Quality Parameters and RAPD-PCR Differentiation of Commercial Baker's Yeast and Hybrid Strains

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Abstract: Baker's yeast, *Saccharomyces cerevisiae*, is a key component in bread baking. Total of 12 commercial baker's yeast and 2 hybrid strains were compared using traditional quality parameters. Total of 5 strains with high leavening power and the 2 hybrid strains were selected and evaluated for their alpha-amylase, maltase, glucoamylase enzymes, and compared using random amplified polymorphic DNA (RAPD). The results revealed that all selected yeast strains have a low level of alpha-amylase and a high level of maltase and glucoamylase enzymes. Meanwhile, the Egyptian yeast strain (EY) had the highest content of alpha-amylase and maltase enzymes followed by the hybrid YH strain. The EY and YH strains have the highest content of glucoamylase enzyme almost with the same level. The RAPD banding patterns showed a wide variation among commercial yeast and hybrid strains. The closely related Egyptian yeast strains (EY and AL) demonstrated close similarity of their genotypes. The 2 hybrid strains were clustered to Turkish and European strains in 1 group. The authors conclude that the identification of strains and hybrids using RAPD technique was useful in determining their genetic relationship. These results can be useful not only for the basic research, but also for the quality control in baking factories.

Keywords: amylase, baker's yeast, differentiation, hybrid strains, RAPD-PCR

Introduction

Yeast is an important microorganism in the field of applied microbiology. In the food industry, yeast acts as an agent for production as well as breakdown products. Strains of the yeast genus *Saccharomyces* are used in some of the largest and oldest biotechnology industries including baking, brewing, distilling, and winemaking (Reed and Nagodawithana 1991). Important biotechnological processes mediated by *Saccharomyces cerevisiae* species are based on the fermentation of starch hydrolysates. Alpha amylase hydrolyzes the alpha- 1, 4 linkage of starch at random and bypasses the alpha-1, 6 linkages resulting in a rapid reduction in the viscosity of starch. Maltose is the predominant sugar of these carbohydrate mixtures, which also contain glucose and maltotriose in considerable amounts. Most yeast strains use maltotriose only after maltose is exhausted, and very often the trisacchride is not completely consumed (Londesborough 2001). Glucomylase is an enzyme able to hydrolyze starch and maltooligosaccharides completely to D-glucose. The enzyme is commonly produced by many strains of yeast (Saito and others 1996) and some yeast strains could produce amylolytic enzymes (Calleja and others 1987).

Improving the performance of the yeast strains used in these processes has come about as a result of developing the strains with novel genotypes. The methods used to obtain these improved genotypes include genetic engineering (Stewart 1981), protoplast fusion, and mutation followed by selection (Jones and others 1977). However, traditional techniques involving mating followed by selection are still effective for strain improvement (Evans 1990). Classical hybridization techniques have been applied successfully for the breeding of new yeast strains for baking (Rose and Vijayalakshmi 1993).

Recent techniques have been developed for the identification of yeast, such as serologic testing (Campbell 1971, 1972) and fatty acid chromatographic profiling (Oosthuizen and others 1987). However, the most acceptable methods of taxonomy to species level are based on physiology (Barnett and others 1983) and morphology (Kreger-van Rij 1984). The characterization of yeast strains has recently been made possible with the advent of molecular techniques. These include chromosome karyotyping (Johnston and Mortimer 1986), RFLP (Espinosa and others 2002), PCR (de Barros Lopes and others 1996; Meroth and others 2003; Manzano and others 2004; Legras and others 2005). RAPD-PCR first introduced by Williams and others (1990), relies on the amplification of fragments with only a single short primer present. RAPD-PCR recognition of species or even individual production strains involves producing arbitrary DNA sequences using single primers of arbitrary nucleotide sequences (Baleiras and others 1994; Molnar and others 1995; Giusto and others 2006; Powell and Diacetis 2007). Laidlaw and others (1996) reported that the RAPD-PCR method confirmed the classical identification of 45 wild yeast strains isolated from 5 traditional Greek sourdoughs. Romano and others (1996) used RAPD and RFLP of mitochondrial DNA to evaluate the genetic diversity of 27 *Candida zeylanoides* strains and 28 *Debaryomyces hansenii* strains isolated from Roncal and Idiazabal cheese at different stages of manufacture in different dairies. Boekhout and others (1997) suggested that the combination of PFGE and RAPD allowed the differentiation of

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clinical, saprobic, and veterinary isolates of *C. neoformans* within each taxon. Meroth and others (2003) concluded that the 3 different yeast strains present in rye flour and detected by RAPD analysis were dominated by the strain added to the process as baker's yeast. The objective of the study is to evaluate the quality of commercial yeast strains commonly used by the international baker's industries and hybrid strains. As well, random primers were used to correlate RAPD-PCR profiling with enzyme activity of selected yeast strains and hybrids.

