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Full Length Research Paper

Molecular and biochemical studies of some yeast strains

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This work focuses on detecting the level of polymorphisms among eleven KSA and Egyptian yeast strains, as efficient tools to assess the genetic relationships and development of yeast strain-specific molecular fingerprints. Moreover, estimation of the amino acid concentration was efficient in selecting the protein rich strains for animal feeding source. To detect the polymorphism among the yeast strains at the molecular level, 8 random amplified polymorphic DNA (RAPD), 5 inter simple sequence repeat (ISSR), and 8 specific simple sequence repeat (SSR) pair of primers were used. The total number of fragments produced by RAPD primers was 46 fragments and represented 52% of polymorphism. Also, number of fragments produced by ISSR and SSR primers was 45 fragments and recorded 63 and 77% of polymorphism among the strains, respectively. The amino acid analysis showed that yeast strains, *Rhodotorula glutinis* (Y.1); *Schwanniomyces occidentalis* (Y.2); *Debaryomyces hansenii* (Y.8); *Kluyveromyces lactis* (Y.9) and *Pichia jadinii* (Y.10) contained almost double the amount of total amino acids compared to the rest of the eleven strains used. The different molecular markers have confirmed each other and supported the biochemical analysis data, because the clustering analysis has shown that the previous five strains, (Y.1); (Y.2); (Y.8); (Y.9) and (Y.10), were falling together in the same sub cluster.

Key words: Yeast, molecular markers, biochemical analysis.

INTRODUCTION

Yeast is a single-cell fungus. There are several species and different varieties of yeast. Differences between yeast varieties are due to the rate of sugar consumption, carbon dioxide and its productions as well as growth environmental conditions (Temperature, oxygen and pH of fermentation process). During the last twenty years, the use of molecular markers reveals polymorphism at the DNA level. Among all, the microsatellite DNA marker has been the most widely used due to its easy use by simple polymerase chain reaction (PCR), followed by a gel electrophoresis for determination of allele size and high degree of information provided by its large number of alleles per locus (Van der Walt, 1965; Naumov, 1986 and 1987; Cai et al., 1996; Kurtzman and Robnett, 1998; Naumov and Naumova, 2002 and Ramadan and Rawia, 2005).

Recently, Kurtzman et al. (2001) reported that, the composition and type of the general species of *Kluyveromyces* were closely identical. Genetic hybridization analysis and bimolecular markers have shown that the species *Kluyveromyces lactis* includes not only lactose-fermenting (Lac $^{+}$) cultured yeasts, but also wild (Lac $^{-}$) strains. This species being represented by the genetically isolated (in part) populations are *krassilnikovii*, *drosophilarum*, *phaseolosporus* and *vanudenii* (Kurtzman 2003). Hybridization analysis has also shown that wild yeasts from the European *krassilnikovii* population are

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Abbreviations: RFLP, Restriction fragment length polymorphism; **ITS,** internal transcribed spacers; **IGS2,** intergenic spacer 2; **YC,** yeast cultures; **RAPD,** random amplified polymorphic DNA; **ISSR,** inter simple sequence repeat; **SSR,** simple sequence repeat; **SDS-PAGE,** sodium dodecyl sulfate polyacrylamide gel electrophoresis; **HPLC,** high performance liquid chromatography.

ancestors of the cultured dairy yeast *K. lactis* var. *lactis*. Hybrids of *Krassilnikovii* × var. *lactis* are highly fertile and exhibit a regular segregation of control markers. Yeasts of the *krassilnikovii* population differ from *Kluyveromyces lactis* var. *lactis* only in their inability to ferment lactose. Among morphological and physiological criteria, the recent systematic of yeasts has widely employed various molecular genetic methods, including sequencing and restriction fragment length polymorphism (RFLP) analysis of various regions of ribosomal DNA. Closely related yeast taxa can be differentiated by an RFLP analysis of the non-coding regions of their rDNA: internal transcribed spacers (ITS1 and ITS2) and intergenic spacer 2 (IGS2) (Esteve-Zarzos et al., 1999; Naumov, 2000; Nguyen et al., 2000; Belloch et al., 2002).

Recently, the use of feed additives containing bacterial and yeast cultures (YC) has been intensive. These probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance. They have been used as growth promoters to replace the widely used antibiotic and synthetic chemical feed supplements. *Saccharomyces cerevisiae* (as live YC) is reported to balance the energy and acid-base metabolism in dairy cattle, which resulted to a significantly higher milk production (Higginbotham and Bath, 1993; Nunes, 1994). An increase in bacterial numbers recovered from the rumen is the most reproducible effect of dietary yeast supplementation and it has been suggested that this increase is central to the action of the yeast in improving ruminant productivity (feed efficiency, milk production and composition) (Brydt et al., 1995; Stacie et al., 2008).

The aim of this study is to improve the taxonomical methods used in classification of different yeast strains by means of molecular markers random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR) and simple sequence repeat (SSR). Moreover, biochemical analysis (total protein profile and total amino acids) were used as tools to select the best yeast strains in Saudi Arabia and Egypt as a rich source of animal protein.

