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Alcohol Fermentation Performance by Novel Genomic Recombinants of *Saccharomyces cerevisiae*

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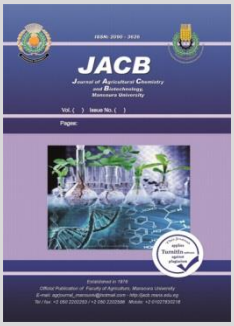

ABSTRACT

This work aimed to describe fermentation characteristics of ethanol productivity by the hybrids of baker's yeast. In this study five parental isolates and 15 genotypic hybrids of *Saccharomyces cerevisiae* derived from three crosses were used for estimating fermentation characteristics during bioethanol production. At 0.02 concentration of sugarcane sub- products the hybrids of P_1 x P_4 increased turbidity and pH value above the mid–parent. In contrast, biomass formation showed decline production. However, at 0.04 and 0.06 sugarcane sub- products some of the hybrids increased significantly pH value above the mid–parent indicating that fermentation profile differs from one concentration of dissolved solids to another. However, ethanol productivity was affected mainly by low pH, high acidity, high alcohol content, high temperature and osmotic pressure. The low pH was the main stress factor on yeast during fermentation. Though, the hybrids derived from the cross between P_2 x P_5 significantly increased pH value above the mid – parent at 0.02 and 0.04 concentration of sugarcane sub–products. The yeast hybrids showed better turbidity and biomass formation at 0.02 concentration if compared with the mid–parents and the higher concentrations of sugarcane sub–products. Most hybrids derived from the cross between P_3 x P_5 showed significant pH values above the mid – parents at all concentrations of sugarcane sub- products. In addition, most hybrids showed significant turbidity above the mid – parents at 0.02 sub–products. However, insignificant differences were shown between yeast genotypes for turbidity and biomass formation at 0.04 and 0.06 concentration of dissolved solids.

Keywords: *Saccharomyces cerevisiae*, hybrids genotypes, ethanol fermentation, biomass, pH, bioconversion, turbidity, sugarcane sub- products.

INTRODUCTION

The two mating types in yeast, a and α leading to genetic recombination such as producing novel combinations of chromosomes. If the two haploid cells are mated they can form diploid cells that can sporulate and undergo meiosis to form another generation of haploid cells, or continue to form diploid cells through mitosis (Herskowitz 1988). *Saccharomyces cerevisiae* genome is composed of about 12.16 Mbp base pairs consisted of 6275 gene organized on 16 chromosomes. About 5800 genes are believed to be functional and at least 31% of the yeast genome are homologs to human genome (Botstein *et al*. 1997).

In Yeast meiosis is a sexual reproduction produced haploid gametes , which can fuse to develop a diploid organism. In baker's yeast, meiosis was directly induced when the environmental conditions are changed such as when starved for carbon and nitrogens then almost cells in the population enter meiosis (Roeder 1995). In yeast meiosis was the first step in sporogenesis leading to the development of haploid ascospores from diploid cells (Winge 1935). Meiosis occurrs when the cells are transfered from a rich growth medium to a poor sporulation medium (De seynes 1868).

Gametogenesis (sporulation) in yeast involves two overlapping steps , meiosis and spore morphogenesis. In which diploid cells respond to nutrient limitation to undergo meiosis to produce four haploid ascospores. Each spore is capable of germinating and fusing with a cell from the opposite mating type (Chu *et al*. 1998). Meiosis in yeast provides a model system for examining developmental process in eukaryotic organism. It was occurrs when a population of yeast cells is transferred to acetate sporulation medium. The result of meiosis and sporulation in yeast is a modified germ cells, the ascus, which contains four haploid ascospores. During this process which occurrs under complete nitrogen starvation, protein degradation happen to be a prerequisite for supplying of amino acids essential for biosynthesis of new sporulation–specific proteins (Betz and Weiser 1976). During ascospores formation in yeast, at least 60–70 % of the pre – existing vegetative protein is broken down until the time of mature asci develop. Proteins synthesized during sporulation is approximately equal the degradation rates as the vegetative proteins (Betz and Weiser 1976). Starvation and progression through meiosis led to marked changes in gene expression (Mata *et al*. 2002).

