aims of this study were to screen and characterize thermotolerant yeast from Thai traditional starters (Loog-pang) for ethanol fermentation. Six samples of Loog-pang were collected from Udon Thani and Nongkai province in the upper north-eastern region of Thailand and screened for thermotolerant yeasts using YM medium (pH 4.5) at 45 °C for 48 hrs. Thirty-three yeast isolates were retrieved and subsequently their growth profiles were investigated in YM broth at 45 °C, 150 rpm for 48 hrs. Ten yeast isolates exhibited the highest growth rate including NK1-4, NK3-5, NK3-6, NK3-8, NK3-10, NK3-13, NK3-14, UD1-1, UD2-2 and UD2-3 were selected for further ethanol fermentation. The study using modified medium supplemented with 20% molasses at 35, 40 and 45 °C for 12 days. The results have revealed that only 4 yeast isolates; NK1-4, NK3-5, NK3-10 and NK3-14 could grow at 35°C and produce ethanol. In addition, these 4 isolates have been grown at 40 and 45 °C but there was no ethanol production observed. The highest ethanol concentration (7.4% v/v) was observed in NK1-4 isolate followed by NK3-10 (2.4% v/v), NK3-14 (2.4% v/v) and NK3-5 (2.3% v/v) on day 4, 12, 12 and 12 respectively. Therefore NK1-4 was used for the up-scale experimental study by using 3L modified medium supplemented with 20% molasses and 10% starter at 35°C for 10 days. The results showed that NK1-4 isolate exhibited the highest ethanol concentration (8.3% v/v) on day 5 with the dry cell mass and specific growth rate (μ) of 35.6 g/L dry and 0.1233h^{-1}, respectively.

Keywords: thermotolorant, yeast, starter, ethanol Fermentation,high temperature

1. Introduction

The increasing demand of ethanol for various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives, has necessarily increased production of this alcohol [1]. Ethanol usually can be produced by chemical synthesis and biosynthesis. Chemical synthesis is occurred by

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hydration of ethylene (C_2H_5). For the biosynthesis, microorganisms especially yeast uses carbohydrates from agricultural products as carbon sources and then converts to ethanol via glycolysis under anaerobic conditions[2].

Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology. An ideal microorganism used for ethanol production has much rapid fermentative potential, improved flocculation ability, appreciable osmotolerance, enhance ethanol tolerance and good thermotolerance [1, 3]. Ethanol fermentation at high temperature is a main necessity for effective ethanol production in tropical counties. The benefits of ethanol fermentation at high temperature are not only decreased risk of contamination but also reduction in cooling costs. At high temperature fermentation, it is necessary to use an efficient yeast strain that can tolerate high temperature[4]. At present, the ethanol production industry uses a mesophilic strain of *Saccharomyces cerevisiae.* However there have been many reports of potential applications of thermotolerant yeast strains in industrial ethanol production, but only a few have been concerned with *S. cerevisiae*[4, 5, 6] Furthermore, various strains of indigenous yeasts that have ability to produce ethanol and have been isolated from different local sources such as silage[3] water samples from sugar factories[4] local fermented foods[7] and fermented pineapple juice[8].

Loog-pang is commonly known as “Chinese yeast cake” to Western people. It is a Thai traditional starter use for food and beverage fermentation, which is a mixed culture of mold, yeast and bacteria[9]. Loog-pang is made from rice flour mixed with a variety of herbs, spices and powdered starter from a previous batch. Herbs and spices serve as an antibacterial medium in alcohol fermentation preventing microbial spoilage or fermentation failure [10].

Sugar cane is cultivated in various parts of Thailand; those crops are utilized for sugar production and blackstrap molasses from sugar industry have used as substrate for ethanol fermentation. Therefore, the purposes of this study were to screen and characterize thermotolerant yeasts from Thai traditional starters (Loog-pang) for ethanol fermentation.

2. Materials and methods

2.1 Isolation of thermotolerance yeast.

Yeasts were isolated from 6 samples of traditional starters (Loog-pang) which were collected from Udon Thani and Nong khai province in the upper north-eastern region of Thailand. Five grams of all samples were stirred in Erlenmeyer flasks containing 95 ml sterile 0.85% (w/v) NaCl and then serially diluted. The serial dilution was made up to 10^{-3} dilution by taking 1 ml of well-shaken suspension and adding into 9 ml water blank tubes. 100 μl from 10^{-2} and 10^{-3} dilutions were plated in triplicates using spread plate technique on YM agar (pH 4.5) incubated at 45 °C for 48 hrs. After incubation, single colony was picked and the cells were observed under microscope. Purified yeast isolates were selected and preserved on YM agar slant (pH 4.5) and stored at 8 °C.