MATERIALS AND METHODS

Yeast strains

Eleven yeast strains were collected from different sources. Seven were collected from Egypt Microbial Culture Collection (EMCC): *Rhodotorula glutinis* (EMCC 175); *Schwanniomyces occidentalis* (EMCC 201); *S. cervisiae* (EMCC 69); *Debaryomyces hansenii* (EMCC 30); *K. lactis* (EMCC 8); *Pichia jadinii* (EMCC 102) and *Kluyveromyces marxianus* (EMCC 76). The other four yeast strains were collected from different sources in Taif-KSA, *S. cervisiae* (Taif 1); *S. cervisiae* (Taif 2); *S. cervisiae* (Taif 3) and *S. cervisiae* (Taif 4).

Isolation and purification of yeast genomic DNA

Genomic DNA was extracted from these strains using DNeasy Mini Kit protocol (QIAGEN).

RAPD and ISSR polymerase chain reaction (PCR)

The DNA amplification performed in a 25 µl reaction volume containing 3 µl (10 ng/µl) genomic DNA, 3 µl primer (Operon Technologies Inc.) and 19 µl master mix (Promega). The PCR temperature profile was applied through a Gene Amp® PCR System 9700 (Perkin Elmer, England). The thermal cycler was programmed with an initial step of 5 min at 94°C, the amplification reaction was carried out using 35 cycles of 40 s at 94°C for denaturation, an annealing step of 1 min at 36°C, an extension step of 1min at 72°C and finally a 7 min extension at 72°C. The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) in 1X TBE buffer at 95 volts. Two different molecular weight markers were used; 100 bp DNA ladder for products of primer OP-A04 and Lambda Hind III / Phix 174 Marker cut by *Hae* III for the rest of primers products (Figure 1). PCR products were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000).

SSR PCR reactions

A total of eight primer pairs were used for SSR markers. The amplification performed in a 25 µl reaction volume containing about 3 µl (10 ng/ul) total DNA, 2 µl primer for each Forward and Reverse (Operon Technologies Inc.). The rest of PCR reaction was performed as explained earlier.

Data scoring

To ensure the absence of artifacts, bands were carefully selected from replicated amplifications. Amplified bands were designated by their primer codes and sizes (base pairs). Data recorded as discrete variables (+) for the presence and (-) for the absence of a similar band. Only intense and reproducible bands appearing on the gel were scored. Jaccard's coefficient was used to calculate the genetic distance (Dps) between yeast strains. MVSP (Kovach, 2002) software was used to calculate the similarity index. These indices were used to generate a dendrogram using the Unweighted pair group method with Arithmetic mean (UPGMA) clustering method.

Amino acid analysis

The total protein of the yeast strains was extracted according to method described by Wang et al. (2007). The system consisted of a LC-6A HPLC (Shimadzu, Kyoto, Japan), and the method was as described by Yamanaka et al. (1994).

The preparation described in this work, is a modification of pharmacopeia JP XII. Different amino acids in 1 g of dried yeast was extracted with 1 ml of 10 % (w/v) hydrochloric acid and 80 ml of water at 80 to 85 for 30 min with frequent shaking. The extract was cooled, diluted to 100 ml with water and filtered through a dry filter paper (Dried Yeast extract A). 5 ml of sodium acetate buffer solution and 1 ml of diastase solution was added to 4 ml of the filtrate extract. The mixture was dried for 3 h at 45 to 50°C (dried yeast extract B). 0.08 ml of 10 % (w/v) hydrochloric acid and 0.6 g anhydrous sodium sulfate was added to 2 ml of extract B and dissolved by heat (50 to 55°C). 1 ml of isobutanol was added to the mixture and after shaken, centrifuged at 12.000 rpm and the isobutanol layer was removed. This washing procedure was repeated for three times (Yamanaka et al., 1994; Helsen et al., 2007).