Materials and Methods

Yeast strains

Total of 12 commercial baker's yeast strains (WY, AGL, HAS, AR, EY, HS, QM, FA, LES, P, MY, AL, and NW) were collected from some Egyptian factories, local and international market. Total of 2 hybridized strains, NW and YH, were produced from HS × LES and HS × EY crosses, respectively.

Media

Medium YEPD (1% yeast extract, 2% glucose, 2% polypeptone) was used for the cultivation and maintenance of the strains. Sporulation medium (0.1% yeast extract, 1% potassium acetate, 0.05% glucose) was used for forcing the vegetative diploid cells to convert into asci formation by meiosis and hence produce haploid spores. For the determination of leavening ability, yeast cells were cultured in molasses medium containing 3% sugar cane molasses, 0.19% urea, and 0.046% KH_2PO_4 (Oda and Ouchi 1989a).

Production of yeast cells

Yeast cells were grown in a test tube containing 15 mL of YEPD medium, then was transferred to 135 mL of molasses medium in shaking flasks (Oda and Ouchi, 1989b), incubated at 28 °C for 24 h with reciprocal shaking at 240 rpm. Cells were harvested by centrifugation, washed twice with distilled water, and the cell pellet adjusted to a moisture content of about 67%.

Sporulation and spore recovery

After 3 to 5 d of growth on the sporulation medium, cells were collected, washed, suspended in 100 mM phosphate buffer, pH 7 (0.6712 moles/L acid and 0.3288 moles/L base), treated with zymolase enzyme (60 μg/mL) and incubated at 30 °C for 30 min (Nakagawa and Ouchi 1994).Then, incubated at 55 °C for 10 min to kill the vegetative cells. After the spore suspension was lightly sonicated twice (30 s at 50% full power, then set on ice 2 min), the spores were spread on YEPD agar medium and incubated at 30 °C. Colonies grown were picked and stored at –70 °C in 15% glycerol.

Determination of mating type

Mating ability was determined by separately mixing each isolate with the 2 tester haploids strains GT160–34B (MATa and MATα). A strain was designated as mating type (a) when sporulation occurred after mixing with the (α) tester strain and was designated as mating type (α) when sporulation took place after mixing with the (a) tester strain.

Construction of hybrids

The isolated haploid strains showing opposite mating type were crossed by mixing in YEPD liquid medium. After 2 d of incubation at 28 °C, the mixtures were streaked on YEPD plates for single colony isolation. The isolated strains were checked for their sporulation ability. Cells showing spore formation were considered hybrids (Nakagawa and Ouchi 1994).

Screening of yeast strains

Dry matter and crude protein were determined according to the method described by the AOAC (1998). Leavening power

Table 1–Evaluation of commercial and hybrid strains of baker's yeast utilized sugar cane molasses 5%.