Industrial ethanol production is dependent on the activity of yeast cells . In some Egyptian locations sugarcane juice industries produce by products running into million of cubic meters per year which has a low value and poses a disposal problems. Today recent industries have developed methods for using the residual sugars containing sugarcane sucker in ethanol productivity (Banat *et al*. 1998). Plasma membrane phospholipids played an

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important role in ethanol tolerance (Ingram and Buttke 1984). Ethanol alters the degree of cell membrane polarity and the polarity of cytoplasm, causing disruption of growth at higher concentration. The disruption may be in the form of increased membrane fluidity at higher concentrations of alcohol (Lioyd *et al*. 1993). Higher contents of cell membrane of unsaturated fatty acids, vitamins and proteins appeared to increase ethanol tolerance (Ingram 1984). Hybrids in *Saccharomyces* have also been described in alcoholic fermentations (Casagerola *et al.* 2001). Therefore, Gonazalez *et al*. (2006) induced hybrids in yeast between *Saccharomyces cerevisiae* and *Saccharomyces kudriavzevii*, the isolated hybrids were grown on decayed leaves in Japan. Moreover, several hybrids in *Saccharomyces* have been found to be well adapted to the environmental stress which occurrs during alcohol fermentation (Belloch *et al*. 2008). When fermentation begins, the yeast cells are affected by osmotic stress due to the high concentration of sugar, as well as, low pH. When fermentation process progresses, other stress appeared such as accumulation of ethanol which become relevant. Similarity, depending on fermentation progresses , temperature is considered as another stresss factor (Cardona *et al*. 2007). The genetic improvement of yeasts can be achieved by hybridization technique via spore conjugation (Winge and Laustsen 1938). The most important fermentation characteristics are ethanol tolerance and efficiency to produce wine without residual sugars (D' Amore *et al*. 1990) .

The aim of this study was to induce new genetically recombinants in yeast via hybridization to investigate fermentation chacteristics of hybrids to stress condition.

MATERIALS AND METHODS

Microbial strains

Five yeast strains are used in this study. These strains, as well as, their references or sources are listed in Table 1.

Table 1. Yeast strains used in this study.

Yeast strains	Source	Designation
Saccharomyces cerevisiae	Baker's yeast , a block of compressed fresh yeast in its wrapper , The Egyptian Starch, Yeast and Detergents Company.	P_1
Saccharomyces cerevisiae	Microbial Genomics and Bioprocessing Research, United States, Department of Agriculture, USA .	P_2
Saccharomyces cerevisiae	Fermented grape juice	P_3
Saccharomyces cerevisiae	Instant yeast supplier silesaffre 59703 marcq, France	P_4
Saccharomyces cerevisiae	Fermented wheat flour juice having a popular name " Buzza "	P_5

Sugarcane juice sub - products

Sugarcane juice wastes were used in this study because of residual sugars containing to be used as a sole source of carbon instead of glucose added to the fermentation medium. It was collected from the local market of sugarcane juice industry in Mansoura city through October 2018. The white fibers inside the stem were collected after remov al of outer surface of the stem. It was cut to separate parts three cm long. The parts containing residual sugars were used as a sole source of carbon in the fermentation medium of ethanol productivity with different concentrations including; 2, 4 and 6% .

Media and growth conditions

Yeast extract peptone dextrose medium (YEPD) was used as a complete medium for growth and maintenance of yeast strains according to Chung *et al*. (1995). Pre Sporulation medium was used to stimulate the cells to sporulate according to Bähler *et al*. (1994). Sporulation medium was also used according to Sherman *et al*. (1982). Fermentation medium used for ethanol production consisted of (g/L), sugarcane juice industrial wastes with the concentration of , 2 % or 4 % or 6 % ; peptone , 10g ; yeast extract , 2 g and distilled water up to 1000 ml.

Antifungal marking agents

Selectable genetic markers are important tools in the construction of yeast hybrids. Ideally, the antifungal markers allow efficient selection of yeast hybrids without affecting any cellular functions. Antifungal resistance markers are alternative to auxotrophic markers. Thus, nine antifungal drugs were used in this study with different concentrations (µg/ml) for genetically marking yeast strains as shown in Table 2.

Table 2. Antifungal drugs and their concentrations used for genetic marking yeast strains.