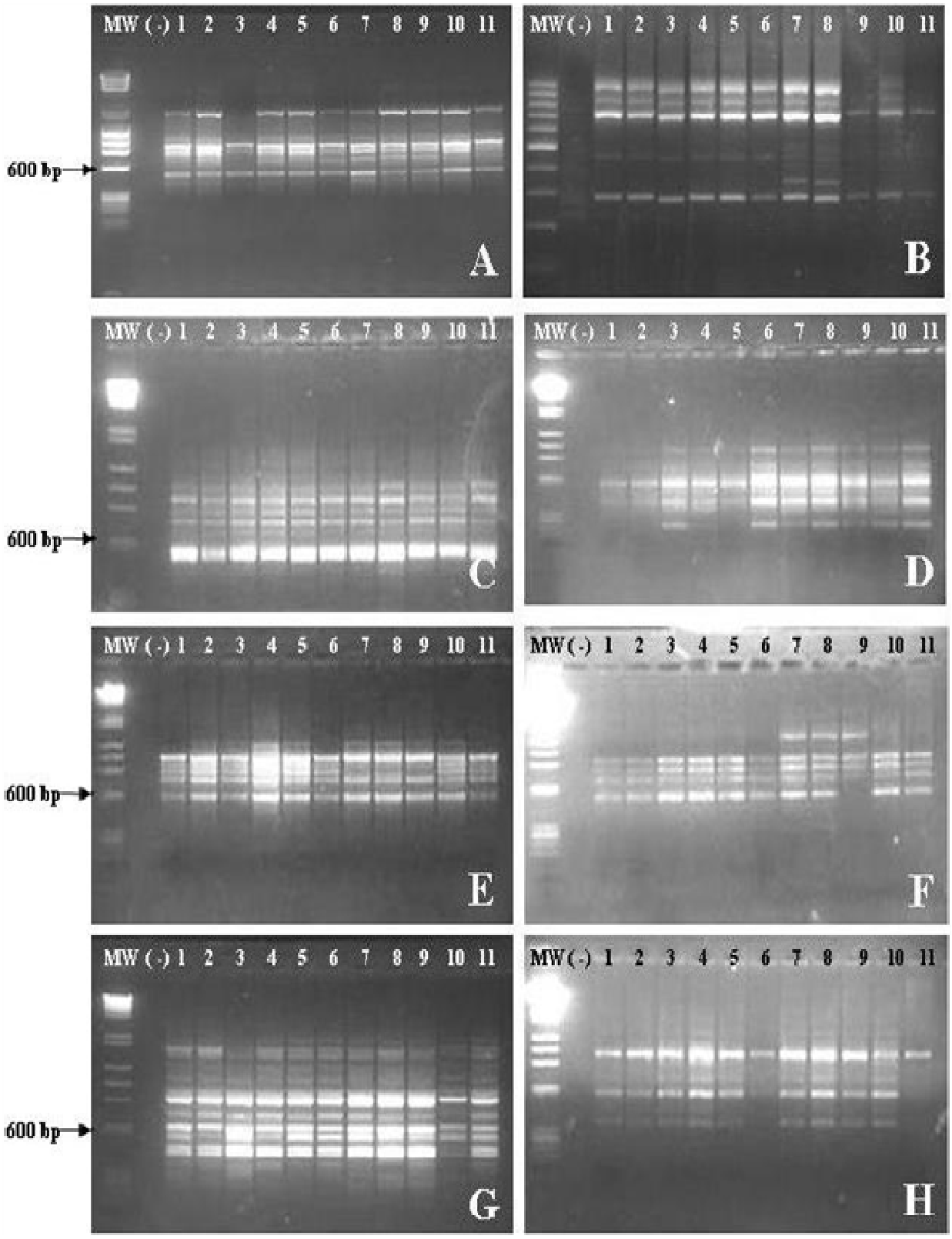

Figure 1. RAPD's fingerprinting of 11 Saudi and Egyptian Yeast strains: MW, is Lambda *Hind* III /Phi X 174 *Hae* III (-), is negative PCR; (1) *R. glutinis* (EMCC 175); (2) *S. occidentalis* (EMCC 201); (3) *S. cervisiae* (EMCC 69); (4) *S. cervisiae* (Taif 1); (5) *S. cervisiae* (Taif 2); (6) *S. cervisiae* (Taif 3); (7) *S. cervisiae* (Taif 4); (8) *D. hansenii* (EMCC 30); (9) *K. lactis* (EMCC 8); (10) *P. jadinii* (EMCC 102); (11) *K. marxianus* (EMCC 76). (A) Primer OP-A02; (B) Primer OP-A04; (C) Primer OP-A07; (D) Primer OP-A09; (E) Primer OP-B05; (F) Primer OP-B06; (G)Primer OP-Z07; (H)Primer OP-Z09.

RESULTS AND DISCUSSION

RAPD fingerprinting

The use of RAPD's as molecular markers was to show fast and reliable discrimination of any variation between the eleven yeast strains. Twenty five random primers were tested, only eight of them showed representative polymorphism, as seen in Figure 1 (scoring data table not shown/supplemented data).The RAPD-PCR results using primer (OP-A02) are illustrated in Figure 1A). The molecular size of five PCR products ranged from 400 to 1500 bp. Two common bands were observed in all strains and exhibited 40% monomorphism, while three fragments showed specific bands and exhibited 60% polymorphism. There is a unique absent band in yeast strain *S. cervisiae* (EMCC 69), but present in other yeast strains.

The results of RAPD's reaction using primer (OP-A04) are shown in (Figure 1B).The results revealed that this primer product were five fragments with size ranged from 330 to 1000 bp. Three bands were polymorphic and exhibited 60%. The PCR production of 300 bp illustrated as specific product with yeast strains *S. cervisiae* (Taif 4) and *D. hansenii* (EMCC 30), while the band of 900 bp was specific for strains number 9 and 11.

A total of six bands produced with random primer (OP-A07) is illustrated in (Figure 1C). The bands size ranged between 150 and 400 bp. Four of the six fragments were not very informative in our study as they did not show any polymorphism level among the different strains, while the other two showed 42.5% polymorphism among the strains.

The RAPD-PCR results using primer (OP-A09) is illustrated in (Figure 1D). The molecular size of five PCR products ranged from 300 to 2000 bp. Two common bands were observed in all strains and exhibited 40% monomorphism, while three fragments showed 60% polymorphism among the yeast strains.