Yeast strain	Fresh yield gm/L	Dry matter %	Leavening power Cm^3 Co_2/h.	Protein %	P_2O_5 %	Trehalose %
WY	30.25 ± 0.62^{CD}	27.12 ± 0.62^{b}	540.0 ± 14.67^{H}	45.83 ± 0.85^{BCD}	2.38 ± 0.08^{G}	4.30 ± 0.18^{G}
AGL	30.00 ± 0.62^{CD}	27.40 ± 0.62^{ab}	592.5 ± 14.67^{G}	44.75 ± 0.85^{CD}	3.43 ± 0.08^{B}	5.01 ± 0.18^{EF}
HAS	32.50 ± 0.62^{BC}	29.10 ± 0.62^{ab}	681.3 ± 14.67^{EF}	$48.2A \pm 0.85^{B}$	3.04 ± 0.08^{C}	5.25 ± 0.18^{F}
AR	30.50 ± 0.62^{CD}	27.13 ± 0.62^{b}	666.3 ± 14.67^{F}	39.56 ± 0.85^{G}	2.53 ± 0.08^{EFG}	5.76 ± 0.18^{DE}
EY	32.38 ± 0.62^{BC}	29.38 ± 0.62^{a}	868.8 ± 14.67^{A}	39.87 ± 0.85^{G}	3.53 ± 0.08^{B}	7.13 ± 0.18^{B}
HS	35.50 ± 0.62^{A}	29.50 ± 0.62^{a}	797.5 ± 14.67^{CD}	41.49 ± 0.85^{FG}	2.47 ± 0.08^{FG}	8.08 ± 0.18^{A}
QM	32.13 ± 0.62^{BCD}	28.58 ± 0.62^{ab}	782.3 ± 14.67^{D}	49.75 ± 0.85^{A}	2.88 ± 0.08^{CD}	6.45 ± 0.18^{C}
FA	32.25 ± 0.62^{BCD}	27.75 ± 0.62^{ab}	711.3 ± 14.67^{E}	43.87 ± 0.85^{DEF}	4.25 ± 0.08^{A}	5.3 ± 0.18^{EF}
LES	31.13 ± 0.62^{CD}	29.35 ± 0.62^{a}	806.3 ± 14.67^{BCD}	44.40 ± 0.85^{CDE}	2.33 ± 0.08^{G}	8.09 ± 0.18^{A}
P	34.88 ± 0.62^{A}	29.4 ± 0.62^{a}	846.3 ± 14.67^{AB}	46.60 ± 0.85^{BC}	2.55 ± 0.08^{EFG}	6.70 ± 0.18^{BC}
MY	30.55 ± 0.62^{CD}	28.35 ± 0.62^{ab}	600.0 ± 14.67^{G}	50.20 ± 0.85^{A}	3.00 ± 0.08^{C}	5.88 ± 0.18^{D}
AL	34.25 ± 0.62^{AB}	29.28 ± 0.62^{a}	859.5 ± 14.67^{A}	48.20 ± 0.85^{AB}	2.65 ± 0.08^{DEF}	7.05 ± 0.18^{B}
NW (Hybrid)	32.25 ± 0.62^{BCD}	29.35 ± 0.62^{a}	796.3 ± 14.67^{CD}	43.40 ± 0.85^{DEF}	2.78 ± 0.08^{CDE}	8.17 ± 0.18^{A}
YH (Hybrid)	35.13 ± 0.62^{A}	29.37 ± 0.62^{a}	830.0 ± 14.67^{ABC}	41.90 ± 0.85^{EFG}	2.95 ± 0.08^{C}	8.23 ± 0.18^{A}

a to b and A to H values in the same column within the same item followed by different superscripts are significantly different at $P < 0.05$ for a to b; $P < 0.01$ for A to C.

Table 2–Enzymatic evaluation of 5 commercial and 2 hybrid strains of yeast.

Yeast strain	Origin	Alpha-amylase (U/gm.dry cell)	Maltase (U/gm.dry cell)	Glucoamylase. (U/gm.dry cell)
EY	Egyptian market	76.25 ± 1.72^{A}	227.50 ± 2.1^{A}	298.56 ± 3.78^{A}
AL	Egyptian market	46.13 ± 1.72^{C}	207.50 ± 2.1^{C}	281.45 ± 3.78^{B}
P	Turkish market	20.19 ± 1.72^{E}	187.44 ± 2.1^{D}	148.28 ± 3.78^{E}
HS	Turkish market	62.75 ± 1.72^{D}	207.75 ± 2.1^{C}	251.29 ± 3.78^{C}
LES	European market	50.23 ± 1.72^{C}	206.25 ± 2.1^{C}	258.97 ± 3.78^{C}
YH (Hybrid)	Mating between HS and EY	61.52 ± 1.72^{B}	220.50 ± 2.1^{B}	297.00 ± 3.78^{A}
NW (hybrid)	Mating between HS and LES	30.50 ± 1.72^{D}	202.75 ± 2.1^{C}	233.47 ± 3.78^{D}

A to E values in the same column within the same item followed by different superscripts are significantly different $P < 0.01$.

was determined according to the Egyptian Standard 191 (2005). Trehalose, alpha-amylase, maltase, and glucoamylase enzymes were determined according to the methods described by Brim (1966), de Moraes and others (1995), Castro and others (1993), and Amirul and others (1996), respectively.