Antifungal Agents	Concentration (mg/ml)	Abbreviation
Flocazole	0.01	*Floz*
Flucoral	0.01	*Fluc*
Fungican	0.01	*Func*
Treflucan	0.01	*Tref*
Lamisil	0.5	*Lami*
Fungisafe	0.5	*Funs*
Itracon	0.01	*Itrc*
Itranox	0.01	*Itrn*
Trosyd	0.01	*Tros*

Methods

Yeast isolation

Yeast isolates were recovered from four sources including (i) fermented grapes, (ii) baker's yeast, (iii) fermented wheat flour juice having a popular name " buzza " and (iv) instant yeast . About one gram of each source was serially diluted in conical flask 100 ml using distilled water. About 100 µl of each of the last two serial dillutions were spread on the top of YEPD medium. Then, the spread yeast cells were further incubated for 72 hours at 30 °C. Single colonies of the expected yeast isolates were picked up and then purified and screened by using a microscope and selective medium (Bonciu *et al*. 2010).

Genetic marking

Antifungal drugs were used in this study for genetic marking yeast strains. Susceptibility to antifungal drugs was measured by plate diffusion method according to Collins and lyne (1985).

Hybridization technique

This technique was done between the cells carrying the opposite genetic markers until the colonies of cells were appeared on sporulation medium which formed asci.

Then each colony formed asci was picked up and grown on YEPD slant agar medium according to Grinsted and Bennett (1990) .

Biomass formation

After the media were centrifugated at 7000 rpm for 10 min, the precipitated yeast cells were then weighted (Walsh *et al*. 1991).

Turbidity assays

The growth of yeast strains and their hybrids was measured spectrophotometrically in the fermentation medium at 660 nm after the fermentation time was exhausted according to (Morales *et al*. 2017).

Testing pH

The pH of the fermentation medium was measured at the end of fermentation time according to (Nasir *et al*. 2017).

Statistical analysis

Experimental data were subjected to the analysis of variance using completely randomized design according to Snedecor and Cochran (1955). Least significant difference (L.S.D.) was used to compare between means.

RESULTS AND DISCUSSION

Genome shuffling is a powerful technique for rapid induction recombinants in industrial yeast. It depends on the recursive fusion of haploid spores released from asci to allow recombination to occurrs. These recombination within the population amplifies to show genetic diversity in the population by creating new genetically recombinants that may expressed hybrid vigor. Genome shuffling directly accelerates evolution by providing recombinants among selected population (patnaik *et al*. 2002). This study was undertaken not only to induce new genetically recombinants in yeast , but also to investigate the efficiency of these recombinants in converting the residual sugars of sugarcane juice industry into ethanol and biomass of cells, in order to demonstrate the applicability of the superior genotype in ethanol productivity using saccharified industrial wastes.

Genetic marking with antifungal drugs

Various antifungal drugs were used against the five isolates of *Saccharomyces cerevisiae* (Table 3). The results indicated that flocazole, flucoral, fungican, treflucan, itracon and itranox showed genetic variations between yeast isolates. However, the other antifungal drugs as lamisil, trosyed and fungisafe had a similar effect of sensitivity among all isolates. One of the most important mechanisms of antifungal drugs resistance leading to resistance to azoles and other classes of drugs in the up regulation of multidrug transporters which belong to either ATP binding cassette (ABC) superfamily or to the major facilitator superfamily (MFS) of transporters (Cowen *et al*. 2014). The AB transporters are not drug transporters but they translocate phosphoglycerides between the two monolayers of lipid bilayer in the plasma membrane (Prasad *et al*. 2017).

Similarly, *S. cerevisiae* was similar to *Candida albicans* for the effect of azole drugs and phosphoglycerides. ATP–binding cassette drug transporters in fungi were located in the plasma membrane. This suggested that plasma membrane localized ATP–binding cassette transporters are significantly related to drug resistance. These transporters may also identify drugs as a substrates. Meanwhile, vacuoles are organelles in the cells are known to be entered in detoxification by sequestrating toxic compounds. It may taken part in fluconazole resistance in *Candida albicans* because resistant isolates were observed to possess numerous large vacuoles if compared with sensitive isolates (Maebashi *et al*. 2002). Recently, Khandelwal *et al*. (2016) found that vacuole localized multidrug resistance protein in *S. cerevisiae* could transport phosphatidylcholine into the vacuolar lumen, however the absence of these transporters leading cells susceptible to methotrexate. This led us to support the hypothesis that *S. cerevisiae* might be involved in drug transport. Furthermore, Khandewal *et al*. (2019) characterize the possibility of vacuolar transporter in yeast and found that the yeast import azoles into the vacuoles and its deletion leading to exhibited suspeptibility to exposed drugs. In addition, Birky and JR(1975) demonstrated that chloramphenicol, erythromycin and oligomycin conferring resistance genes were located on the mitochondrial DNA of *Saccharomyces cerevisiae*.

