SciMedCentral

International Journal of Plant Biology & Research

Research Article

Isolation and Characterization of Effective Yeast Strains for Bioethanol Production

Swe Zin Yu, Nwe Ni Win Htet, Thet Su Hlaing, and San San Yu*

Department of Biotechnology Research, Microbiology Laboratory, Myanmar

***Corresponding author**
San San Yu, Department of Biotechnology Research, Microbiology Laboratory, Myanmar, Tel: 959-2401-379; Email: sakurasan2007@gmail.com

Submitted: 30 May 2018

Accepted: 14 June 2018

Published: 18 June 2018

ISSN: 2333-6668

Keywords
- Ethanol tolerance
- Temperature tolerance
- Effective yeast strains

Abstract

Since recent years, bioethanol has become greatly interested as an alternative to petroleum derived fuel. The recent study aims at searching for effective native yeast strains for bioethanol production. In this study, fourteen yeast strains that were collected from different sources were identified based on carbon and nitrogen assimilation tests and fermentative capacity tests. Among them, five yeast strains could be assumed as *Saccharomyces cerevisiae* according to the conventional identification. The ethanol and temperature tolerant tests were also carried out. According to ethanol and temperature tolerant tests, the isolate Y3 had the highest temperature and ethanol tolerant level (45°C and 10% v/v respectively).

INTRODUCTION

Alcohol was identified as a possible replacement fuel during 1920's when there was a growing demand for gasoline and potential shortage of oil. The two types of alcohol that could be used as a fuel are ethyl alcohol and methyl alcohol. However, when mixed with gasoline, ethyl alcohol is limited to 10% by volume. Methyl alcohol is very corrosive and this limits its use in mixture [1]. Bioethanol is the most promising biofuel and the starting material for various chemicals production [2]. Ethanol has very acceptable properties as fuel such as high biodegradable properties, low volatility and low evaporation. Therefore, bioethanol produced from renewable biomass has received considerable attention in current years. In contrast to this, during the production process of bioethanol a huge amount of carbon dioxide is released which makes its ecological effectiveness close to zero [3]. Ethanol can be produced by bacteria and yeasts. However, yeasts have some advantages over bacteria in having efficiently conversion to ethanol from sugars and some important industrial characteristics of low nutrient requirements, ethanol resistance and tolerance to pH.

Yeasts are unicellular fungi and this enables them to occupy a wide variety of habitats. A typical environment where yeasts are found is one that is moist and has abundant supply of simple, soluble nutrients such as sugars and amino acids [4]. Yeasts, the heart of the fermentation process, are versatile microorganisms which have been used for centuries by man to produce bread and alcoholic drinks. The most well-known and commercially significant yeasts are the related species and strains of *Saccharomyces cerevisiae* because of its ability to produce high ethanol concentration from simple sugars [5].

During ethanol fermentation, yeasts are exposed to various stresses. Among the stresses, ethanol is considered to be major stress responsible for decrease ethanol production [6]. The process of increasing ethanol tolerance of yeast can be done by some mutation technique [7]. High ethanol tolerant strains are able to extend the process of fermentation for longer time and produce distinct products in the presence of ethanol [8].

In addition to ethanol, heat stress which is generated during fermentation process greatly affects ethanol production and decreases the specific growth rate of yeast strains [9,10]. Moreover, ethanol production at high temperatures has gained much interest due to several advantages, including reduced cooling costs and a reduced risk of contaminations [11]. Hence, the ability to temperature tolerance of the isolates becomes one of the most affecting factors in ethanol fermentation.

Therefore, the aim of this study is to search for the effective native strains of *Saccharomyces cerevisiae* which have ethanol and thermo tolerant activities.

MATERIALS AND METHODS

Isolation of *saccharomyces cerevisiae*

The strains of *S. cerevisiae* were isolated from various fruits purchased from the market, Kyaukse, Mandalay Division, Myanmar. PYG medium (20g/L peptone, 10g/L yeast extract, 20g/L glucose) was used for the isolation of *S. cerevisiae*. After 72 hours cultivation at 30ºC, single morphologically well-formed colonies were isolated. The appropriate ones were re- cultivated several times until they were purified.

Conventional identification of isolated yeasts

Yeasts, unlike moulds, cannot usually be identified by morphological data alone [7]. The yeast strains were identified according to the procedures described Barnett and Payne, Kurtzman and Lodder and Kreger [12-14].

