Research Article

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Isolation and characterization of xylose-utilizing yeasts for ethanol production

Abstract

In this research work, twenty two xylose-utilizing yeasts were isolated from various sources. Although all isolates could assimilate all tested sugars, they have variations in sugar fermentation pattern. In temperature tolerant activity, almost all yeast isolates could grow well at 40°C. Weak growth of seven yeast isolates (YP3, YP4, YP7, YP8, YP11, YP12 and YP15) was occurred at 45°C. Yeast isolates could grow at pH range (pH3 to pH6) and their optimum growth was occurred at pH3 and pH4. Moreover, isolated yeast strains were tolerant to ethanol concentration of 5%. Some yeast isolates could grow at 7% ethanol concentration. Among all isolates, YP5 and YP14 could produce 1.1% and 1.5% of ethanol concentration respectively at 14 days incubation period and YP17 could produce 0.6% at 3 days incubation period.

Keywords: assimilate, fermentation, temperature, pH, optimum growth, ethanol concentration

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Introduction

Bioethanol or biofuel as an alternative to fossil fuels has been expanded in the last few decades in the whole world.[1] Ethanol is a clean and renewable type of fuel which can be produced economically and environmentally friendly by reducing greenhouse gas emissions from fossil fuels. Many agricultural by-products can be used as potential raw materials for bioethanol production. The production of bioethanol from agricultural by-products is very prospective because the raw materials do not compete with other food-source materials which contain sugar and starch.[2]

D-xylose, the predominant pentose sugar in plant hemicelluloses, is second only to D-glucose in natural abundance. These sugars represent the majority of all carbohydrates obtained from the hydrolysis of renewable plant biomass, and their efficient utilization is essential for the development of viable biomass to energy conversion processes.[3] Although glucose is more abundant than xylose in nature, fermentation of D-xylose is of interest in enhancing the yield of ethanol. Yeasts such as *Pichia stipitis, P. segobiensis, Candida shehatae* and *Pachysolen tannophilus* are of industrial interest because they can ferment D-xylose to ethanol (Kurtzman, 1998). In using yeast strains in fermentation process, there are some factors important to achieve high yield of ethanol.

Ethanol fermentation at relatively high temperature is an important target for effective ethanol production in tropical countries where average day-time temperatures are usually high throughout the year. The advantages of rapid fermentation at high temperature are not only to decrease the risk of contamination but also to reduce the cooling costs.[4] To achieve high temperature fermentation, it is necessary to use an efficient yeast strain that can tolerate high temperature.[5]

Moreover, ethanol is known to act as an inhibitor to yeast cells, inducing loss of cell viability which results in less efficient fermentation process and thus to reduce ethanol yield and inhibition of yeast growth.[6] Thus, ethanol tolerant yeast strains are beneficial, in order to achieve high fermentation efficiency and finally a high yield of ethanol.[7]

Therefore, tolerance to high temperatures and high ethanol concentrations become important properties of microorganisms of interest to industry.[8] The ability of yeast to produce ethanol depends on many factors such as strains, growth factors and optimum environmental conditions.[9,10] Thus, this study focused on isolation of ethanol producing yeast strains which can tolerate to high temperature and high ethanol concentrations to get high yield of ethanol by agricultural waste fermentation.

Materials and Methods

Isolation of yeast strains

To isolate yeast strains, samples were collected from various fruits, animal feces and agricultural soil. The fruits were purchased from the market in Kyaukse, Myanmar. Animal feces and agricultural soil were also collected from Kyaukse Township of Mandalay Region in Myanmar. Each sample was mixed with 10ml of sterilized 0.9% NaCl solution in sterilized test tube and the mixture was kept standing for 1hour. About 0.5ml of upper clear portion of each sample was added to Yeast Nitrogen Base-Xylose (YX) medium broth (0.67% yeast nitrogen base, 6% D-xylose) supplemented with 0.25% sodium propionate[11] and incubated at 37°C for 72hours. Then, isolated yeast strains were cultured on YX agar medium and the crossed purification was processed until the strains with the same morphology were examined by microscope. Yeast extract-Malt extract (YM) medium was used to maintain the isolated strains.

Characterization of yeast isolates

Study on some characteristics for yeast identification was carried out by conventional technique. The colonies on YX medium and YM medium were observed for colonial morphology. Cellular morphology of yeast isolates from these media was also examined by Gram staining method under microscope using high power objective lens (100x).