Table 3. Genotypes of different *Saccharomyces cerevisiae* isolates based on sensitivity or resistance to antifungal drugs.

Antifungal drugs	Symbol	Yeast isolates				
		P_1	P_2	P_3	P_4	P_5
Flocazole	*Floz*	-	+	+	+	+
Flucoral	*Fluc*	+	+	+	+	-
Fungican	*Func*	-	-	-	+	+
Treflucan	*Tref*	+	-	-	+	-
Lamisil 0.5	*Lami*	-	-	-	-	-
Lamisil 0.1	*Lami*	-	-	-	-	-
Itracon	*Itrc*	-	-	-	+	-
Itranox	*Itrn*	+	+	-	+	-
Trosyd	*Tros*	-	-	-	-	-
Fungisafe 0.5	*Funs*	-	-	-	-	-
Fungisafe 0.1	*Funs*	-	-	-	-	-

+ , - : Resistance and sensitive to antifungal drugs , respectively.

Genome shuffling

Genome shuffling is a powerful tool for rapid improvment of ethanol productivity in yeast. The starting population was generated by hybridization as shown in Table 4. The parental isolates involved in each conjugation were carrying the opposite genetic markers depending on antifungal drugs. For each hybridization, the cells were transferred from complete medium (YEPD) to presporulation.

Table 4. Hybridization between different genotypes of *Saccharomyces cerevisiae* carrying the opposite genetic markers of antifungal drugs.

Mating	Parental genotypes	Recombinant genotypes	Hybrids
P_1 X P_4	*Floz*$^+$ *Func*$^+$ *Itrc*$^-$ X *Floz*$^+$ *Func*$^+$ *Itrc*$^-$	*Floz*$^+$ *Func*$^+$ *Itrc*$^+$	H_1 H_2 H_3 H_4 H_5
P_2 X P_5	*Fluc*$^+$ *Func*$^+$ *Itrn*$^-$ X *Fluc*$^+$ *Func*$^+$ *Itrn*$^-$	*Fluc*$^+$ *Func*$^+$ *Itrn*$^+$	H_6 H_7 H_8 H_9 H_{10}
P_3 X P_5	*Fluc*$^+$ *Func*$^-$ X *Fluc*$^+$ *Func*$^-$	*Fluc*$^+$ *Func*$^+$	H_{11} H_{12} H_{13} H_{14} H_{15}

Medium for 48 hr prior to transfer to sporulation medium containing selectable markers of antifungal drugs. Cultures were maintained in sporulation medium for at least 30 days until single colonies developed on selective media. The positive colonies were picked up to be evaluated for ethanol production. The population of cells transferred to sporulation medium which in its diploid state were taken the direction to sporulation by starvation on sodium acetate sporulation medium. Since, sporulation in yeast as in bacteria may be dependent on acetate utilization (Nurse 1985). During sporulation state meiosis occurred to be resulted a modified cell named asci which contains four haploid ascospores. Mating occurred between two spores differed in their mating types (a x α) from different asci to reform diplophase from which the positive genotypes were appeared on sporulation medium. During this process the cells undergo change in genetic constitution from diploid through meiosis to haploid cells carrying meiotic recombination. In this respect, Roth and Fogel (1971) reported that only heterozygous diploid cells (a x α) at the locus of the mating type are able to undergo DNA replication, meiotic recombination and sporulation. In contrast, homozygous diploid cells (a / a or α / α) in the mating type locus were not capable of these processes (Roth and Lusnak 1970). Though, sporulation can occur only in the cells carrying the opposite mating types a / α , it is usually initiated by transferring the cells to sporulation medium containing sodium or potassium acetate (McCusker and Haber 1977). Respiration is necessary for sporulation because of the fact that petites mutants can not sporulate (Sherman and Slonimski 1964). After the cells are transfered to acetate medium, glycogen was initiated only by the cells to accumulate after sporulation, eventually, the acetate can be exhausted and glycogen be used as a source of carbon (Kane and Roth 1974). The results obtained in this study agreed with Hopper *et al.* (1974), who reported the degree of correlation between each biochemical event such as DNA synthesis, meiotic recombination and sporulation by carrying out the measuring on cultures of both sporulating (a / α) and otherwise isogenic (a / a or α / α) homozygous diploids of the mating type. Furthermore, Kassir and Simchen (1976) reported that the meiotic process can start only when the cells are heterozygous for the mating type locus as a / α which will accomplish meiosis and spore formation. Hawthorne and mortimer (1963) demonstrated that haploids from the same mating type were able to mate but enabled to sporulate. This indicated that diploid cells used in this study are heterozygous for the mating type (a / α) because they are sporulate and generate new meiotic recombinants.