Morphological study: The colonies on PYG medium were determined for colonial morphology (surface, margin, color, shape, etc.). Cellular morphology was examined under compound microscope using high power objective lens (1000x).

Assimilation of carbohydrates: To test the aerobic assimilation of carbon source by yeast, the synthetic medium (0.5% $(NH_4)_2$ SO_4, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 2% agar) was used. The growth of cells for utilization of different sugars was examined up to 3 days incubation.

Carbohydrate fermentation: The ability of anaerobic assimilation (fermentation) of some carbohydrates was determined by using peptone water broth with Durham glass tubes or by adding Bromocresol Purple (2% solution) as indicator. The result was observed daily up to 10 days incubation.

Nitrate assimilation: For investigating the assimilation of different sources of nitrogen, the synthetic medium (2% glucose, 0.1% KNO_3, 0.1% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 2% agar) was used. The results were observed after the 3rd, 7th and 14th day incubation.

Utilization of organic acid: Simmon's medium was used to determine the utilization of citric acid of the isolated strains. After 3 days incubation at 30ºC, the results were observed.

Temperature studies: Temperature studies were carried out using PYG broth. The tube containing broth was inoculated with respective yeast isolate at initial optical density value of 0.1and incubated at 37ºC, 42ºC, 45ºC and 47ºC respectively. After 48 hour incubation period, the growth of each isolate was studied by serial dilution method on PYG agar media.

Ethanol tolerant test: To test ethanol tolerance, both PYG liquid medium and PYG agar medium were used. After the respective yeast isolate has been inoculated into liquid medium supplemented with different ethanol concentrations (5%,10%,15% and 20%) at 30ºC for 48 hrs, the growth of yeast isolates at different ethanol concentrations was checked by streaking out on PYG agar medium.

RESULTS AND DISCUSSION

Isolation and characterization of yeast strains

Fourteen strains of yeasts were isolated from various fruits. Of these, the morphological characteristics of five yeast isolates that were similar as that of *S. cerevisiae* are described in Figure 1a,b and Table 1.

Assimilation patterns of these five isolates on some carbohydrates are presented in Table 2 and fermentation patterns in Table 3.

In this work, it was observed that all five isolates had similar carbon assimilation and fermentation patterns under three times experiments. All strainsY1, Y2, Y3 and Y4 could assimilate and ferment galactose. However, Y5 could not assimilate and ferment galactose and the fermentation of maltose sugar by Y5 is weak. Ann Vaughan-Martini and Alessandro Martini demonstrated that the assimilation and fermentation of galactose sugar by *S. cerevisiae* was variable [15].

In addition to this, *S. cerevisiae* is able to ferment to 6 carbon sugar but not able to ferment to 5 carbon sugars such as arabinose and xylose. In this research, these five isolates were not able to ferment arabinose and xylose.

According to morphological and standard biochemical methods, these five isolates (Y1 from grape, Y2 from loquat, Y3 from mangosteen, Y4 from lychee and Y5 from pineapple) were found to be assumed as *S. cerevisiae.*

However, morphological, physiological and biochemical tests have commonly been used for phenotypic characterization of yeast species. These methods are complex and time consuming and can lead to incorrect classification at species level [16]. Hence, in this research work, molecular identification is needed for classification at species level.

Determination of temperature and ethanol tolerant activity

The heat stress during fermentation process greatly affects the ethanol production and decreases specific growth rate of the strain. Salvado Z et al., reported that *S. cerevisiae* was the yeast best adapted to grow at high temperatures within the *Saccharomyces* genus, with the highest optimum (32.3°C) and

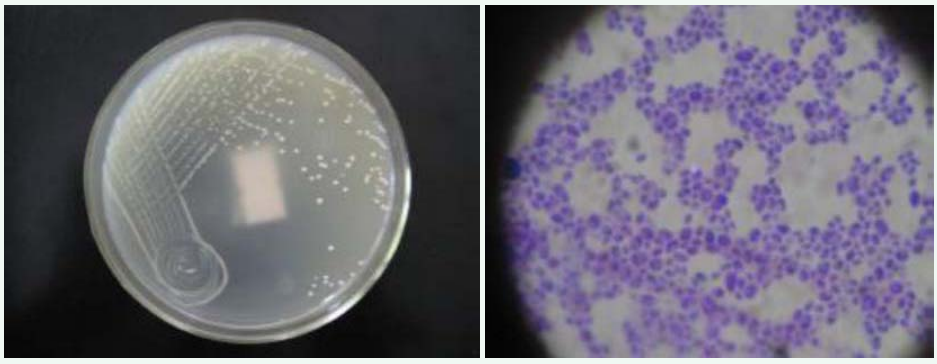

Figure 1 (a) Colony Morphology of yeast isolates (b) Microscopic Morphology of yeast isolate.