The results of RAPD reaction using primer (OP-B05) is shown in (Figure 1E). The results revealed that the products of this primer were six fragments exhibiting 50% polymorphism and 50% monomorphism among the strains. A total of five bands produced in the eleven yeast strains with random primer (OP-B06) are illustrated in Figure 1F. The bands size ranged between 600 and 1200 bp indicating 60% polymorphism. The fragments No. 1 (1200 bp) was very informative in our study as it is presented only in the three strains *S. cervisiae* (Taif 4), *D. hansenii* (EMCC 30) and *K. lactis* (EMCC 8), respecttively. The two smaller fragments were absent only in strain *K. lactis* (EMCC 8).

The results of RAPD's reaction using primers (OP-Z02 & OP-Z09) are shown in (Figures 1G and H). The results revealed that, primer product OP-Z02 were nine fragments presenting specific polymorphism for the yeast strain *P. jadinii* (EMCC 102). The results of primer product OP-Z09 were four fragments, exhibiting specific polymorphism for the yeast strains *S. cervisiae* (Taif 3) and *K. marxianus* (EMCC 76) respectively.

ISSR fingerprinting

The use of ISSR molecular markers was also to confirm the RAPD's and show fast and reliable discrimination of any variations. In this kind of molecular markers, the DNA of eleven yeast strains were used as templates for five pairs of ISSR primers, as shown in Figure 2 (scoring data table not shown/supplemented data).

The ISSR-PCR results using primer (ISSR1) are illustrated in Figure (2A). The molecular size of six PCR products ranged from 200 to 900 bp. All the six bands were polymorphic among all strains, exhibiting 100% polymorphism. There is a 530 bp unique band, exhibiting in yeast strain *S. cervisiae* (Taif 1). A total of six bands with primer (ISSR5) are illustrated in Figure 2B, producing bands with molecular size ranging between 650 and 1000 bp, five of the six fragments were very informative in our study as they did show a high polymerphism level among the different strains (~83.3%), while the sixth fragment was common among all strains. This primer produced a specific product in yeast strains *R. glutinis* (EMCC 175), *S. cervisiae* (EMCC 69), *S. cervisiae* (Taif 3), *S. cervisiae* (Taif 4).

A total of nine bands with primer (ISSR7) are illustrated in (Figure 2C), producing bands with molecular size ranging between 300 and 1000 bp. Three of the nine fragments were not very informative in our study as they did not show any polymorphism level among the strains, while the other six products showed ~ 60% polymorphism among the eleven strains. This primer was very informative for the yeast strain *D. hansenii* (EMCC 30) (gel lane No. 8) because it has a unique band of ~ 300 bp.

The ISSR-PCR results using primer (ISSR8) are illustrated in Figure 2D. The molecular size of four PCR products ranged from 300 to 900 bp. All the four bands were common among all strains, except the PCR fragment of 900 bp which was polymorphic only in strain *K. marxianus* (EMCC 76).

The results of ISSR's reaction using primer (ISSR10) are shown in Figure 2E. The results revealed that, this primer products were six fragments, four exhibiting monomorphism among the eleven yeast strains, while the other two bands ranging between 200 and 400 bp exhibiting specific polymorphism for the yeast strains *R. glutinis* (EMCC 175) and *K. marx*ianus (EMCC 76).

SSR fingerprinting

The use of SSR molecular markers were used to show fast and reliable discrimination of any variations. The DNA of the eleven yeast strains (Figure 3A, B, C, D, E,

