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Enhancing Bioethanol Production from Sugarcane Molasses by *Saccharomyces cerevisiae* Y17

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THIS STUDY aims to investigate the recycling of agro-industrial wastes as sugarcane and beet molasses for ethanol production using many yeast isolates. Out of fifteen tested isolates of yeasts, seven isolates showed high ethanol production contributing more than or equal 7% from sugarcane molasses with productivity ranging between 1.45 and 1.78$gL^{-1}h^{-1}$. Interestingly, isolate Y17 was the highest ethanol producer (8.55%) and it was chosen for the further optimization experiments. Isolate Y17 was identified phenotypically and genotypically as *Saccharomyces cerevisiae* and then was deposited in the GenBank with accession number KP096551. There was a significant enhancement in the ethanol production (9.55%) using *S. cerevisiae* Y17 from sugarcane molasses fermentation at 18% sugar, 30°C and pH 4.5 for 96h incubation period. These results suggest that *Saccharomyces cerevisiae* Y17 may be subjected to genetic engineering improvement and used as a promising candidate for economical ethanol fermentation by utilization of sugarcane molasses as a renewable and low cost-effective substrate.

Keyword: Bioethanol, molasses, Fermentation, Genotypic, *Saccharomyces*, 18S rRNA gene sequence.

Introduction

Bioethanol is considered a promising type of biofuel that produced by fermentation of sugars and used as a partial gasoline replacement in different areas of the world (Sunggyu & Shah, 2012; Bhatia et al., 2012; Sadik & Halema, 2014 and Rasmey et al., 2017). The sugarcane and beet molasses are by-products of sugar industries in Egypt and are cheap raw materials, readily available, and ready for conversion with limited pretreatments as compared with starchy or cellulosic materials for bioethanol fermentation on industrial scale. Most of sugars in molasses are present in a readily fermentable form (Razmovski & Vucurovi, 2011). Sugarcane molasses is a dark viscous fluid, and rich in nutrients required by most microorganisms such as carbon, nitrogen, phosphorus, sodium, potassium and non-nitrogenous compounds. Beet molasses also is a commonly used feedstock (Dodić et al., 2009). One metric ton of sugarcane is capable of producing approximately 106kg of sugar and 46kg of molasses. A typical molasses to ethanol conversion rate is 4kg of molasses/L ethanol (Lavarack, 2001). However, this rate can vary based on production practices and sugar content of the molasses (Nguyen et al., 2009 and Silalertruksa & Gheewala, 2010).

Molasses composition is usually influenced by the variety and maturity of the cane and beet, soil, climate and the processing conditions in the factory (El-Gendy et al., 2013). High yielding and efficient fermentation of molasses with varying composition requires selection of special yeast strains having high tolerances to inhibitory conditions as well as ability of fast fermentation. Other natural requirements like tolerance to high alcohol, sugar and temperature are also necessary (Bazmi et al., 2007)

Several microorganisms (bacteria and fungi)

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are ethanologenic microbes. Despite the evolving trend of using bacteria for ethanol production, yeast is still the primary choice for fermentation (Chandra & Panchal, 2003). Yeast is extensively used and well-liked microorganisms for wine and fuel ethanol fermentations due to their various unique characteristics like high growth rates (anaerobically or aerobically), proficient ethanol fermentation, and capability to tolerate various stresses (Piskur et al., 2006). *Saccharomyces cerevisiae* is one of the well-known ethanol producers (Izmirlioglu & Demirci, 2012).

The main goal of the present work was to maximize bioethanol production from sugarcane molasses in batch fermentation process using *Saccharomyces cerevisiae* Y17.

Materials and Methods

Feedstock and chemical analysis

Five kilograms of sugarcane molasses was purchased from Sugars and Integrated Industries Egyptian Distillation Plants in Hawamdeia City, Giza, Egypt, while five kilograms of beet molasses was purchased from Delta Sugar Company (DSC), Kafr El-Sheikh, Egypt. The molasses was transferred to the laboratory into sterilized plastic bottles and stored at 4ºC.

The chemical properties of the sugarcane (El-Samman, 2010) and beet molasses (El-Tantawy, 2012) used in this study are shown in Table 1.

Culture medium

The yeast extract peptone dextrose agar (YPDA) medium of the following composition (g/L dist. H_2O): Yeast extract, 10.0; peptone, 20.0; dextrose, 20.0 and agar-agar, 20.0 had been used for maintenance and inoculum preparation according to Wickerham (1951).

Yeast isolates and inocula preparation

Fifteen yeast isolates, previously isolated from different substrates and examined for ethanol production (Hawary, 2014), were used in this study. Active cultures for fermentation experiments were prepared by growing the tested yeast isolates on YPD broth medium for 24h at 30°C and 150rpm.

Sugarcane and beet molasses pretreatment

The clarification of molasses was conducted according to Shashank (1994). The molasses were diluted using distilled water to prepare molasses with 18 % sugar concentration and the pH of the diluted molasses was adjusted to 4.5 using concentrated sulfuric acid. The molasses is heated to about 95°C in water bath for 15min. The hot diluted molasses was left to settle for 2h to precipitate the sludge and the cleared supernatant was transferred into the fermentation bottles.

Fermentation of molasses

Twenty four hours old yeast inoculum was used to inoculate the pretreated molasses at the rate of 10%. Production of ethanol was conducted in 100ml glass bottles that included 45ml of pretreated molasses and 5ml of 24h old culture. The bottles were incubated on a rotary shaker (150rpm) at 30°C for 5h at aerobic conditions and continued the fermentation period to 48h under anaerobic conditions by replacing the cotton plaque by rubber cover according to Gurav & Geeta (2007).