Alcohol fermentation characteristics

The results of fermentation products of sugarcane juice wastes ranged from 20 g to 60 g per litre are shown in Table 5. At 0.02 concentration the hybrids showed vigorous turbidity in relation to the mid parents. However, biomass yield showed decline production. In addition, all hybrids significantly increased pH value above the mid parents, in terms of sugar fermentation pattern which converted to ethanol. The fermentation profile was not consistent at higher concentrations of sugars because none of the hybrids showed significant turbidity at 0.04 concentration, but two hybrids out of five showed significant turbidity at 0.06 concentration above the mid parents .

Table 5 . Fermentation results of sugarcane juice industrial wastes by the hybrids of *Saccharomyces cerevisiae* resulted from the mating between P_1 X P_4.

Strains	Concentration of sugarcane refuse (g / 100 ml)								
	2			4			6		
	Turbidity	pH†	Biomass (g/l)	Turbidity	pH††	Biomass (g/l)	Turbidity	pH†††	Biomass (g/l)
P_1	2.09	4.97	24.36	1.68	8.85	16.55	1.06	6.75	16.83
P_4	1.93	4.97	31.57	1.77	4.96	12.35	1.87	7.75	22.34
MP	2.01	4.97	27.96	1.72	6.92	14.45	1.47	7.43	19.59
H_1	2.09	8.73	25.51	1.78	7.70	13.32	2.23	8.50	21.46
H_2	2.19	8.73	19.81	1.80	8.25	21.99	1.98	8.70	22.95
H_3	2.15	8.67	21.27	1.60	8.10	19.63	1.96	8.45	22.66
H_4	2.21	8.23	25.03	1.06	4.15	16.13	1.34	6.85	10.90
H_5	2.13	8.50	22.53	1.90	7.95	17.85	2.00	8.00	15.97
F - test	NS	**	NS	**	**	*	**	**	NS
LSD 0.05	-	0.38	-	0.20	0.78	6.83	0.53	0.98	-
LSD 0.01	-	0.53	-	0.27	1.13	9.58	0.74	1.45	-

***, ** = Significance at 0.05 and 0.01 levels of probability, respectively , NS = Insignificant differences.**
† Initial PH = 5.5, †† Initial PH = 5.6, ††† Initial PH = 6.1.

In addition, two other hybrids out of five produced significant weight of biomass at 0.04 concentration above the mid parents, but not the same hybrids showed significant turbidity. This indicated that these hybrids fermented vigorously the sugars to be forming biomass and ethanol, as well with reduced the quantities of secondary metabolites that have a marked effect on turbidity, all of which contribute to the product aroma (Steensels *et al.* 2014).

On the other hand, four hybrids and three hybrids, out of five for each, increased significantly pH value above the mid parents at the concentration of 0.04 and 0.06 sugarcane juice industrial wastes, respectively. This may be due to high alcohol production increasing pH value. The result obtained herein indicated that fermentation profile of sugarcane sugars differs from one concentration to another. The existence of natural hybrids between different species or within the same species has already been suggested by several authors (Masneuf *et al.* 1998).

The results obtained in this study agreed with Romano *et al.* (1985), who achieved the genetic improvement of wine yeasts with homothallic and self – diploidizing strains by the technique of Winge and Laustsen (1938) via hybridization by spore conjugation.