To determine the aerobic assimilation of carbon sources, the synthetic media[12] was used. The ability of anaerobic assimilation (fermentation) of some carbohydrates was determined by using

peptone water broth (20g Peptone and 5g NaCl/l). The quantity of the tested carbohydrates was 2%. The results were observed daily up to 3 days incubation period for assimilation test and 7 days incubation period for fermentation test. Other additional tests such as gelatin liquefaction, citrate utilization, nitrate utilization, nitrate reduction, starch hydrolysis, were also performed.[13]

Thermo tolerance of yeast isolates

For determination of thermo tolerance, YX liquid medium was prepared. Then, each tube in which the broth was inoculated by yeast isolate with the initial optical density value of 0.1 and incubated at 25°C, 30°C, 37°C, 40°C and 45°C. After 48 hour incubation period, the growth of each isolate was studied by serial dilution method on YX agar media.[14]

pH tolerance of yeast isolates

To study pH tolerance, YX broth at different pH (3, 4, 5, 6 and 5.49 in original media pH) was prepared. The initial inoculum of each yeast isolate was adjusted to the optical density value of 0.1 and then incubated at 37°C. The YX broth was used as control. After incubation for 48hours, each cell concentration was measured using spectrophotometer at 600nm.[15]

Ethanol tolerance of yeast isolates

Ethanol tolerance of yeast isolates was determined by using YX liquid medium supplemented with different ethanol concentration (0%, 3%, 5% and 7%). With the initial optical density of 0.1, yeast isolates were inoculated and then incubated at 37°C for 48hours and the medium was used as control for respective concentration. Each cell density was measured by spectrophotometer at 600nm.[16]

Ethanol producing activity of yeast isolates

Ethanol producing activity of yeast isolates was determined by potassium dichromate oxidation method.[17] Ethanol standards were made by using ethanol-water solution in the range of 0-10 % ethanol (v/v). Standard curve was prepared by taking 1ml of each concentration of the standard solution [0-10% (v/v)] in a 100ml volumetric flask containing 25ml of potassium dichromate solution. The samples were heated at 60°C for 20 minutes in a water bath and then cooled and diluted to 50ml with distilled water. Absorbance was recorded at a wavelength of 600nm using Spectrophotometer. The amount of ethanol in each sample was determined by using the standard curve of ethanol.

Results and Discussion

Isolation of yeast strains

Twenty two yeast strains were totally isolated from different sources (Y1 from soil source, Y2 from pig's feces and other twenty yeast strains from various fruits including lychee, loquat, rambutan, mangosteen, banana, grape, papaya, pineapple, apple, pear, guava, jackfruit, orange, damson, custard apple and persimmon).

Characterization of yeast isolates

The morphological characteristics of yeast isolates on YX medium and YM medium were studied. Although their colonial morphological characteristics were mostly similar, there were some variations in their microscopic morphology.

Sugar assimilation and fermentation patterns of yeast isolates were described in Table 1 and Table 2. Sugar assimilation patterns of yeast isolates showed the same result in particular carbon sources. But, they have variations in sugar fermentation patterns. All yeast isolates could ferment glucose and sucrose and could not ferment lactose. In xylose fermentation test, most of yeast isolates could ferment the sugar. Some biochemical characteristics of yeast isolates were also studied and they gave the same result in each test as shown in Table 3. According to these characteristics, yeast isolates could not be identified exactly.

Some factors influenced in bioethanol production

There are many factors that influence the etha¬nol yield and fermentation rate in the fermentation process from sugar with microorganism, such as pH, temperature and the sugar content.[18] All yeast isolates were therefore tested for some important factors required in ethanol production.

One of the important factors was temperature tolerance. Cooling costs during the process of ethanol production are expensive; hence, by using thermo tolerant yeasts, cooling and distillation costs can be reduced.[19,20] Thermo tolerant yeasts were able to grow and ferment during the summer months in non-tropical countries as well as under tropical climates. Thermo tolerant activities of yeast isolates were therefore studied. In this study, seven yeast isolates (YP3, YP4, YP7, YP8, YP11, YP12 and YP15) could tolerate 45°C and other eleven isolates were able to grow well at 40°C. Fakruddin reported that their selected yeast strains were thermo tolerant and able to grow up to 46°C temperature.[21] Moreover, Thanonkeo found that three yeast isolated among all isolates obtained from their research exhibited their ability to grow at high temperature 45°C.[10] Hence, yeast isolates in this study had thermo tolerant activity. It is expected that the cooling cost can be reduced when ethanol fermentation is performed with these strains.