Ethanol determination

Ethanol produced in the fermentation medium was estimated by potassium dichromate ($K_2Cr_2O_7$) oxidation method according to Balasubramanian et al. (2011) and the volumetric ethanol productivity (Qp) was calculated according to Onsoy et al. (2007).

Characterization and identification of the selected yeast isolate Y17

Phenotypic characterization

The morphological characteristics such as colony shape, color, and texture as well as the microscopic features such as the cells shape, budding form of the selected isolate Y17 were conducted on YPDA, acetate agar and 5% malt extract broth while the formation of pseudohyphae was studied on corn meal agar medium using the cover slip method (Lodder & kreger-van, 1952).

Genotypic identification

DNA extraction and amplification: DNA extraction was conducted according to Kumar et al. (2010). The 18S rRNA encoding gene was amplified by the polymerase chain reaction (PCR) from purified genomic DNA using the two primers, 18SF: 5′-TTAAGCCATGCATGTCTAAG-3′ (forward) and 18SR: 5′-GACTACGACGGTATCTAATC-3′ (reverse) according to the methods of Hall et al. (1999). The PCR amplification was performed by using Qiagen Proof-Start Tag Polymerase Kit (Qiagen, Hilden, Germany). The following substrates were combined in 25µl including about

50ng of template DNA, 12.5µl PCR Master Mix, 5pmol (0.5µl) each of forward and reverse primers and the total reaction volume was completed by 11.5µl of water DNAase free water. The reaction mixture was incubated at an automated thermal cycle (Master cycler, Eppindorff, Germany) under the following conditions: temperature cycling comprised 35 cycles of DNA denaturation at 94°C for 30sec, followed by annealing at 52°C for 30sec and extension at 72°C for 3min. Negative control was very important by addition of all above solutions without template DNA. Reactions were stopped by chilling at 4°C. PCR products were analyzed by electrophoresis on 1% agarose TBE-gels (Tris-base Boric EDTA-gel) and the gels were visualized and pictured under UV light. PCR products were purified from gel with the QIA quick gel extraction kit (Qiagen, Hilden, Germany).

DNA sequencing: The amplified PCR products were sequenced (Macrogen Inc.) in both directions using an automatic DNA sequencer (3500 Genetic Analyzer, Applied Biosystems). The obtained sequence was aligned with most closely related taxa retrieved from GenBank using CLUSTALAW program (Thompson et al., 1997). Evolutionary tree was inferred using the neighbour-joining method using Treeviewx Program (Saitou & Nei, 1987).

Optimized conditions for bioethanol production

Different environmental and nutritional parameters were conducted to maximize bioethanol value produced by the tested isolate from sugarcane molasses. The effects of different initial pH values (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5) were assayed. Different molasses sugar concentrations (5% - 35%) with 5% intervals at the primary screening and then with 1% intervals at the secondary screening of the same experiment were tested. The cultures were fermented at different incubation temperatures (25 - 45^{o}C) with 5^{o}C interval. The effect of different fermentation periods (24, 48, 72, 96, 120 and 144h) on ethanol production was estimated. The effect of different nitrogen sources (peptone, yeast extract, ammonium phosphate, ammonium sulfate and urea) on ethanol production was investigated.

Statistical analysis

Analysis of variance (ANOVA) was performed using CoStat V. 6.311 (CoHort software, Berkeley, CA94701). Ethanol production mean values were compared at 5% significance level using Tukey's test. Least significant difference (LSD) test was used to test the significant differences between the whole means of different groups and compared with the critical difference at the 5% level.

Results

Fifteen yeast isolates were screened for their potentiality to produce bioethanol from sugarcane and beet molasses. The results presented in Table 2 showed all the tested yeast isolates were low ethanol producer from beet molasses with production and yield reaching 2.72% and 0.55gL^{-1}h^{-1}, respectively. On the other hand, these isolates were significantly able to produce considerable amount of ethanol from sugarcane molasses. Out of the 15 tested yeast isolates, 7 isolates were able to produce more than or equal 7% ethanol during the fermentation of sugarcane molasses with productivity ranged between 1.45 and 1.78gL^{-1}h^{-1}. It was worth mentioned that the highest ethanol producer isolate on sugarcane molasses was Y17 amounting 8.55% and thus it was selected for further experiments.

TABLE 1. Composition of sugarcane and beet molasses used in this study.

Molasses type	Water content %	Ashes %	Total sugar %	Total nitrogen %	Mineral substances %	pH
Sugarcane molasses	20	11	52	3.4	6.2	5.2
Sugar beet molasses	16	13	49.8	2.0	6.5	8.9

TABLE 2. Ethanol production by the tested 15 yeast isolates at 18 % of sugar sugarcane and beet molasses for 48h.