The recombinant hybrids derived in this study are a considerable improvement over the parental strains and possess characteristics making them suitable for the fermentation of residual sugars in sugar crop wastes. The results obtained herein agreed with Wilkins *et al.* (2007), who found that fermentation at initial pH 6.0 produced most ethanol, but at pH 4.4 produced the least ethanol. This indicated that the residual glucose concentrations increased as pH decreased which are due to the inability of baker's yeast to ferment glucose at lower pH values. The same authors found that final pH linearly correlated with glucose concentration in the fermentation (r^2 = 0.97). The results suggested that the increasing media pH can alleviate some of the inhibitory effect of acidity on fermentation (Casey *et al.* (2010). However, Novak *et al.* (1981) demonstrated that produced ethanol was more toxic for yeast cells affecting the growth to be stopped. The decline in pH was caused by dissolved carbon dioxide or to the production of organic acids (Jayaram *et al.* 2013). This indicated that pH is a key factor that affects ethanol fermentation (Kasemets *et al*. 2007). The lower pH was related to yeast inhibition because of undissociated organic acids enters the cells by simple diffusion method through plasma membrane (Cassio *et al.* 1987). The lower pH caused acidification of intracellular pH which stimulate H^+-ATPase of plasma membrane to eliminate intracellular H^+. The pH less than 4.0 leading to increased H^+- ATPase activity three times with expenditure of ATP resulting more stressed in yeast cells under this condition (Eraso and Gancedo 1987). Turbidity, pH values and biomass production by all yeast hybrids were significantly increased above the mid parents at 0.02 concentration of sugarcane juice by-products (Table 6). However, insignificant differences were shown between yeast genotypes for turbidity and biomass production at 0.04 and 0.06 sugarcane juice sub- products.

In contrast, at 0.04 concentration of substrate all hybrids significantly increased pH value above the mid parents. In addition, four hybrids out of five significantly increased pH value above the mid parents at 0.06 concentration. This may be due to vigorous alcohol production by these hybrids above the mid parents. These results indicated that yeast hybrids might have the capability to growth and utilize the sugars in the wastes of sugar crops to produce biomass and higher ethanol productivity which affected to increase pH value. Therefore, yeast hybrids showed significantly better growth at the lower concentration of sugar (0.02 g) than at the higher concentration, which showed insignificant results between yeast genotypes for turbidity and biomass production. Accordingly, yeast hybrids might be also better adapted to metabolize the carbon sources in the wastes of sugar crops. These results agreed with Gonzalez *et al.* (2007), who found that natural yeast hybrids are not only well adapted to fermentation conditions but they produce higher amounts of aromatic compounds than the parental strains. Consequently, the construction of laboratory hybrids imitating the natural evolution process represents a promising method for genetic improvement of baker's yeast. These results open a new trend in the construction of new hybrids with biotechnological approach to be used to convert carbon sources in the wastes of sugar crops into economical products

Table 6. Fermentation results of sugarcane juice industrial wastes by the hybrids of *Saccharomyces cerevisiae* resulted from the mating between P_2 X P_5.

Strains	Concentration of sugarcane refuse (g / 100 ml)								
	2			4			6		
	Turbidity	pH†	Biomass (g/l)	Turbidity	pH††	Biomass (g/l)	Turbidity	pH†††	Biomass (g/l)
P_2	2.06	4.80	31.12	1.52	8.30	16.97	2.06	8.03	13.67
P_5	1.73	4.7	21.92	1.46	6.40	15.29	1.55	6.10	12.57
MP	1.89	4.75	26.52	1.49	7.35	16.13	1.80	7.07	13.12
H_6	2.80	8.45	35.64	1.29	9.15	13.60	1.91	8.23	10.78
H_7	2.65	8.25	41.18	1.38	9.05	15.69	1.92	7.87	12.32
H_8	2.49	8.45	34.21	1.66	9.75	16.42	2.10	8.03	15.80
H_9	2.76	8.35	36.86	1.61	8.65	14.21	2.23	8.33	18.58
H_{10}	2.54	8.85;..	35.54	1.65	8.80	13.01	1.97	8.33	25.10
F - test	**	**	*	NS	**	NS	NS	**	NS
LSD 0.05	0.51	0.26	11.86		0.78			1.07	
LSD 0.01	0.71	0.39	16.63		1.15			1.48	

***, ** = Significance at 0.05 and 0.01 levels of probability, respectively . NS = insignificant differences.**
† Initial PH = 5.1, †† Initial PH = 5.6, ††† Initial PH = 6.1.