Table 1: Morphological characteristics of yeast isolates.

Yeast Isolates	Morphological Characteristics					
	Surface	Margin	Size (mm) and Color	Cell Shape	Cell Size (µm)	Vegetative Reproduction
Y1	Smooth	Entire	1.3, Cream and dull	Spheroidal to ovidal	5-7 x 4-10	Budding
Y2	Smooth	Entire	1.3, Cream and dull	Spheroidal to ovidal	5-7 x 4-10	Budding
Y3	Smooth	Entire	1.2, Cream and dull	Spheroidal to ovidal	5-7 x 4-10	Budding
Y4	Smooth	Entire	1.5, Cream and dull	Spheroidal to ovidal	5-7 x 4-10	Budding
Y5	Smooth	Entire	1.3, Cream and dull	Spheroidal to ovidal	5-7 x 4-10	Budding

Table 2: Carbohydrate assimilation patterns of yeast isolates.

Carbon Source	Yeast Isolates				
	Y1	Y2	Y3	Y4	Y5
Glucose	+	+	+	+	+
Galactose	+	+	+	+	-
Maltose	+	+	+	+	+
Sucrose	+	+	+	+	+
Lactose	-	-	-	-	-
D -Arabinose	-	-	-	-	-
D -Xylose	-	-	-	-	-
D- Ribose	-	-	-	-	-
L-Rhamnose	-	-	-	-	-
Raffinose	+	+	+	+	+
Soluble Starch	-	-	-	-	-
Ethanol	+	+	+	+	+
Methanol	-	-	-	-	-
Citrate	+	+	+	+	+
Nitrate	-	-	-	-	-

+ Can Assimilate - Cannot Assimilate

Table 3: Carbohydrate fermentation patterns of yeast isolates.

Carbon Source	Yeast Isolates				
	Y1	Y2	Y3	Y4	Y5
Glucose	+	+	+	+	+
Galactose	+	+	+	+	-
Maltose	+	+	+	+	+/ Weak
Sucrose	+	+	+	+	+
Lactose	-	-	-	-	-
Arabinose	-	-	-	-	-
Xylose	-	-	-	-	-
Raffinose	+	+	+	+	+

+ Can Ferment; - Cannot Ferment

Table 4: Growth patterns of isolated yeasts at various temperatures.

Yeast Isolates	Growth Patterns at Different Temperatures			
	37ºC	42ºC	45ºC	47ºC
Y1	+++	++	-	-
Y2	+++	++	-	-
Y3	+++	+++	++	-
Y4	+++	++	-	-
Y5	+++	++	-	-

+++ can grow well; ++ can grow; - cannot grow

maximum (45.4°C) growth temperatures [17]. In this study, only isolate Y3 could grow up to 45°C but the other four isolates could grow only up to 42°C as described in Table 4. Hence, Y3 isolate is expected to be performed ethanol fermentation while cooling cost is reduced.

Ethanol counts as a toxin for yeast cells and tolerance to it is closely related to ethanol productivity which is major factor in industrial ethanol production [18]. The microorganisms should grow and produce ethanol in the presence of at least 4% ethanol (v/v) [19]. Ellyastono et al., found that the wild type of *S. cerevisiae* had ethanol tolerance only 2.5% (v/v) ethanol concentrations [20]. In this research work, it was observed that the two isolates (Y3 and Y4) could tolerate up to 10% ethanol concentration and the other isolates, Y1, Y2 and Y5 could tolerate to 5% ethanol concentration.

In the report of Atiya Techaparin, five thermotolerant yeasts, designated *Saccharomyces cerevisiae* KKU-VN8, KKU-VN20, and KKU-VN27, *Pichia kudriavzevii* KKU-TH33 and P. *kudriavzevii* KKU-TH43, demonstrated high temperature and ethanol tolerance levels up to 45ºC and 13% (v/v), respectively [21].

In comparison with other wild type strains, it can be found that five isolates of this research work have higher level in temperature and ethanol tolerance. However, temperature and ethanol tolerant levels of these five isolates were not as high as that of the other designated strains.