Isolates code	Ethanol production			
	Sugarcane molasses		Beet molasses	
	EC% (v/v)	Productivity ($gL^{-1}h^{-1}$)	EC% (v/v)	Productivity ($gL^{-1}h^{-1}$)
Y1	7.12 ± 0.01^{c}	1.50	0.80 ± 0.10^{g}	0.17
Y7	7.00 ± 0.10^{c}	1.45	2.72 ± 0.10^{a}	0.57
Y8	4.80 ± 0.02^{d}	1.00	1.94 ± 0.04^{c}	0.40
Y9	8.06 ± 0.03^{b}	1.68	1.72 ± 0.20^{cd}	0.36
Y11	$4.22 \pm 0.02d^{de}$	0.88	2.45 ± 0.05^{b}	0.51
Y13	2.98 ± 0.14^{g}	0.62	2.66 ± 0.02^{a}	0.55
Y17	8.55 ± 0.01^{a}	1.78	1.69 ± 0.01^{ce}	0.35
Y19	3.90 ± 0.10^{f}	0.81	1.80 ± 0.10^{cd}	0.37
Y30	4.00 ± 0.08^{de}	0.83	2.33 ± 0.30^{b}	0.49
Y31	3.72 ± 0.10^{f}	0.78	1.40 ± 0.20^{f}	0.29
Y33	4.11 ± 0.45^{de}	0.86	0.80 ± 0.1^{g}	0.17
Y36	4.77 ± 0.07^{d}	0.99	1.73 ± 0.01^{cd}	0.36
Y38	7.06 ± 0.03^{c}	1.47	2.33 ± 0.10^{b}	0.49
Y40	6.80 ± 0.20^{c}	1.42	1.56 ± 0.06^{ce}	0.32
Y45	6.67 ± 0.20^{c}	1.38	1.60 ± 0.20^{ce}	0.33

Values are means of three replicates ± standard deviation and the values with the same letters are not significantly different.

Identification of the yeast isolate Y17

The identification of yeast isolate Y17 was based on phentotypic characterization and genotypic analysis. Phenotypic characterization included the morphological growth characters and biochemical characters revealed that the isolate Y17 is *Saccharomyces cerevisiae* as shown in Table 3. The shape of cells and budding of the isolate on YPDA, acetate agar, malt extract broth and corn meal agar media are shown in Fig. 1. Subsequently, the alignment analysis of 18S rRNA gene sequence of the isolate Y17 with related sequences obtained from GenBank database revealed that the yeast isolate Y17 is closely similar to *Saccharomyces cerevisiae* strain DAOM 216365 (JN938990) with 99% nucleotides identity (Fig. 2). The selected isolate Y17 sequence was deposited in the GenBank under the accession number KP096551.

Optimization conditions for alcoholic fermentation process

Parameters such as pH, temperature, substrate concentration and nitrogen source were investigated to obtain maximum yield of bioethanol by *Saccharomyces cerevisiae* Y17 from sugarcane molasses.

Effect of pretreatment process on bioethanol production

The pretreatment of sugarcane molasses using sulfuric acid gave an effective impact on the fermentation process by *S. cerevisiae* Y17. Whereas, high ethanol production (8.55%) was obtained after the pretreatment of molasses by sulfuric acid. On the other hand, the using of nitric and phosphoric acids, in the pretreatment process caused in ethanol production by 8.06 and 7.51% , respectively (Fig. 3).

Effect of different hydrogen ion concentrations

Bioethanol concentration gradually increased along with the increase in pH and reached the maximum percentage of bioethanol production at pH 4.5 to be 8.55 (v/v) (Fig. 4). The optimum pH value 4.5 was selected for completing the further experiments.

Effect of different molasses sugar concentrations

Figure 5a showed that the concentration of bioethanol enhanced along the increase in sugar concentration. The maximum ethanol production at sugar concentration was obtained at 15 - 20% of molasses. Furthermore, screening on molasses

sugar concentration was conducted using 1% intervals between 15 - 20% to determine the exact sugar concentration. Figure 5b showed that the highest concentration of bioethanol was determined at 18% sugar. Further increasing in sugar molasses concentration resulted in decrease the bioethanol production.

TABLE 3. Morphological and biochemical characteristics of the isolate Y17.

Characteristics			Obvious
Morphological		Yeast extract peptone dextrose agar	Colonies is butyrous and light cream-colored with smooth surface, raised and opaque.
		5% Malt broth	Cells are subglobose to ovoidal, budding is monobloar, bipolar and multilateral.
		Acetate agar	Cultures are smooth and white to cream in color. Cells are large and subglobose to ovoidal with large nuclei. Vegetative cells are transformed into asci with four ascospores
		Corn meal agar	Pseudohyphae are rudimentary bearing chains of ovoidal blastoconidia.
Biochemical	Fermentation	Glucose	+ve
		Sucrose	+ve
		Lactose	-ve
		Raffinose	+ve
		Trehalose	-ve
	Assimilation	Glucose	+ve
		Galactose	+ve
		Sucrose	+ve
		Raffinose	+ve
		Maltose	+ve
		Lactose	-ve
		Cellobiose	-ve
		Trehalose	+ve
		Soluble starch	-ve
		L-Arabinose	-ve
		L-Rhamnose	-ve
		D-Mannitol	-ve
		citrate	-ve
	Catalase production		+ve
	Urease production		-ve
	Nitrate reduction		-ve
	Growth at 37°C		+ve
	Growth at 2% NaCl		+ve