Statistical analysis

Analysis of variance (ANOVA) was computed using the General Linear Model procedure of statistical analysis system (SPSS 1999). Variable means for treatments indicating significant differences in the ANOVA were compared and the differences were indicated using Duncan's multiple range testes (Duncan 1955).

RAPD-PCR

DNA was extracted according to Powell and Diacetis (2007) by transferring a large colony of cells into 660 μL TE-SDS buffer, produced by mixing 200 mL of 50× TE buffer (7.44 g/L EDTA, 6.06 g/L Tris, pH 7.5 with 20 mL of a 10% SDS solution). RAPD-PCR was performed using primers OPA03, OPA12, OPA13, OPA15, OPA16, OPA17, OPA18, OPA19, and OPA20 (Operon Technologies Inc., Alameda, Calif., U.S.A.). PCR was carried out in a reduced volume of a 25 μL reaction mixture containing Tris HCl 10 mM, KCl 50 mM, $MgCl_2$ 3.5 mM, dNTP 0.3 mM, Primer 1μM, AmpliTaq 0.5 U, 100 ng DNA. The amplification started with a denaturation step of 5 min at 94 °C, followed by 40 cycles consisting of 1 min at 94 °C, 1 min at 36 °C, 2 min at 72 °C and ended with a final elongation step of 10 min at 72 °C. PCR products were separated in a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light. Computer assisted analysis of the RAPD-PCR fingerprinting patterns was performed using RAPDistance software package, version 1.4 (Armstrong and others 1994). Only strong and clearly random amplified polymorphic DNA (RAPD) bands were scored as present (1) or absent (0) for each of the primer-strain combinations. Pair-wise comparisons of the strains, based on the presence or absence of unique and shared bands, were used to generate similarity coefficients (Excoffier and others 1992). The strains were then clustered using the unweighted pair-group method with arithmetic average (UPGMA). A dendrogram was generated from the similarity data following the method of Sokal and Sneath (1963).

Results and Discussion

Baker's yeast strain evaluation

Total of 12 baker's yeast strains were evaluated for their quality parameters, for example, fresh yield, dry matter, leavening power, protein content, P_2O_2, and trehalose (Table 1). The values of fresh yield of different tested strains were between 30 and 35.50 gm/L, whilst the values of dry matter were between 27.12% to 29.50%. Randez and others (1999); Trivedi and others (1984) reported that the most important quality parameters of baker's yeast were leavening power and trehalose content, which could be used in differentiating different baker's yeast strains. Based on the highest value of leavening power and trehalose content, 5 yeast strains could be selected to follow the present investigation. These strains were Egyptian strain (EY), Turkish strain (HS), European strain (LES), Turkish strain (P), and Egyptian strain (AL). The leavening power and trehalose content of these strains were 868.8, 797.5, 806.3, 846.3, and 859.5 cm^3CO_2/h, and 7.13, 8.08, 8.09, 6.70, and 7.05, respectively (Table 1). The HS, EY, and LES strains were