The pH of the fermentation medium could highly affect the rate of ethanol production by microbes. Yeast preferred acidic condition and its optimum pH was 5.0 to 5.2, but brewing and distilling strains were capable of good growth at pH range approximately 3.5 to 6.[22] The yeast isolates in this research work were able to grow at pH range from 3 to 6 and their maximum growths were observed at pH 3 and pH 4. So, they were suitable for use in fermentation that was usually performed at acidic condition (Figure 1) (Figure 2).

One of the important factors for ethanol production was the ethanol tolerance of the microbe used in fermentation process. The microorganism should grow and produce ethanol in the presence of at least 4% (v/v) ethanol.[23] Ethanol tolerance of yeast cell is closely related to ethanol productivity.[24] The growth rates of many organisms decrease markedly with increasing ethanol concentrations. Ekunsanmi & Odunfa[25] reported that the ethanol tolerance is an advantage when a yeast is being considered for industrial use especially where ethanol is being produced. Ellyastono et al. found that two mutant strains of flocculant Saccharomyces cerevisiae from 0.8KGy dose that could survive in the presence of 5% (v/v) ethanol concentration, while the wild type could survive in the presence of 2.5% (v/v) ethanol concentration.[26] The yeast isolates were able to grow in the medium containing up to 10% ethanol. In this research work, isolated yeast strains could grow well at 5% ethanol concentration.[10] Their growth was weak at the medium containing 7% ethanol. As yeast isolates could tolerate ethanol in suitable concentration, they were able to use in xylose fermentation to ethanol.

Table 1 Sugar assimilation patterns of yeast isolates

Yeast Isolates	Sugar (carbon sources)										
	Glucose	Galactose	Lactose	Sucrose	Ribose	Rhamnose	Arabinose	Trehalose	Cellobiose	Xylose	Mannitol
YP1	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP2	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP3	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP4	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP5	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP6	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP7	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP8	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP9	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP10	+	+	+(w)	+	+	+(w)	+	+	+	+	+
Yp11	+	+	+(w)	+	+	+(w)	+	+	+	+	+
Yp12	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP13	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP14	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP15	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP16	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP17	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP18	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP19	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP20	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP21	+	+	+(w)	+	+	+(w)	+	+	+	+	+
YP22	+	+	+(w)	+	+	+(w)	+	+	+	+	+

+(w), weak growth

Table 2 Sugar fermentation patterns of yeast isolates

Yeast isolates	Sugar (carbon sources)				
	Glucose	Galactose	Lactose	Sucrose	Xylose
YP1	+	+	-	+	+
YP2	+	+	-	+	+
YP3	+	+	-	+	+
YP4	+	-	-	+	+
YP5	+	+	-	+	+
YP6	+	+	-	+	+
YP7	+	+	-	+	+
YP8	+	+	-	+	+
YP9	+	+	-	+	+
YP10	+	-	-	+	+
Yp11	+	+	-	+	+
Yp12	+	+	-	+	-
YP13	+	+	-	+	+
YP14	+	+	-	+	+

Citation: Htet NNW, Hlaing TS, Yu SZ, et al. Isolation and characterization of xylose-utilizing yeasts for ethanol production. *J Bacteriol Mycol Open Access.* 2018;6(2):109–114. DOI: 10.15406/jbmoa.2018.06.00186

Table Continued

YP15	+	+	-	+	+
YP16	+	-	-	+	+
YP17	+	-	-	-	+
YP18	+	-	-	+	-
YP19	+	-	-	+	-
YP20	+	-	-	+	+
YP21	+	+	+	+	-
YP22	+	-	-	+	+

Table 3 Biochemical characteristics of yeast isolates

Yeast isolates	Gelatin liquefection	Citrate utilization	Nitrate utilizatioin	Nitrate reduction	Starch hydrolysis
YP1	-	+	-	-	+
YP2	-	+	-	-	+
YP3	-	+	-	-	+
YP4	-	+	-	-	+
YP5	-	+	-	-	+
YP6	-	+	-	-	+
YP7	-	+	-	-	+
YP8	-	+	-	-	+
YP9	-	+	-	-	+
YP10	-	+	-	-	+
Yp11	-	+	-	-	+
Yp12	-	+	-	-	+
YP13	-	+	-	-	+
YP14	-	+	-	-	+
YP15	-	+	-	-	+
YP16	-	+	-	-	+
YP17	-	+	-	-	+
YP18	-	+	-	-	+
YP19	-	+	-	-	+
YP20	-	+	-	-	+
YP21	-	+	-	-	+
YP22	-	+	-	-	+