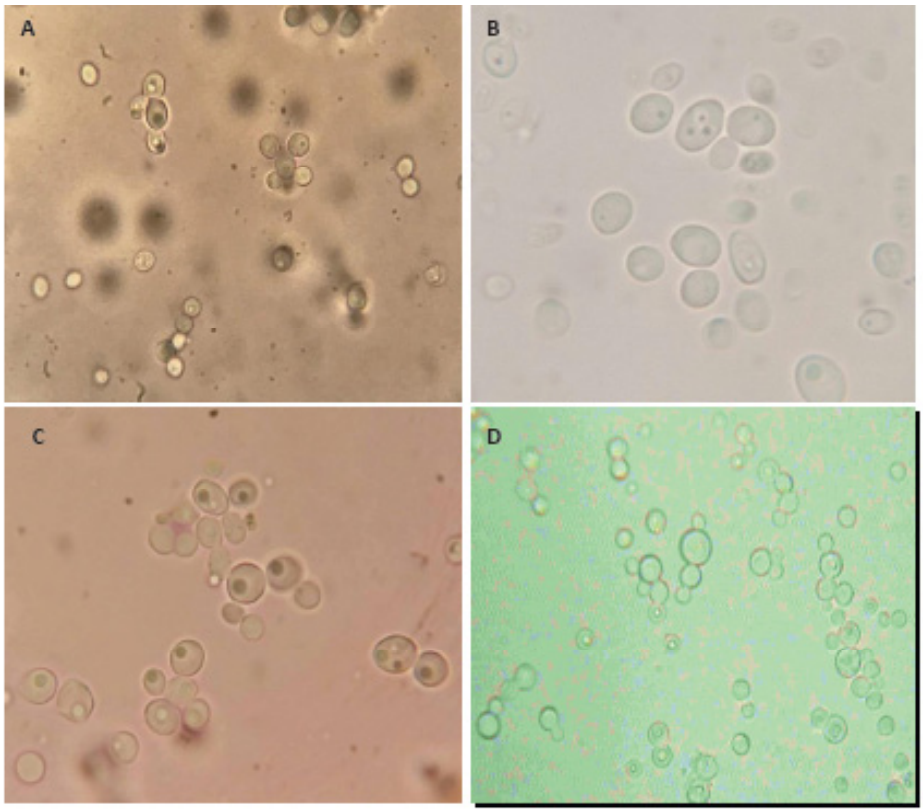

Fig. 1. ***Saccharomyces cerevisiae*** **Y17 (KP096551) on (A) YPDA, (B) Acetate agar, (C) Malt extract broth and (D) Corn meal agar.**

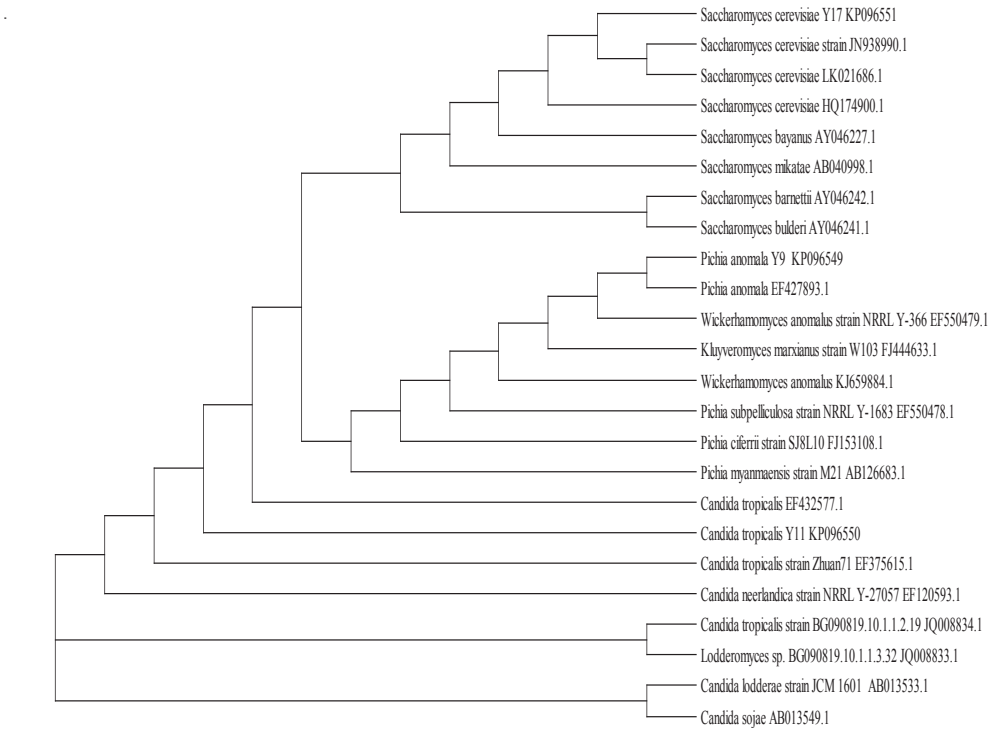

Fig. 2. Phylogenetic tree of nucleotide sequence of ***Saccharomyces cerevisiae*** **Y17 matching with different neighbor sequences.**