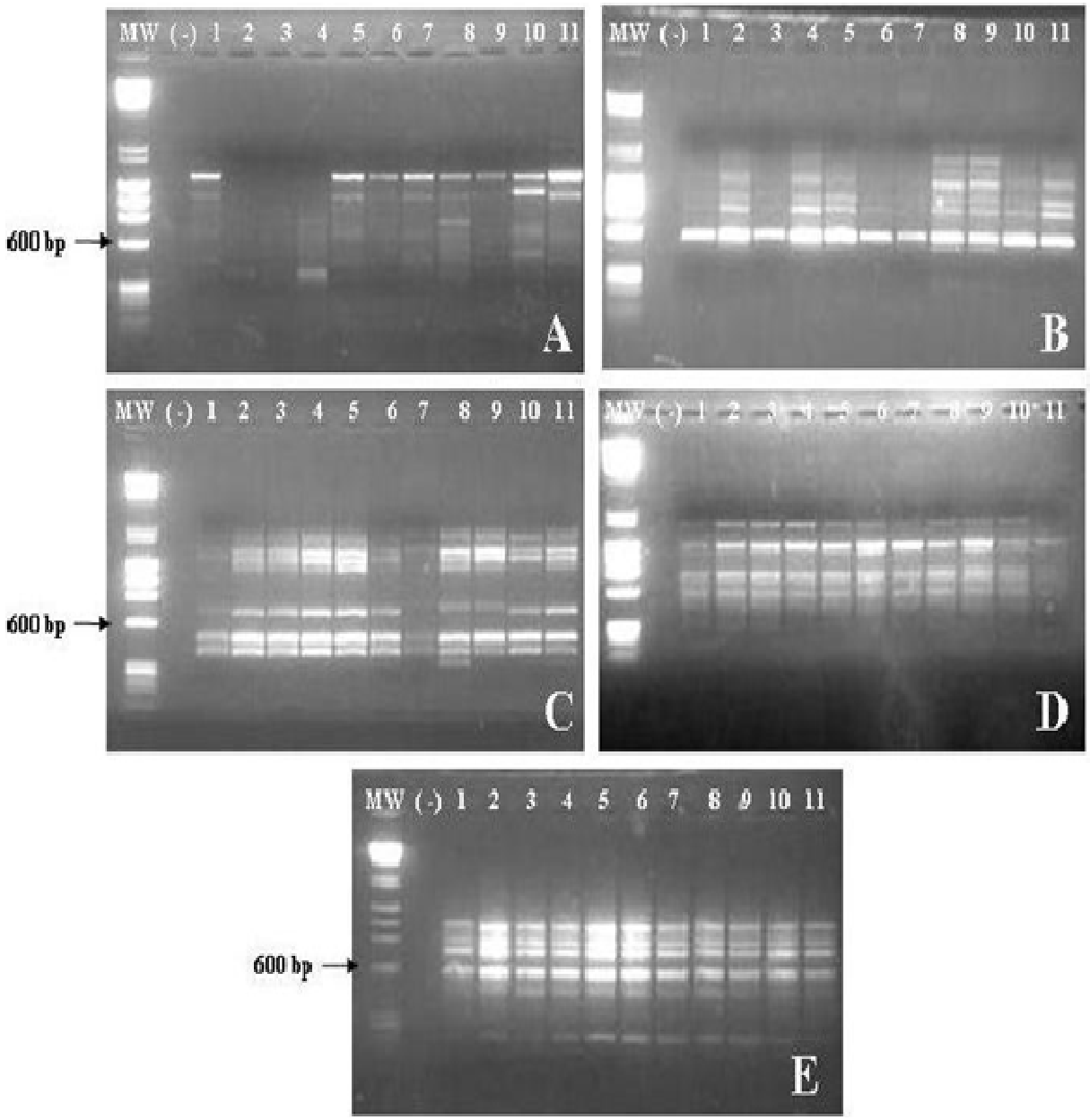

Figure 2. ISSR's fingerprinting of 11 Saudi and Egyptian Yeast strains. MW, is Lambda *Hind* III /Phi X 174 *Hae* III; (-), is negative PCR; (1) *R. glutinis* (EMCC 175); (2) *S. occidentalis* (EMCC 201); (3) *S. cervisiae* (EMCC 69);(4) *S. cervisiae* (Taif 1); (5) *S. cervisiae* (Taif 2); (6) *S. cervisiae* (Taif 3); (7) *S. cervisiae* (Taif 4); (8) *D. hansenii* (EMCC 30); (9) *K. lactis* (EMCC 8); (10), *P. jadinii*(EMCC 102); (11) *K. marxianus* (EMCC 76). (A)Primer ISSR1; (B) Primer ISSR5; (C) Primer ISSR7; (D) Primer ISSR8; (E) Primer ISSR10.

and F) and 10 strains (excluding *S. cervisiae* (Taif 4)) (Figure 3G and H) were used as templates for eight yeast specific primer pairs, (scoring data table not shown/ supplemented data). The SSR-PCR results using primer pair (ScAAT1) are illustrated in Figure 3A. The PCR product was two informative fragments (260 and 280 bp. respectively) and exhibited 100% polymorphism among the 11 strains. This pair of primers showed specific product for the yeast strains *R. glutinis* (EMCC 175); *S. occidentalis* (EMCC 201); *S. cervisiae* (EMCC 69); *S. cervisiae* (Taif 1) and *S. cervisiae* (Taif 3), gel lanes No. 1, 2, 3, 4, 5 and 6).

The SSR-PCR results using primer pair (ScAAT3) are illustrated in Figure 3B. The molecular size of the two PCR products is 400 and 100 bp, respectively. The 400 bp fragment was polymorphic and appeared only in yeast strains *S. cervisiae* (Taif 3); *S. cervisiae* (Taif 4); *D. hansenii* (EMCC 30); *K. lactis* (EMCC 8); *P. jadinii* (EMCC 102) *K. marxianus* (EMCC 76) and gel lanes (No. 6, 7, 8, 9, 10 and 11).