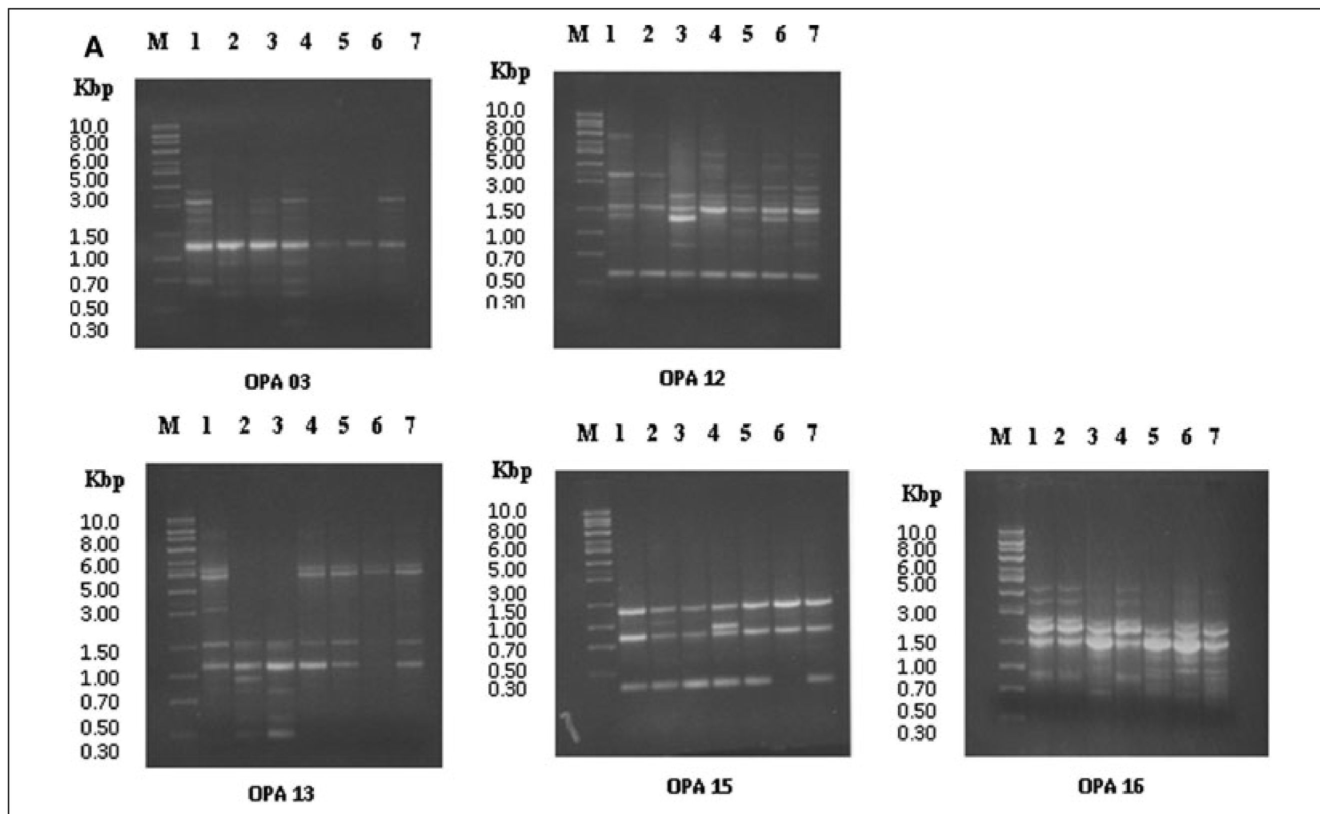

Figure 1a–RAPD profiles of the 5 yeast strains and 2 hybrid strains amplified with RAPD primers, OPA03, OPA12, OPA13, OPA15, and OPA16. M: molecular weight marker (1 kb DNA ladder). Lanes from 1 to 7 represent: EY, HS, YH, AL, LES, NW, and P, respectively.

selected for the mating process: HS × LES and HS × EY. The quality parameters of 2 hybrid strains (NW and YH) were recorded in Table 1, where, their leavening power and trehalose content were 796.3, 830 cm^3 CO_2/h and 8.17, 8.23%, respectively.

Alpha-amylase, maltase, and glucoamylase contents in selected yeast strains

The objective of many research laboratories is to develop strains of *Saccharomyces* sp. that are able to synthesize and secrete alpha amylase and glucoamylase. Selective technique of Higgins and others (1999) was used as a preliminary strain generation and screening tests for strong maltose utilization. The 5 selected baker's yeast strains and the 2 hybrid strains were analyzed for their content of alpha-amylase, maltase, and glucoamylase (Table 2). The Egyptian yeast strain EY had the highest content of the evaluated enzymes, where its contents were 76.25, 227.5, and 298.56 U/gm dry cells for alpha-amylase, maltase, and glucoamylase, respectively. On the other hand, the Turkish strain P recorded the lowest values for the same determined enzymes, where its contents were 20.19, 187.44, and 148.28 U/gm dry cells, respectively. Regarding the 2 hybrid yeast strains (YH and NW), it could be noticed that, their contents of the determined enzymes were 61.52, 220.5, 297 and 30.50, 202.75, and 233.47 U/gm dry cell for alpha-amylase, maltase, and glucoamylase enzymes, respectively. Addition of alpha-amylase during bread production increased the specific volume of bread and affects on starch retro gradation, improved gas production, and modifies the rheological properties of bread dough (Armero and Collart 1998; Calleja and others 1987). Therefore, the Egyptian yeast strain EY and YH hybrid strain have important industrial applications as good alpha-amylase production strains.