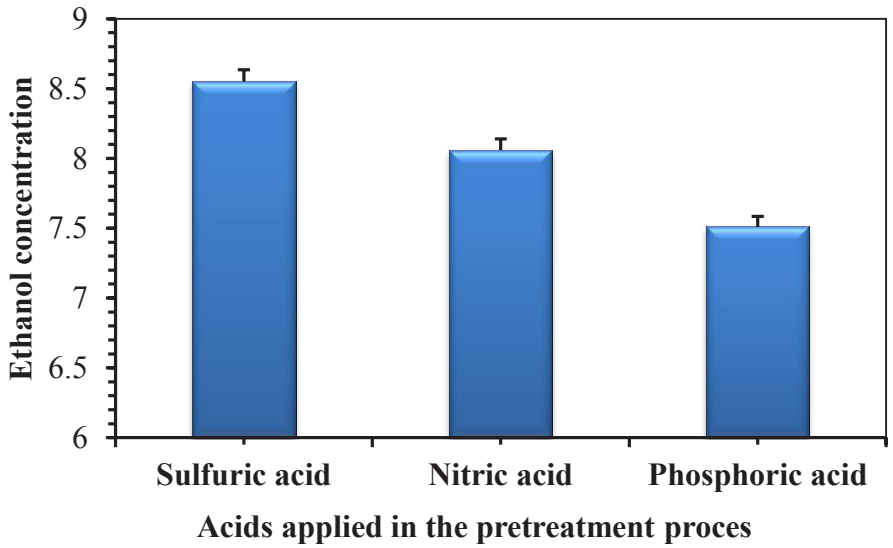

Fig. 3. Ethanol production from 15% molasses by *S. cerevisiae* Y17 during the application of different acids in the pretreatment process.

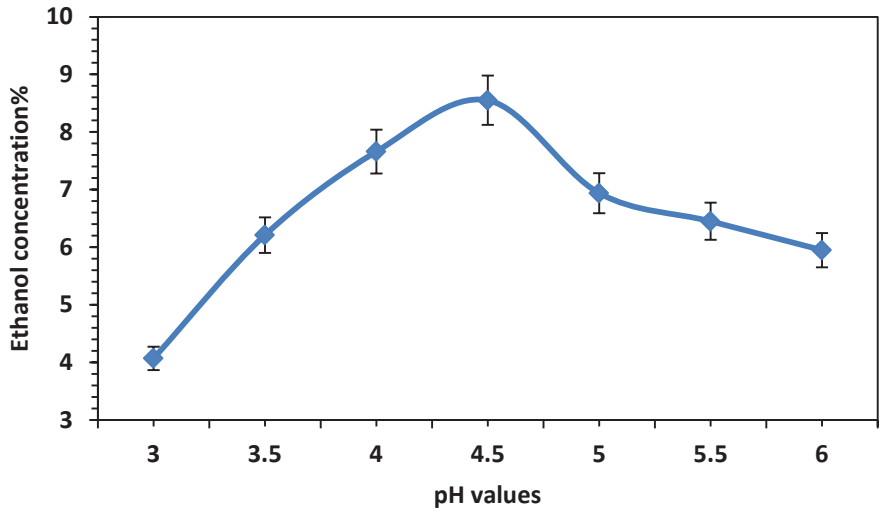

Fig. 4. Ethanol production level by *S. cerevisiae* Y17 as affected with different initial pH values.

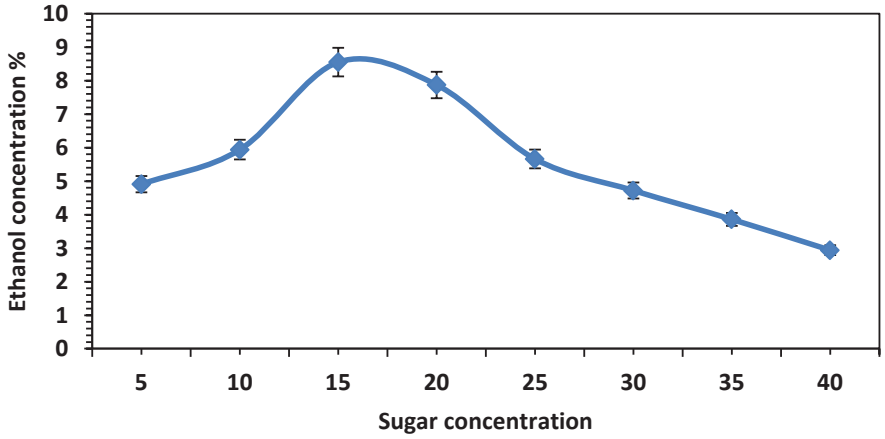

Fig. 5a. Ethanol production level by *S. cerevisiae* Y17 as affected with different initial molasses sugar concentration with 5% intervals.

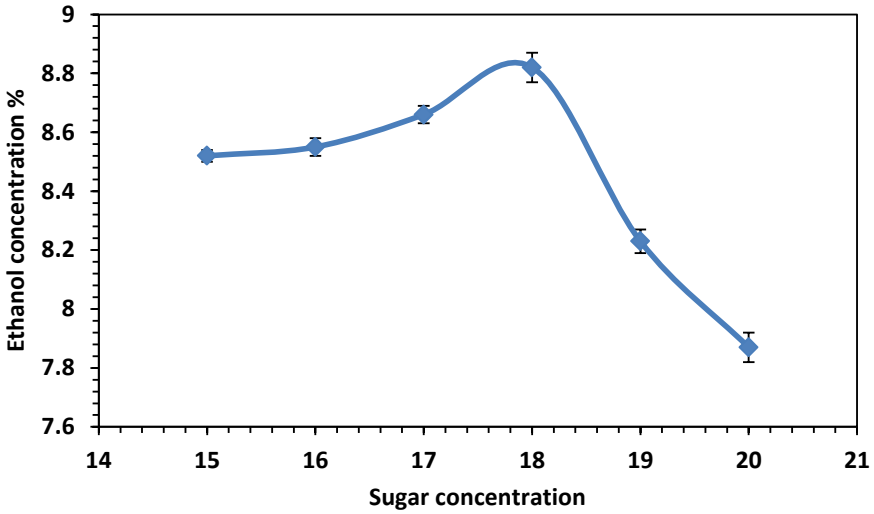

Fig. 5b. Ethanol production level by *S. cerevisiae* Y17 as affected with different initial molasses sugar concentration with 1% intervals.