CONCLUSION

This study showed that Y3 isolate to be assumed as *S. cerevisiae* had the highest temperature and ethanol tolerant level (45ºC and 10% v/v respectively) among five isolates. Therefore, it can be concluded that the indigenous isolate Y3 had some reliable conditions to use in ethanol production.

ACKNOWLEDGEMENTS

The author would like to acknowledge Biotechnological Research Department, Kyaukse, Myanmar.

REFERENCES

1. Lachke A. Biofuel from D- xylose the second most abundant sugar. 2002; 1-58.
2. Naveen Kumar KJ, Thippeswamy B, Krishnappa M. Bioethanol Fermentation from Fungal Pretreated Lignocellulosic Areca nut (*Areca catechu* L.) Husk using Yeasts and *Zymomonas mobilis* NCIM 2915. Int J Curr Microbiol App Sci. 2016; 5: 571-582.
3. Bioethnol. 2011.
4. Lachance MA, Starmer WT. Ecology and Yeasts. In: Kurtzman CP, Fell JW, editors. The Yeasts, a Taxonomy Study. Netherlands: Elsevier Science. 1998; 21-30.
5. Ma K, Minato W, Kenji S, Yoshihito S. Flocculation phenomenon of a mutant flocculent Saccharomyces cerevisiae strain: Effects of metal ions, sugars, temperature, pH, protein denaturants and enzyme treatments. Afr J Biotechnol. 2010; 9: 1037-1045.
6. Ellyastono EY, Harsojo, Wardani AK. Screening of flocculant *Saccharomyces cerevisiae* (NCYC 1195) for high tolerance of ethanol concentration. IJTRA. 2014; 2: 26-28.
7. White J. Yeast Technology. Norwich: Jarrold and Sons Limited. 1954.
8. Aboserreh NA, Soliman EA, El-Khalek BA. Mutation induction for genetic improvement of Saccharomyces boulardii which used as probiotic yeast. Res J Agr Biol Sci. 2006; 2: 478-482.
9. Patrascu E, Rapeanu G, Hopulele T. Current approaches to efficient biotechnological production of ethanol. Innovative Romanian Food Biotechnol. 2009; 4: 1-11.
10. Sree NK, Sridhar M, Suresh K, Banat IM, Venkateswar Rao L. Isolation of thermotolerant, osmotolerant, flocculating Saccharomyces cerevisiae for ethanol production. Bioresour Technol. 2000; 72: 43-46.
11. Abdel-Banat BM, Hoshida H, Ano A, Nonklang S, Akada R. High-temperature fermentation: how can processes for ethanol production at high temperatures become superior to the traditional process using mesophilic yeast? Appl Microbiol Biotechnol. 2010; 85: 861-867.
12. Barnett JA, Payne RW, Yarrow D. Yeasts: Characteristics and Identification. UK: Cambridge University Press. 1990.
13. Kurtzman C. The Yeasts. A Taxonomic Study. 4th edn. Netherland: Elsevier Science Publishers. 1998.
14. Lodder J, Kreger VR. The Yeasts. A Taxonomic Study. 1953; 45: 147-150.
15. Vaughan-Martini A, Martini A. Description of telemophic ascomycetous genus and species, The Yeasts, A Taxonomic Study. 4th edn.
16. www.blackwell-syngery.com/doi/pdf/
17. Salvadó Z, Arroyo-López FN, Guillamón JM, Salazar G, Querol A, Barrio E. Temperature Adaptation Markedly Determines Evolution within the Genus Saccharomyces. Appl Environ Microbiol. 2011; 77: 2292-2302.
18. Jones RP. Biological Principles for the effects of ethanol. Enume Microb Technol. 1989; 11:130-153.
19. Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund MF, Lidén G, Zacchi G. Bio-ethanol the fuel of tomorrow from residues today. Trends Biotechnol. 2006; 24: 549- 556.
20. Ellyastono EY, Harsojo, Wardani AK. Screening of flocculant *Saccharomyces cerevisiae* (NCYC 1195) for high tolerance of ethanol concentration. IJTRA. 2014; 2: 26-28.
21. Atiya Techaparina, Pornthap Thanonkeo, Preekamol Klanrit. High-temperature ethanol production using thermotolerant yeast newly isolated from Greater Mekong Subregion. Braz J Microbiol. 2017; 48: 461-475.

Cite this article

Yu SZ, Win Htet NN, Hlaing TS, Yu SS (2018) Isolation and Characterization of Effective Yeast Strains for Bioethanol Production. Int J Plant Biol Res 6(4): 1094.