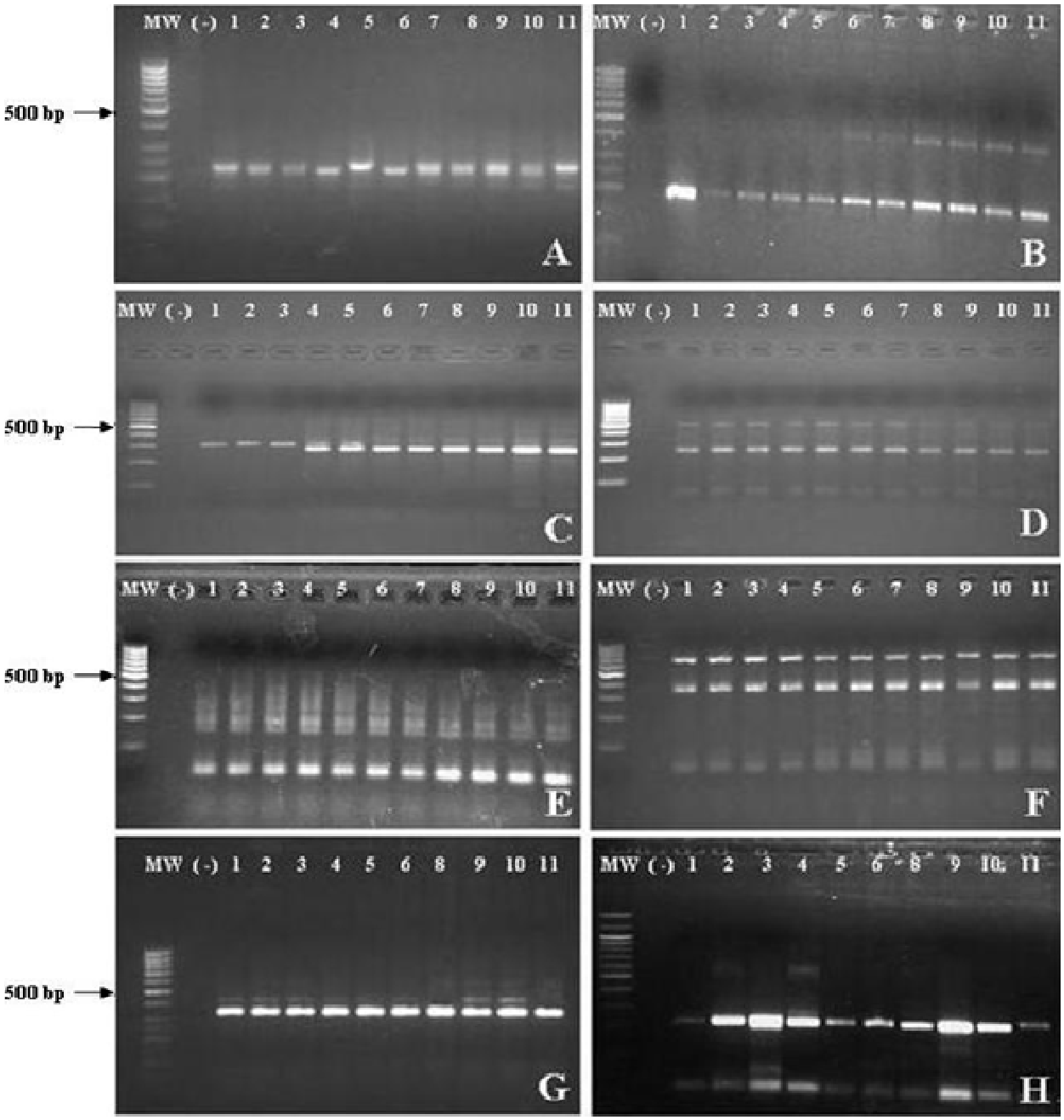

Figure 3. SSR's fingerprinting of 11 Saudi and Egyptian Yeast strains. MW, is 100 bp ladder (ferments); (-), is negative PCR; (1) *R. glutinis* (EMCC 175); (2) *S. occidentalis* (EMCC 201); (3) *S. cervisiae* (EMCC 69);(4) *S. cervisiae* (Taif 1); (5) *S. cervisiae* (Taif 2); (6) *S. cervisiae* (Taif 3); (7), *S. cervisiae* (Taif 4); (8) *D. hansenii* (EMCC 30); (9) *K. lactis* (EMCC 8); (10) *P. jadinii* EMCC 102); (11) *K. marxianus* (EMCC 76). (A) Primer ScAAT1; (B) Primer ScAAT3; (C) Primer ScAAT6; (D) Primer ScAAT7; (E) Primer ScAAT8 (F) Primer ScAAT9; (G) Primer ScAAT4; (H) Primer ScAAT5.

Two bands in the 11 strains using the primer (ScAAT6) are illustrated in Figures 3C, its molecular size ranging between 300 and 250 bp, respectively. This pair of primers showed a specific product for yeast strains: *R. glutinis* (EMCC 175); *S. occidentalis* (EMCC 201) and *S. cervisiae* (EMCC 69), gel lanes (No. 1, 2 and 3). While the SSR-PCR results using primer pairs (ScAAT7) and (ScAAT8) are illustrated in Figure 3D and E. The PCR products exhibited no polymorphism among the 11 strains.

A total of four bands among the yeast strains with the pair of primer (ScAAT9) are illustrated in Figure3F, producing bands with molecular size ranging between 50 and 700 bp. Only one fragment showed 25% of

Table 1. Genetic similarity (GS) of matrix computed according to Dice coefficient from RAPD, ISSR and SSR markers Yeast. (Y.1), *R. glutinis* (EMCC 175); (Y.2), *S. occidentalis* (EMCC 201); (Y.3), *S. cervisiae* (EMCC 69); (Y.4), *S. cervisiae* (Taif 1); (Y.5), *S. cervisiae* (Taif 2); (Y.6), *S. cervisiae* (Taif 3); (Y.7), *S. cervisiae* (Taif 4); (Y.8) *D. hansenii* (EMCC 30); (Y.9), *K. lactis* (EMCC 8); (Y.10*), P. jadinii* (EMCC 102); (Y.11*) K. marxianus* (EMCC 76).