As shown from the results summarized in Table (7), significant differences were shown between genotypes for pH values at all concentrations of sugars. However, significant differences were shown between genotypes for turbidity and biomass production at the concentration of 0.02 and 0.04 sugarcane juice industrial by- products, respectively. All hybrids showed significant pH values above the mid parents at 0.02 concentration of the substrate. In addition, four hybrids out of five genotypes significantly increased pH value above the mid parents at 0.04 concentration of sugarcane juice sub- products. However, at 0.06 concentration of the substrate, three hybrids out of five showed significantly increase the pH value above the mid parents. Fermentation conducted in this study has been used low-cost resources like sugarcane juice industry by-products for production of alcoholic beverages and biomass of yeast as a source of single cell protein (Romano *et al.* 2006). Therefore, the hybrid yeast must be well adapted to the stress conditions occurring during the alcoholic fermentation.

At the beginning of fermentation, *Saccharomyces cerevisiae* cells are affected by osmotic stress due to high sugar concentration, as well as, low pH. Therefore, when the fermentation was in progress, other stress conditions appeared, as

ethanol accumulation (Cardona *et al.* 2007). The results obtained in this study are in harmony with Zuzuarregui and Olmo (2004), who found a variation in yeast growth with sugar concentration.

Table 7. Fermentation results of sugarcane juice industrial wastes by the hybrids of *Saccharomyces cerevisiae* resulted from the mating between P_3 X P_5.

Strains	Concentration of sugarcane refuse (g/100 ml)								
	2			4			6		
	Turbidity	pH†	Biomass (g/l)	Turbidity	pH††	Biomass (g/l)	Turbidity	pH†††	Biomass (g/l)
P_3	1.92	4.85	31.12	1.82	7.16	13.71	2.06	8.10	23.42
P_5	1.73	4.70	21.92	1.46	6.40	15.29	1.55	6.00	12.57
MP	1.82	4.78	26.52	1.64	6.78	14.50	1.80	7.05	17.99
H_{11}	2.06	8.65	17.32	1.75	5.66	18.61	1.58	7.90	18.51
H_{12}	2.42	8.50	10.32	1.61	6.70	14.16	2.15	8.50	22.20
H_{13}	2.20	8.35	19.74	1.48	8.40	9.75	2.23	8.10	23.74
H_{14}	2.18	8.55	21.61	1.76	8.53	12.78	2.29	8.60	27.94
H_{15}	2.23	8.35	27.41	1.57	8.23	9.22	2.30	8.50	23.25
F - test	**	**	NS	NS	*	**	NS	**	NS
LSD 0.05	0.28	0.38			1.55	7.35		1.09	
LSD 0.01	0.40	0.57			2.16	10.21		1.51	

***, ** = Significance at 0.05 and 0.01 levels of probability, respectively , NS= Insignificant differences.**
† Initial PH = 5.1, †† Initial PH = 5.6, ††† Initial PH = 6.1.

In addition, Lafon- Lafourcade (1983) found that growth rate and the completeness of fermentation by baker's yeast were decreased as the initial concentration of sugar in grape increases above 200g per liter. Fleet and Heard (1993) observed that growth rate and fermentation by *S. cerevisiae* were decreased as the pH was decreased from 3.5 to 3.0. However, a recent study of pH influence on growth of *Saccharomyces bayanus* var. *uvarum* showed that pH does not have a significant effect on growth (Serra *et al.* 2005). The primary role of recombinant genome in yeast hybrids was the production of alcoholic beverages via efficient conversion of residual sugars in sugarcane juice sub- products to ethanol, carbon dioxide and other minor metabolites (Pretorius 2000). Charoenchai *et al.* (1998) observed that variation in pH did not affect the growth rate of baker's yeast. In addition, Serra *et al.* (2005) also observed that pH does not have a significant effect on yeast growth. In this study yeast genotypes converted sugars into biomass and alcohol. Therefore, alcohol produced in the fermentation medium was affected on the pH values. Along the fermentation process, the production of ethanol from sugar crop wastes for use as a transportation fuel is a mature biotechnology (Bothast and Schlicher 2005).