Effect temperatures and fermentation period on bioethanol production

Bioethanol production by *Saccharomyces cerevisiae* Y17 increased with the increase in temperature and reached the maximum value at 30°C (Fig. 6). Further increase in temperature reduced the percentage of ethanol production.

The results showed that the bioethanol production increased as the fermentation period progressed (Fig. 7). After 24h, the bioethanol concentration was 6.67% with productivity of 2.78$gL^{-1}h^{-1}$. The bioethanol production increased after 48h to 8.82% (v/v) with productivity of 1.83$gL^{-1}h^{-1}$. After 72h, the bioethanol production percentage gradually increased to be 9.18 but with clear decrease in the productivity to 1.28$gL^{-1}h^{-1}$. The optimum ethanol production (9.55%) was achieved after 96h with a further decrease in the productivity (0.99$gL^{-1}h^{-1}$). also, the further increase in the fermentation period showed a gradual decrease in both of the bioethanol concentration and the productivity.

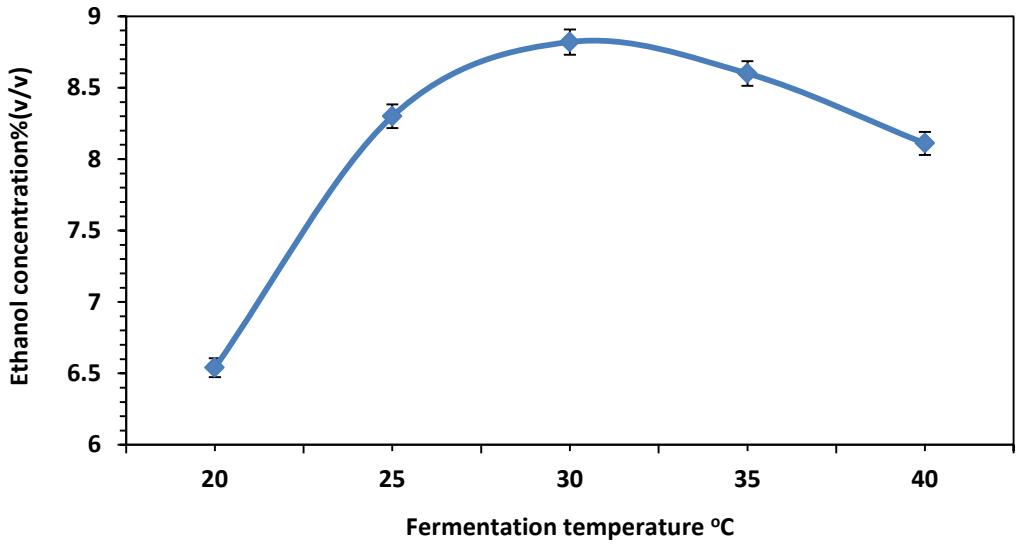

Fig. 6. Ethanol production level % (v/v) by *S. cerevisiae* Y17 at different fermentation temperatures.

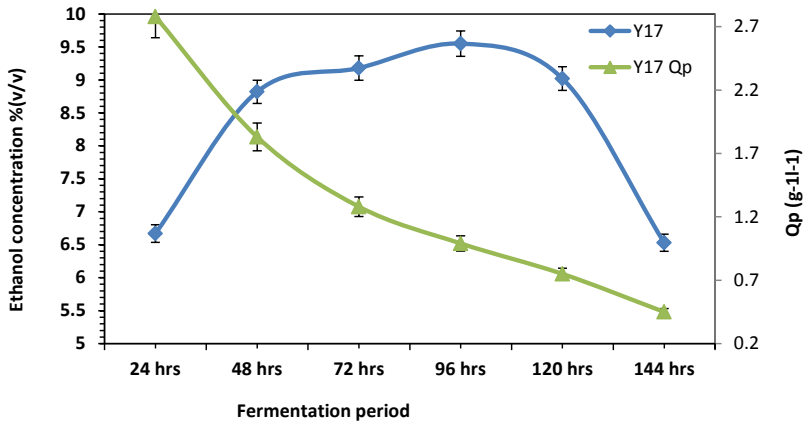

Fig. 7. Ethanol production level and volumetric ethanol productivity by *S. cerevisiae* Y17 at different fermentation periods.

Effect of different nitrogen sources on bioethanol production

This study demonstrated that the supplementation of different organic and inorganic nitrogen sources (peptone, yeast extract, ammonium phosphate, ammonium sulfate and urea) significantly stimulated the ethanol production by *S. cerevisiae* Y17 (Table 4). The maximum ethanol production was associated with addition of yeast extract as nitrogen source in the fermentation medium reaching 9.84%. Urea represented the second nitrogen source that enhanced the ethanol production to 9.82%. Also, peptone improved the ethanol production to 9.72%. On the other hand, the inorganic nitrogen sources ammonium sulfate and ammonium phosphate caused considerable increase in ethanol production to 9.76 and 9.67%, respectively. The ethanol production in the control was 9.55% (Table 4).

TABLE 4. Ethanol production by *S. cerevisiae* Y17 as affected with different nitrogen sources at optimum fermentation conditions.