2.2 Studying of yeast growth curve

Each yeast isolate was cultivated in YM broth (pH4.5) and then incubated in a rotary shaker with shaking speed of 150 rpm at 45 °C for 48 hrs. Yeast growth curves were studied by measured the optical density (OD_{660}) of the culture every 2 hr using spectrophotometer. Successful cultures were collected and screened further for their ethanol production efficiency at high temperature.

2.3 Screening of thermotolerane yeasts for ethanol production at high temperature

Screening for high ethanol production was conducted at 35 40 and 45 °C in 500 ml Erlenmeyer flasks containing 270 ml of modified medium supplemented with 20% molasses 1.2 g/L Na_2HPO_4 0.8 g/L KH_2PO_4 0.4 g/L $MgSO_4$•$7H_2O$ and 0.1 g/L $MnSO_4$•H_2O with pH adjustment to 4.5 with 1N NaOH. The inocula were prepared by transferring one full loop of 24 hrs culture grown on a slant of YM agar into an Erlenmeyer flask containing 30 ml of modified molasses medium as above. After incubation on a rotary shaker for 24 hrs, the inocula were transferred at the rate of 10% to the screening medium, followed by incubation on a rotary shaker at 35 40 and 45 °C for 12 days

2.4 Ethanol fermentation in large scale

Batch fermentation was carried out in a 5L plastic jar with 3L of modified molasses medium as earlier described after that inoculated at 35 °C for 10 days by using 10% starter size of the isolation which gave the highest ethanol production.

2.5 Analysis of Fermentation parameters

Ethanol concentration; The percentage ethanol by volume was determined from the table correlating percentage volume of ethanol with specific gravity at 20°C according to the methods of A.O.A.C (1990) [11].

Reducing sugar; The cultured supernatant was analyzed for reduced sugar using DNS method [12].

3. Results

3.1 Isolation and growth study of thermotolerance yeast

Thirty-three isolates of thermolerant yeast were isolated from 6 samples of traditional starters (Loog-pang). The growth ability at 45 °C of all yeast strains was studied. The results found that there were 10 isolates with highest specific growth rate as shown in table 1. In addition, the result in table 1 indicates that differences were found on specific growth rate among yeast isolations at the significant level of .05.

Table 1. Cell dry weight and specific growth rate of ten yeast isolates when incubated at 45 °C

Yeast Isolations	Specific Growth Rate (h^{-1})
NK1-4	0.1233
NK3-6	0.0818
UD2-3	0.0564
NK3-13	0.0530
NK3-8	0.0513
NK3-10	0.0504
NK3-14	0.0499
UD2-2	0.0423
NK3-5	0.0034
UD1-1	0.0266

3.2 Screening of thermotolerant yeasts for ethanol production at high temperature

Ten yeast isolates exhibited the highest growth rate including NK1-4, NK3-5, NK3-6, NK3-8, NK3-10, NK3-13, NK3-14, UD1-1, UD2-2 and UD2-3 were selected for further ethanol fermentation at high temperature (35 40 and 45°C) for 12 days. The result revealed that only 4 isolates NK1-4, NK3-5, NK3-10 and NK3-14 were able to grow and produce ethanol at 35°C (fig. 1) while other yeast isolates could grow but no ethanol produced at 35 40 and 45 °C therefore their results were not showed.

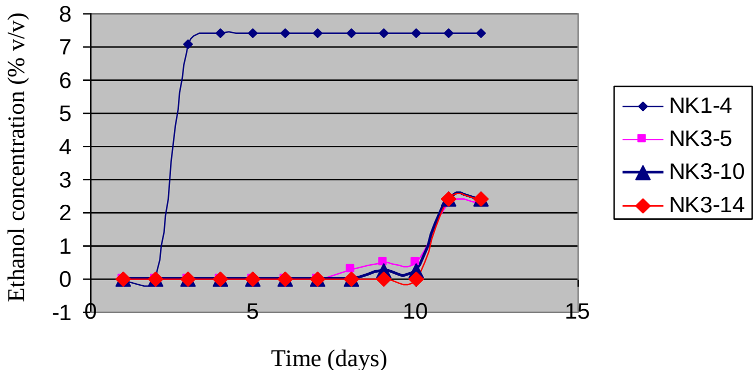

Fig. 1. Ethanol fermentation pattern at 35 °C using NK1-4, NK3-5, NK3-10 and NK3-14 yeast isolates