Table 4 Growth of yeast isolates at different temperatures

Yeast Isolates	Different Temperatures				
	25°C	30°C	37°C	40°C	45°C
YP1	++	++	+++	+++	-
YP2	++	++	+++	+++	-
YP3	++	++	+++	+++	+
YP4	++	++	+++	+++	+
YP5	++	++	+++	+++	-
YP6	++	++	+++	+++	-
YP7	++	++	+++	+++	+
YP8	++	++	+++	+++	+
YP9	++	++	+++	+++	-

Citation: Htet NNW, Hlaing TS, Yu SZ, et al. Isolation and characterization of xylose-utilizing yeasts for ethanol production. *J Bacteriol Mycol Open Access.* 2018;6(2):109–114. DOI: 10.15406/jbmoa.2018.06.00186

Table Continued

YP10	++	++	+++	++	-
Yp11	++	++	+++	+++	+
Yp12	+++	+++	+++	+++	+
YP13	++	++	+++	+++	-
YP14	++	++	+++	+++	-
YP15	++	+++	+++	+++	+
YP16	++	++	+++	+++	-
YP17	+++	++	+++	+++	-
YP18	++	++	+++	+	-
YP19	+++	+++	+++	++	-
YP20	+++	+++	+++	+++	-
YP21	+++	+++	+++	+++	-
YP22	+++	+++	+++	++	-

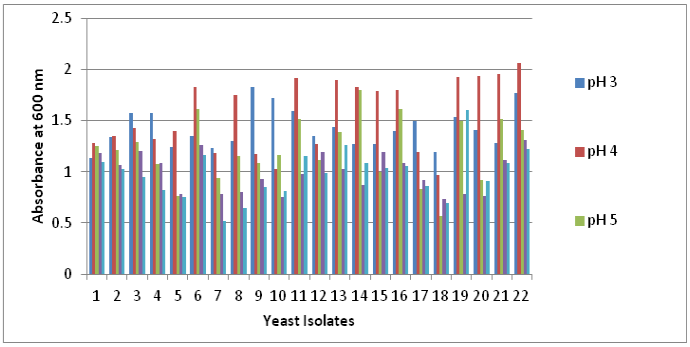

Figure 1 pH tolerance activity of yeast isolates

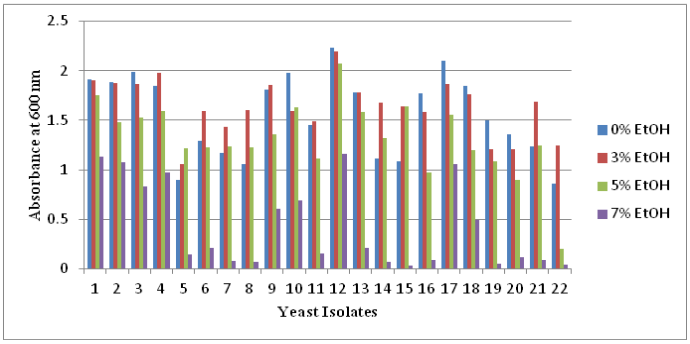

Figure 2 Ethanol tolerance activity of yeast isolates

Ethanol producing activity of yeast isolates

In ethanol production, eighteen yeast isolates could produce ethanol (0.01%-1.5%). The amount of ethanol concentration produced by YP5 and YP14 were 1.1% and 1.5% after 14 day incubation at 37°C. Likewise, YP17 could produce 0.6% after 3 days incubation at 40°C and 0.5% after 14 days incubation at 37°C. In the research of Limtong, *P. stipitis* CBS5773 produced the highest ethanol concentration 1.51% from fermentation of xylose after 36hours.[27,28] According to this research work, three isolates (YP5, YP14 and YP17) could therefore be able to use for ethanol production from xylose fermentation.

Conclusion

In this research work, total of twenty two xylose-utilizing yeast strains were isolated from various sources. Among all yeast isolates, most yeast isolates could tolerate high temperature and wide range of pH. Moreover, they could grow well at 5% ethanol concentration and some isolates could tolerate 7% ethanol. In determining ethanol producing activity, YP5, YP 14 and YP17 could produce 1.1%, 1.5% and 0.6% of ethanol concentration respectively. Therefore, the research of this study could be a source for application of industrial fermentation uses.

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Conflict of interest

None.

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