Nitrogen source	Ethanol production	
	EC%	Productivity ($gL^{-1}h^{-1}$)
Control	9.55^{a} ±.05	1.99
Amm. phosphate	9.67^{b} ±.02	2.01
Amm. sulphate	9.76^{c} ± .03	2.03
Peptone	$9.72b^{c}$ ± .02	2.02
Urea	9.82^{d} ± .07	2.04
Yeast extract	9.84^{d} ± .06	2.05

Discussion

The industrial revolution has been accompanied by an increase in energy needs. Today, fossil fuel is the backbone of the industrial revolution and world energy demand. Alternative energy-related research currently receives immense attention, mostly in relation to the cost of petroleum oil, global warming issue and increased depletion of fossil fuel reserves (Foley & Olabi, 2017). Consequently, interest in liquid biofuels fermentation, which declined following the development of the petrochemical industry, has been retrieved (Abd-Alla et al., 2015). However, a major challenge hampering recommercialization of the ABE process is lack of economic competitiveness due to the absence of inexpensive, sustainable and easily fermentable substrates capable of generating high ABE yields. In Egypt, these are no clear economic and environmental strategies for the utilization of molasses. Therefore, this study aims to explore the possibility of using these wastes as a renewable and inexpensive substrate for ethanol production.

The current study demonstrated that the fifteen tested yeast isolates were able to utilize sugarcane molasses producing considerable amount of ethanol. However, these isolates were lower ethanol producers from beet molasses. The inhibition of some yeast isolates during bioethanol fermentation of molasses might be due to the presence of different toxic substances that can affect yeast growth. Variable amounts of herbicides, insecticides, fungicides, fertilizers and heavy metals applied to beet or cane crops can be found in molasses and in different stocks. Moreover bactericides, which are added during sugar production in refinery plants, can be found (Reed & Nagodawithana,

1988). All these toxic substances can decrease yeast performance by inhibiting their growth (Pérez-Torrado et al., 2009). Patrascu et al. (2009) reported that the ethanol yield from molasses was differed depending on the yeast strain used for fermentation. In this study, seven isolates out of the 15 tested yeast isolates were able to produce more than 6% (v/v) ethanol concentration in the fermented media. Other seven yeast isolates were moderate ethanol producer and only one isolate was low ethanol producer with less than 3% (v/v) concentration from sugarcane molasses. On the other hand, none of the 15 selected isolates had the capability to produce more than 3% (v/v) ethanol concentration from beet molasses. However, Zohri et al. (2012) used Egyptian beet molasses and recorded that the highest level of ethanol production was 9.2 (v/v) at 20% molasses sugar for 72h. This difference in the results might be due to the difference in beet molasses composition, hydrogen concentration, the inhibitors that might present in the molasses and the strains that used.

The conventional phenotypic methods as well as the traditional physiological and biochemical identification tests are not reliable, time consuming and not so precise (Garner et al., 2010). Molecular approaches for characterization and identification, have in part, replaced the traditional methods and are based on DNA base composition, genome association, gene sequencing and PCR based methods (Baleiros-Couto et al., 1994 and Nisiotou & Gibson, 2005). Therefore, the genotypic identification based on phylogenetic analysis was used to identify the selected isolate in this study. Our results showed that the tested isolate Y17 was belonging to family Saccharomycetaceae and identified as *Saccharomyces cerevisiae*. *S. cerevisiae* strains have been identified as the yeast of choice for efficient bioethanol fermentation due to their ability to convert hexose sugars to high concentrations of ethanol despite the presence of inhibitory compounds in medium (Lin & Tanka, 2006).

During industrial scale production of ethanol from molasses fermentation, there were various factors that affect yeast growth as well as fermentative metabolism. The culture conditions such as pH, temperature, sugar concentration, etc. together with effect of additives had profound effect on ethanol production (Jones et al., 1981). So, these factors were investigated to obtain the maximum yield of bioethanol using sugarcane molasses. The *S. cerevisiae* Y17 was selected for the optimization due to its high ability to produce ethanol (8.55% (v/v)) from sugar cane molasses. The selected isolate produced the highest ethanol concentration when the medium pH was adjusted using sulfuric acid. This is supported by Lazaridou et al. (2002) who found that sulfuric acid reduced the harmful compounds found in molasses. The results in this study showed that the bioethanol concentration gradually increased along with the increase in molasses pH and reached a maximum percentage of bioethanol production at pH 4.5 and then it started to decline. An optimum pH of 4.5 was also reported by many investigators (Nadeem, 1992; Yadav et al., 1997; Bhandari, 1999 and Reed, 2001).

Increasing sugar concentration gave a high yield of ethanol during fermentation. However, Jones et al. (1994) found that the very high sugar concentrations are inhibitory to fermentation due to increasing osmotic stress. Borzani et al. (1993) demonstrated the logarithmic relationship between time of fermentation and initial concentrations of sugar. Javid (1994) reported that at the fermentation with 17% sugar concentration, a saturation limit was reached and the sugar repression of the enzymes in fermentative pathway became more significant causing the slower conversion rate. This could be the explanation of the results obtained in this study that the bioethanol yield increased along with the increase in sugar concentration and reach maximum ethanol production at sugar concentration of 18% and then decreased with increasing sugar concentration. Zohri & Mostafa (2000) found that increasing sugar concentration lead to increase the viscosity in fermentation medium which had an inhibitory effect on yeast growth and their ethanol production capability. Also, Reddy & Reddy (2006) reported that increasing in sugar concentration will lead to a decrease in sugar utilization, which results in reduction of the total ethanol production.