3.3 Ethanol fermentation in large scale

Batch fermentation was carried out in a 5L plastic jar with 3L of modified molasses medium as earlier described after that inoculated with 10% starter size, 35 °C for 10 days. The result shown that NK1-4 isolate exhibited the highest ethanol concentration (8.3% v/v) on day 5 with the dry cell mass and specific growth rate (μ) of 35.6 g/L dry and 0.1233 h^{-1}, respectively.

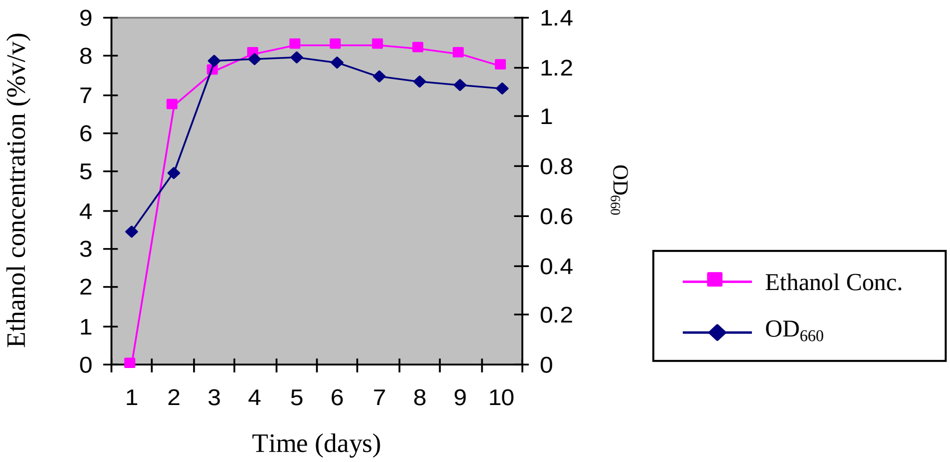

Fig. 2. Growth and ethanol fermentation pattern at 35 °C using NK1-4 yeast isolate

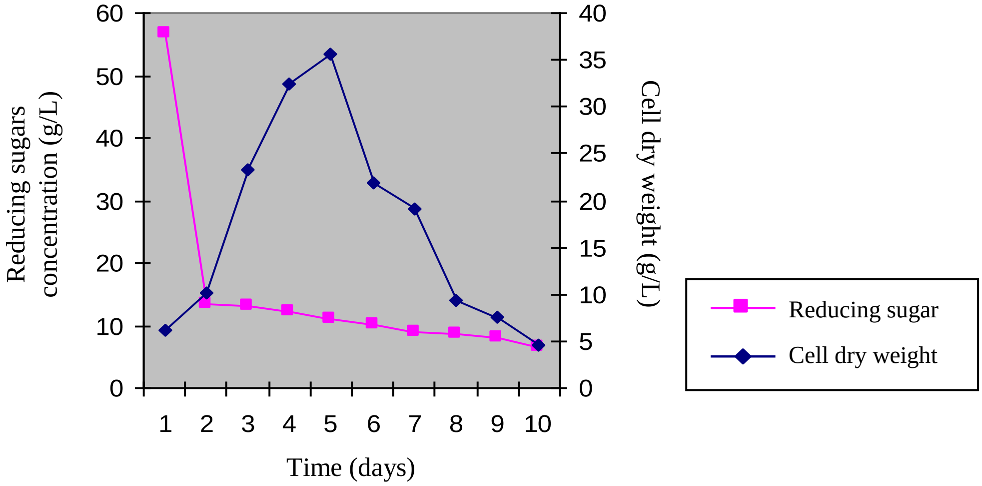

Fig. 3. The relation pattern between cell dry weight and reducing sugars concentration of large scale fermentation at 35 °C using NK1-4 yeast isolate

The result in fig.3 shows the relation between cell dry weight and reducing sugars concentration of large scale ethanol fermentation using NK1-4 yeast isolate at 35 °C. Yeast increasingly grew in first 5 days, at the same time reducing sugars concentration rapidly decreased. The minimum reducing sugars concentration and cell dry weight were 6.458 and 4.98g/L, respectively on the day 10.