In conclusion, it is possible that the ability of *Saccharomyces cerevisiae* to produce high values of biomass was not related to its turbidity because of secondary metabolites produced in the media which may affect turbidity. Furthermore, some hybrids increased the pH of the fermentation medium which may be resulted from large amounts of alcohol produced. More generally, these hybrids resulted a higher pH in the fermentation medium than the parental strains. Consequently, they will screened in a continued work for ethanol productivity to evaluate the improved genotype for practical traits. Meanwhile, the inhibiting effect on ethanol on *Saccharomyces cerevisiae* may affect on biomass formation of some genotypes. The possible inhibition may be due to the toxicity effect, as well as, sensitivity of some genotypes to ethanol produced in the fermentation medium. During fermentation, if the pH dropps below the initial value, this decreased could be is probably linked to acetic acid production which is one of different products of yeast metabolism, because acetic acid acts synergistically with ethanol. During fermentation, many of contaminated microorganisms are able to produce acetic acid and modified the activity of *Saccharomyces cerevisiae*.

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تعبير التخمر الإيثانولى بواسطة تراكيب وراثية جديدة من خميرة الخباز .

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يهدف هذا البحث إلى دراسة خصائص عملية التخمر الإيثانولى بواسطة تراكيب وراثية هجينة من خميرة الخباز. تم فى هذه الدراسة إستخدام خمس تراكيب وراثية أبوية من الخميرة بالإضافة إلى ١٥ تركيب وراثى هجين تم عزلها من ثلاث تهجينات أجريت بين السلالات الأبوية وذلك لتقدير خصائص عملية التخمر أثناء عملية إنتاج الإيثانول . أظهرت الهجن الناتجة من التهجين بين الآباء $P_1 \times P_4$ زيادة فى قيم pH ،العكارة الخلوية– جعلتها أعلى من متوسط الآباء . وعلى العكس من ذلك ظهر إنخفاض فى معدل تكوين المادة الخلوية الحية وذلك عند تركيز ٠,٠٢ من مخلفات عصير قصب السكر . بينما أظهرت بعض الهجن عند التركيزات ٠,٠٤ ، ٠,٠٦ من هذه المخلفات زيادة معنوية فى قيمة pH عن متوسط الآباء ، مما يعكس أن سلوك التخمر يختلف من تركيز إلى آخر من مخلفات صناعة عصير قصب السكر . ومع ذلك يتأثر إنتاج الإيثانول أساساً بقيمة pH المنخفضة ، الحموضة المرتفعة ، محتوى البيئة من الكحول المرتفع ، درجة الحرارة المرتفعة ، الضغط الأسموزى . وعليه فإن قيمة pH المنخفضة تعتبر عامل إجهاد أساسى على خلايا الخميرة أثناء عملية التخمر . وعليه فإن التراكيب الوراثية الهجينة الناتجة عن التهجين بين الآباء $P_2 \times P_5$ قد أحدثت زيادة معنوية فى قيمة pH عن متوسط الآباء عند التركيزات ٠,٠٢ ، ٠,٠٤ من مخلفات صناعة عصير قصب السكر . كما أظهرت التراكيب الوراثية الهجينة عكارة خلوية جيدة عند تركيز ٠,٠٢ من هذه المخلفات إذا ما قورنت بمتوسط الآباء وبمستوى آداء الهجن عند التركيزات السكرية الأعلى من هذه المخلفات . أظهرت معظم الهجن الناتجة عن التهجين بين الآباء $P_3 \times P_5$ قيم معنوية للـ pH مقارنة بمتوسط الآباء عند كل تركيزات المخلفات الصناعية لعصير قصب السكر المستخدمة فى هذه الدراسة . وبالإضافة إلى ذلك أظهرت معظم الهجن عكارة خلوية معنوية مقارنة بمتوسط الآباء عند تركيز ٠,٠٢ من هذه المخلفات . ومع ذلك ، أوضحت النتائج إختلافات غير معنوية بين التراكيب الوراثية المختلفة للخميرة بالنسبة للعكارة الخلوية وإنتاج المادة الخلوية الحية عند تركيزات ٠,٠٢ ، ٠,٠٤ من المواد الصلبة القابلة للذوبان (السكريات) الموجودة بهذه المخلفات .