	Y.1	Y.2	Y.3	Y.4	Y.5	Y.6	Y.7	Y.8	Y.9	Y.10	Y.11
Y.1	100.00										
Y.2	94.30	100.00									
Y.3	80.99	81.98	100.00								
Y.4	84.10	85.77	80.96	100.00							
Y.5	66.23	65.09	65.33	66.88	100.00						
Y.6	81.00	80.99	88.03	81.11	65.33	100.00					
Y.7	83.00	81.40	88.68	82.24	66.11	91.30	100.00				
Y.8	92.00	95.08	82.09	84.83	67.00	82.66	82.93	100.00			
Y.9	93.31	96.00	83.90	83.77	66.60	81.81	81.58	93.50	100.00		
Y.10	93.86	98.03	82.00	83.98	66.80	81.00	82.40	94.44	83.55	100.00	
Y.11	66.20	67.11	65.33	67.32	84.00	64.88	66.91	66.20	65.40	67.82	100.00

polymorphism. In panels G and H, only 10 Yeast strains were used, excluding strain *S. cervisiae* (Taif 4). A total of two bands in the ten used strains with primer pair (ScAAT4) are illustrated in Figure 3G, producing bands with molecular size ranging between 300 and 400 bp, showing 50% polymorphism. The SSR-PCR results using primer (ScAAT5) are illustrated in Figure 3H. The molecular size of five PCR products ranged from 50 to 200 bp. Four out of the five bands were polymorphic among the used yeast strains, exhibiting 80% polymorphism.

Genetic similarity estimation based on RAPD, ISSR and SSR markers

The RAPD's, ISSR's and SSR's in this study have showed a genetic similarity ranging from 64.88 to 98.03 (Table 1). The highest genetic similarity revealed by this molecular marker analysis was between *S. occidentalis* (EMCC 201) (Y.2) and *P. jadinii* (EMCC 102) (Y.10). The lowest genetic similarity was illustrated among *S. cervisiae* (Taif 3) (Y.6) and *K. marxianus* (EMCC 76) (Y.11).

Cluster analysis based on SSR markers

Results in Figure 4 showed that, in the dendrogram for the 11 yeast strains cluster, comprises of two major clusters with 35% dissimilarity. One cluster includes 2 yeast strains *S. cervisiae* (Taif 2) (Y.5) and *K. marxianus* (EMCC 76) (Y.11) strains with 84% genetic similarity. The second cluster including the other 9 Yeast strains (Y.1), *R. glutinis* (EMCC 175); (Y.2), *S. occidentalis* (EMCC 201); (Y.3), *S. cervisiae* (EMCC 69); (Y.4), *S. cervisiae* (Taif 1); (Y.5), *S. cervisiae* (Taif 2); (Y.6), *S. cervisiae* (Taif 3); (Y.7), *S. cervisiae* (Taif 4); (Y.8) *D. hansenii* (EMCC 30); (Y.9), *K. lactis* (EMCC 8); (Y.10*), P. jadinii* (EMCC 102); (Y.11) *K. marxianus* (EMCC 76), with similarity of 81% to more than 98%. The second cluster is divided into two sub clusters. One includes five strains (Y.1), *R. glutinis* (EMCC 175); (Y.2), *S. occidentalis* (EMCC 201); (Y.8) *D. hansenii* (EMCC 30); (Y.9), *K. lactis* (EMCC 8) and (Y.10), *P. jadinii* (EMCC 102), with similarity of 92% and higher. These results demonstrated that, these five strains share genetic backgrounds. Despite this, the five strains collected from different KSA regions showed the highest similarity. This indicates that, environmental and biotic conditions are not of much importance to cause genetic variations among the yeast strains. Moreover, the classification of these strains might be based on morphological traits which are not so reliable compared to molecular markers. The second sub cluster includes three strains: *S. cervisiae* (EMCC 69) (Y.3); *S. cervisiae* (Taif 3) (Y.6) and *S. cervisiae* (Taif 4) (Y.7), with similarity of 88% and higher.

Amino acid analysis of yeast using high-performance liquid chromatography (HPLC) technique

This analysis aimed at selecting yeast strains with high concentration of essential amino acids in order to be used in animal feeding programs. The biochemical analyses (amino acids estimation) are shown in Table 2. The amino acid analysis showed that yeast strains, *R. glutinis* (EMCC 175), *S. occidentalis* (EMCC 201), *D. hansenii* (EMCC 30), *K. lactis* (EMCC 8) and *P. jadinii* (EMCC 102) contained almost double the amount of amino acids compared with *S. cervisiae* (EMCC 69), *S. cervisiae* (Taif 1), *S. cervisiae* (Taif 2), *S. cervisiae* (Taif 3), *S. cervisiae* (Taif 4) and *K. marxianus* (EMCC 76) as illustrated in Figure 6. These results confirmed the logic results, where these five strains were in the same sub cluster (Figure 5), but some biotics or abiotics stresses