Random amplified polymorphic DNA (RAPD)

Total of 9 random primers were used in the present study to identify 5 strains of commercial yeast and 2 hybrid strains. These primers generated a number of amplified DNA fragments ranging from 5 to 13 amplicons and the size of amplified fragments ranging from <300 to 4900 bp with different primers, (Figure 1a and 1b). Moreover, primer OPA18 amplified the highest number of amplicons (13) among yeast strains, while the lowest number was 5 amplicons with the primer OPA14 or OPA19. The number of polymorphic amplicons ranged from 3 amplicons from primer OPA14 to 9 amplicons from primer OPA13. As shown in Table 3, the level of genetic similarity among the 7 strains ranged between 60% and 85%. The highest genetic similarity 85% was observed between P strain and hybrid strain (NW), followed by 83% between strains EY and AL. This was followed by 77% similarity index between hybrid strain (YH) and hybrid strain (NW) also between strains LES and NW, while the lowest genetic similarity (60%) was observed between strains EY and LES. The applicability of the method for determining genome similarities among yeast strains was investigated by performing cluster analysis on the RAPD data. The UPGMA dendrogram generated from the

Table 3–Similarity coefficient percentage among 5 commercial and 2 hybrid strains of yeast based on RAPD-PCR.

Yeast Strain	EY	HS	YH (Hybrid)	AL	LES	NW (Hybrid)	P
EY	100						
HS	67	100					
YH (Hybrid)	68	71	100				
AL	83	66	69	100			
LES	60	69	72	63	100		
NW (Hybrid)	65	64	77	72	77	100	
P	72	61	74	75	74	85	100