The fermentation process accompany with evolution of heat that raises the fermentor temperature and it becomes necessary to cool the large fermentors in the industry, this major operation is of a high necessity and a cost factor in the production of ethanol (Zohri et al., 2012). It is well known that temperature affect growth, metabolism and bioethanol production capability of the fermenting organism. During

this investigation, bioethanol production by the selected isolate increased with the increase in temperature and reaches maximum value at 30°C. These results were consistent with Sedha et al. (2000) and Ali (2010). Further increase in temperature reduced the ethanol production and this might be due to changing the transport activity or saturation level of soluble compounds and solvents in the cells, which might increase the accumulation of toxins including ethanol inside cells (Phisalaphong et al., 2006). The indirect effect of high temperature might also be ascribed to the denaturation of ribosomes and enzymes and the problems with the fluidity of membranes (Gao & Fleet, 1988).

During ethanol production on the industrial scale, yeast may be confronted with a variety of environmental stresses that can cause the loss of yeast cell viability, reduction of yeast growth and increasing fermentation times which in turn decreased fermentation rates (Graves et al., 2007). Fermentation period is a very important factor from an economic point of view in ethanol production. Our results revealed that the ethanol yield was reaching its maximum after 96h (9.55% (v/v) for *S. cerevisiae* Y17. However, the volumetric productivity of the selected yeast isolate was dramatically decreased during this fermentation time, so from an economic point of view, it could be estimated that the optimum ethanol production had been achieved after 48h as both ethanol production and the volumetric productivity were at very high levels. On the other hand, Suryawati et al. (2008) and Faga et al. (2010) reported that the appropriate time for different strains of *Kluyveromyces marxianus* to produce the highest amount of ethanol was 72h. So it is clear that the appropriate fermentation time for the optimum ethanol production depend on the organism as well as the conditions of production.

Nitrogen deficiency slows down yeast growth and the fermentation possibly due to the inhibition of the protein synthesis that transports sugars to the interior of the cells through cell membrane (Butzke & Dukes, 1996). It has been shown that adequate nitrogen increases yeast growth provided that the other essential yeast nutrient is not lacking (Nofemele et al., 2012). Different organic nitrogen sources (peptone, urea, yeast extract) and inorganic nitrogen sources (ammonium phosphate and ammonium sulphate) were investigated as possible nitrogen supplements for the molasses. These results showed that all nitrogen sources investigated had a positive effect on the ethanol yield. The maximum ethanol production was obtained with the organic nitrogen source "yeast extract" where the recorded ethanol concentration was 9.84% (v/v). Yeast extract provides convenient growth factors for microbial growth (Nancib et al., 2001 and Ortiz-Muniz et al., 2010). These results were in agreement with Zayed & Foley (1987) and El-Refai et al. (1992) who found that the addition of urea significantly improved the ethanol yield. Junior et al. (2008) found that peptone improved the fermentation performance of the yeast which was in agreement with the results obtained in this study. On the other hand, it was observed that ammonium sulphate was a good inorganic nitrogen source that stimulated the ethanol production. Ammonium sulphate had been chosen as inorganic nitrogen source for future experiments as it is a simple source that can enter the cell directly (Mendes-Ferreira et al., 2004). Ammonium sulphate is a common and efficient nitrogen source for microbial growth; it is cheap and does not produce a toxic effect towards the microbial enzymes (Bamforth, 2005).

Conclusion

The *Saccharomyces cerevisiae* Y17 KP096551 could be very effective and is potential microbial inoculant for production of bioethanol from sugarcane molasses on large scale.

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تحسين انتاجية الايثانول الحيوى بواسطة خميرة سكارومايسيس سيرفيسيا (*Saccharomyces cerevisiae*) رقم Y17 من مولاس قصب السكر

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تهدف هذه الدراسة إلى محاولة استخدام المخلفات الصناعية الزراعية مثل قصب السكر ودبس البنجر لإنتاج الإيثانول الحيوى باستخدام العديد من عزلات الخميرة. أظهرت النتائج ان عدد سبع من بين خمسة عشر معزولة مختبرة من الخمائر قادرة على إنتاج الإيثانول بنسبة عالية من مولاس قصب السكر بأكثر من أو يساوي 7% بإنتاجية تتراوح بين 1.45 و 1.78 جرام/لتر/ساعة. ومن المثير للاهتمام أن المعزولة Y17 كانت الأعلى انتاجا للإيثانول (8.55٪) وتم اختيارها للتجارب الأخرى لتحسين الإنتاج. تم تعريف المعزولة Y17 بالطرق التقليدية والوراثية على انها سكارومايسيس سيرفيسيا *Saccharomyces cerevisiae* ثم تم إيداعها في بنك الجينات تحت رقم تسلسل KP096551. تم زيادة الإنتاج للإيثانول بعد اجراء تجارب التحسين إلى نسبة 9.55% باستخدام المعزولة Y17 للسكارومايسيس سيرفيسيا من تخمير مولاس قصب السكر تحت ظروف تخمير بنسبة 18% من السكر و تحضين عند 30 درجة مئوية ودرجة الحموضة 4.5 لمدة 96 ساعة فى التحضين. لذلك تشير هذه النتائج إلى أن معزولة الخميرة رقم Y17 للسكارومايسيس سيرفيسيا قادرة على انتاج الإيثانول بنسبة عالية، ومن الممكن أن تخضع لتجارب التحسين من الهندسة الوراثية من اجل رفع كفائتها على إنتاج الإيثانول، واستخدامها كمعزولة ميكروبية ناجحة وقوية لتخمير الإيثانول بشكل اقتصادى عن طريق استخدام مولاس قصب السكر كمصدر متجدد ومنخفض التكلفة.