4. Discussion

This investigation is to describe the indigenous thermotolerant yeast isolated from six samples of Thai traditional starter (Loog-pang), which collected from Udon Thani and Nongkai Province. Thirty-three isolates of thermotolerant yeasts were screened from those samples, using YM medium (pH 4.5) at 45 **°C**. This result is similar to recent reports, Chaijamrus and Mouthung (2011) achieved to select yeasts from Loog-pang for ethanol production utilizing malted rice from waste paddy [9]. Limtong et.al., (2002) accomplished to isolate various yeasts from 38 samples of Loog-pang. Furthermore, they mentioned that in Asian countries, they normally knew that traditional starters are apparently mixed cultures of molds, yeasts and bacteria grown on rice or other cereals [13]. Therefore, Loog-pang is always available in abundance in Thailand and serves as readily available raw materials for the isolation of yeasts. Capability of ethanol fermentation at high temperature of all isolates was investigated. There were thirty-three isolates of thermotolerant yeasts grown on YM medium at 45 °C but only ten isolates expressed the highest growth rate and they were selected for further ethanol fermentation study using modified medium supplemented with 20% molasses at 35, 40 and 45 °C. The results showed that only 4 isolates (NK1-4, NK3-5, NK3-10 and NK3-14) were able to grow and produce ethanol at 35°C. However, these 4 isolates were able to grow at 40 and 45 °C but no ethanol production was observed. This occurrence was caused from many factors. Stress or environmental stimuli can cause structural changes and/or metabolic changes in an organism acting as expression activator for genes involved in the synthesis of specific compounds that protect the organism. So that temperature is one of an importance abiotic factors that influence on growth and ethanol production [15, 16].

NK1-4 was then used for the up-scale experimental study using 3L modified medium supplemented with 20% molasses with 10% starter at 35°C for 10 days. The results showed that NK1-4 isolate exhibited the highest ethanol concentration (8.3% v/v) on day 5 with the dry cell mass and specific growth rate (μ) of 35.6 g/L dry and 0.1233 h^{-1}, respectively. The previous study, Brady et.al., (1994) discovered the thermotolerant yeast strain, *Kluyveromyces marxianus* IMB3 was capable of growth and ethanol production on lactose containing media at

45°C. On media containing 4% (w/v) lactose, ethanol production increased to 6.0 g/l within 50 hrs and this represented 29% of theoretical yield [14]. Those reports showed that *Kluyveromyces marxianus* IMB3 produced ethanol at higher temperature but lower productivity than this report.

Further studies in the ethanol fermentation at high temperature by thermotolorant yeasts could be performed to learn more about the essential nutrients for those yeasts to enhance growth and ethanol formation. Also, economic potentiality of ethanol production should be considered in future fermentation plan.

5. Conclusion

Six samples of Loog-pang were collected from Udon Thani and Nongkai province in the upper north-eastern region of Thailand and screened for thermotolerant yeasts using YM medium (pH 4.5) at 45 °C for 48 hrs. Thirty-three isolates were retrieved and subsequently their growth profiles were investigated in YM broth at 45 °C, 150 rpm for 48 hrs. Ten isolates exhibited the highest growth rate including NK1-4, NK3-5, NK3-6, NK3-8, NK3-10, NK3-13, NK3-14, UD1-1, UD2-2 and UD2-3 were selected for further ethanol fermentation study using modified medium supplemented with 20% molasses at 35, 40 and 45 °C for 12 days. The results showed that only 4 isolates NK1-4, NK3-5, NK3-10 and NK3-14 were able to grow and produce ethanol at 35°C. However, these 4 isolates were able to grow at 40 and 45 °C but no ethanol production was observed. The highest ethanol concentration (7.4% v/v) was observed in NK1-4 isolate followed by NK3-10 (2.4% v/v), NK3-14 (2.4% v/v) and NK3-5 (2.3% v/v) on day 4, 12, 12 and 12, respectively. NK1-4 was then used for the up-scale experimental study using 3L modified medium supplemented with 20% molasses with 10% starter at 35°C for 10 days. The results showed that NK1-4 isolate exhibited the highest ethanol concentration (8.3% v/v) on day 5 with the dry cell mass and specific growth rate (μ) of 35.6 g/L dry and $0.1233h^{-1}$, respectively.

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