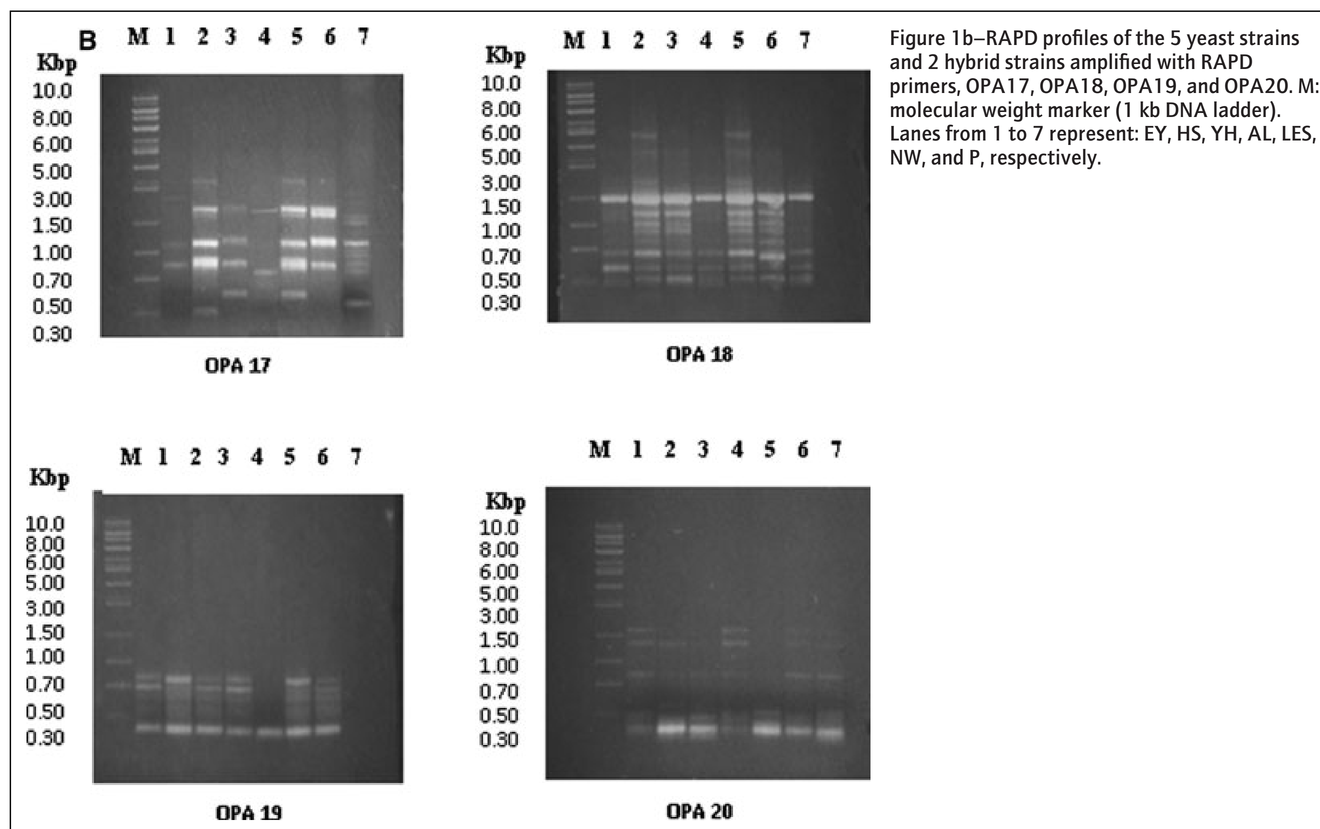

Figure 1b–RAPD profiles of the 5 yeast strains and 2 hybrid strains amplified with RAPD primers, OPA17, OPA18, OPA19, and OPA20. M: molecular weight marker (1 kb DNA ladder). Lanes from 1 to 7 represent: EY, HS, YH, AL, LES, NW, and P, respectively.

similarity values is shown in Figure 2. This dendrogram grouped the 2 hybrid and 5 strains into 2 main clusters, the first cluster contained 2 Egyptian strains, EY and AL. On the other hand, the second cluster contains the strains HS, YH (hybrid), LES, NW (hybrid), and P. This cluster was divided into 2 main sub clusters; the first one contained strain HS, while the 2nd subcluster contained the other strains in 2 groups. The 1st group contained strain YH (hybrid) only and the 2nd group was divided into 2 subgroups, 1 containing strain LES and the other contained strains NW (hybrid) and P. The close relationships between the 2 Egyptian yeast strains EY and AL suggesting close similarity of each of the 2 genotype. On the other hand, the relationship between strain HS and strain YH (hybrid) in the same cluster might be expected, since the strain YH was derived from mating between the strain HS and the strain EY. Our results of RAPD marker are similar with those of previously reported (Laidlaw and others 1996; Meroth and others 2003). They showed the usefulness of RAPD-PCR technique in yeast strain differentiation and identification. In addition, RAPDs marker has been used in yeast microbiology to evaluate genetic diversity (Romano and others 1996) and to assess a differentiation of clinical, saprobic, and veterinary isolates (Boekhout and others 1997). According to the results obtained from RAPD-PCR, it could be noted that very different strains to those of leavening power, trehalose content, and enzyme activity have highest similarity coefficient and were grouped into clades.

Conclusions

The results of the present study show that the RAPD-PCR technique is applicable for the identification of baker's yeast strains and may be useful in baker's yeast breeding and in the study of the dynamics of yeast populations during bread making.

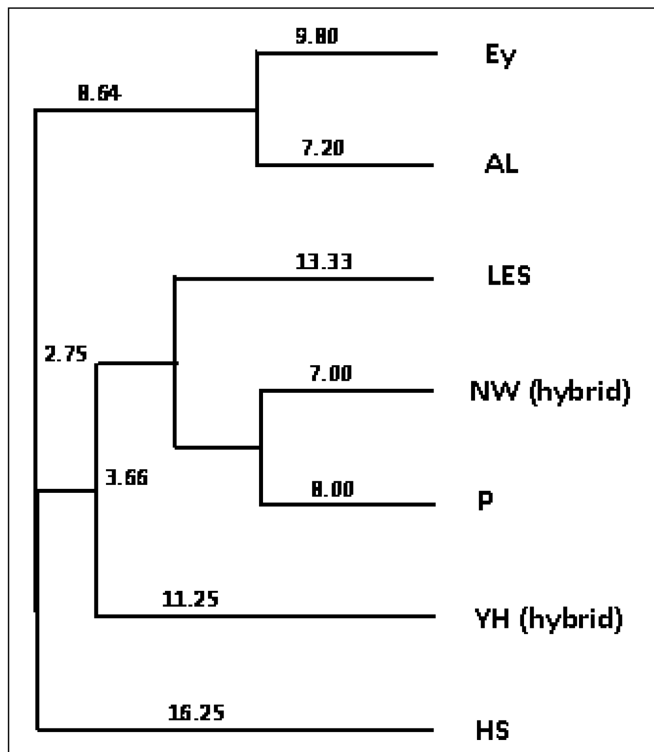

Figure 2–Dendrogram for 5 commercial and 2 hybrid strains of yeast constructed from RAPD's data using UPGMA and similarity matrices.

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