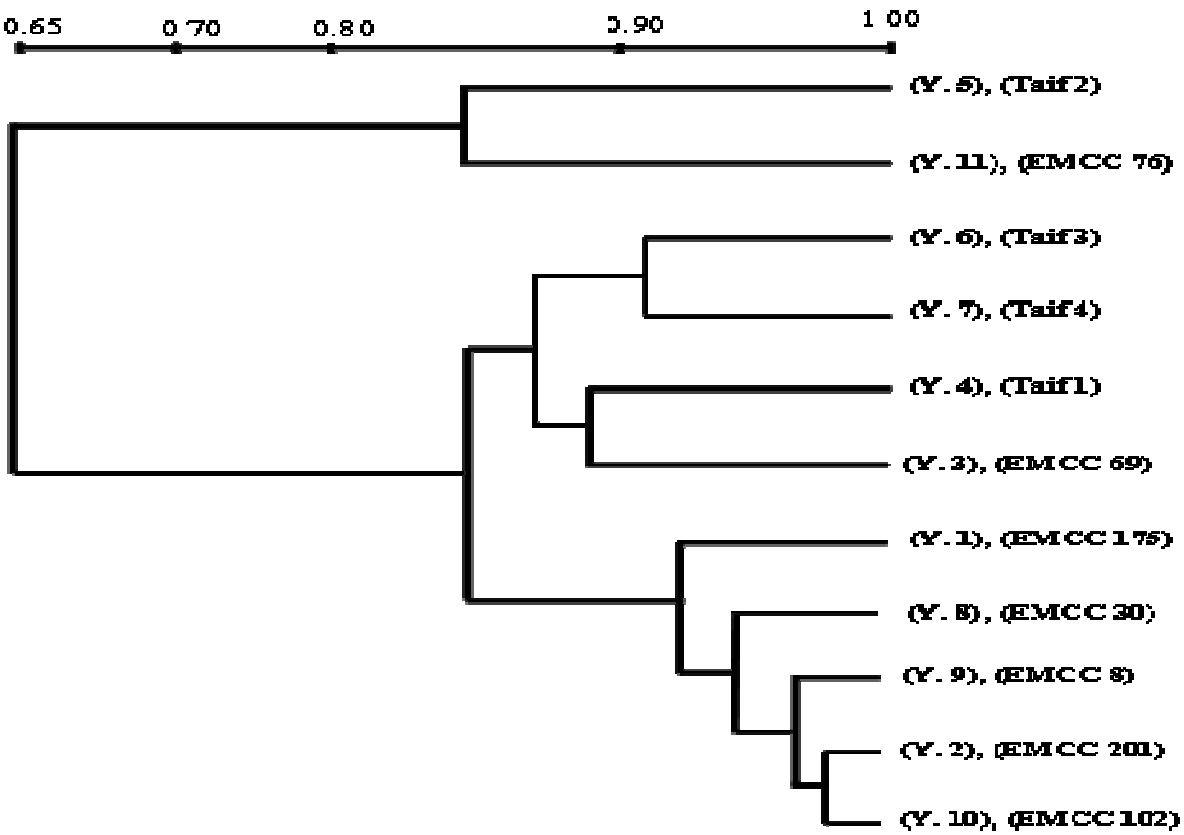

Figure 4. Combined RAPD's, ISSR's and SSR's phylogenetic analysis among eleven Yeast strains. (1) *R. glutinis* (EMCC 175); (2) *S. occidentalis* (EMCC 201); (3) *S. cervisiae* (EMCC 69); (4) *S. cervisiae* (Taif 1); (5) *S. cervisiae* (Taif 2); (6) *S. cervisiae* (Taif 3); (7) *S. cervisiae* (Taif 4); (8) *D. hansenii* (EMCC 30); (9) *K. lactis* (EMCC 8); (10) *P. jadinii* (EMCC 102); (11) *K. marxianus* (EMCC 76).

Table 2. Amino acid contents analysis using HPLC technique of eleven Yeast strains. (Y.1), *R. glutinis* (EMCC 175); (Y.2), *S. occidentalis* (EMCC 201); (Y.3), *S. cervisiae* (EMCC 69); (Y.4), *S. cervisiae* (Taif 1); (Y.5), *S., cervisiae* (Taif 2); (Y.6), *S. cervisiae* (Taif 3); (Y.7), *S. cervisiae* (Taif 4); (Y.8) *D. hansenii* (EMCC 30); (Y.9), *K. lactis* (EMCC 8); (Y.10), *P. jadinii* (EMCC 102); (Y.11) *K. marxianus* (EMCC 76).

Yeast strain	Total essential amino acids	Total aromatic amino acids	Total of other amino acids	Total amino acids
(Y.1), *R. glutinis*	176.8	30.4	142	349.2
(Y.2), *S. occidentalis*	189.4	30.4	138.1	357.9
(Y.3), *S. cervisiase*	60	29.5	97.8	187.3
(Y.4), *S. cervisiase*	86.7	30.4	90.9	208
(Y.5), *S. cervisiase*	48.9	66.4	99.5	214.8
(Y.6), *S. cervisiase*	98.4	30.4	92.9	221.7
(Y.7), *S. cervisiase*	65.2	36	95.6	196.8
(Y.8), *D. hansenii*	199.5	30.6	140.9	371
(Y.9), *K. lactis*	187.6	30.9	154.5	373
(Y.10), *P. jadinii*	208.3	32.4	146.9	387.6
(Y.11), *K. marxianus*	92.7	33.3	105.7	231.7

could cause the little differences between these strains.

The total protein profile analysis will clear the ideas about the correlation between the total amino acids (post transcriptional/pre translational status and post translation of the protein total amount), because it does not mean that the presence of high concentration of free

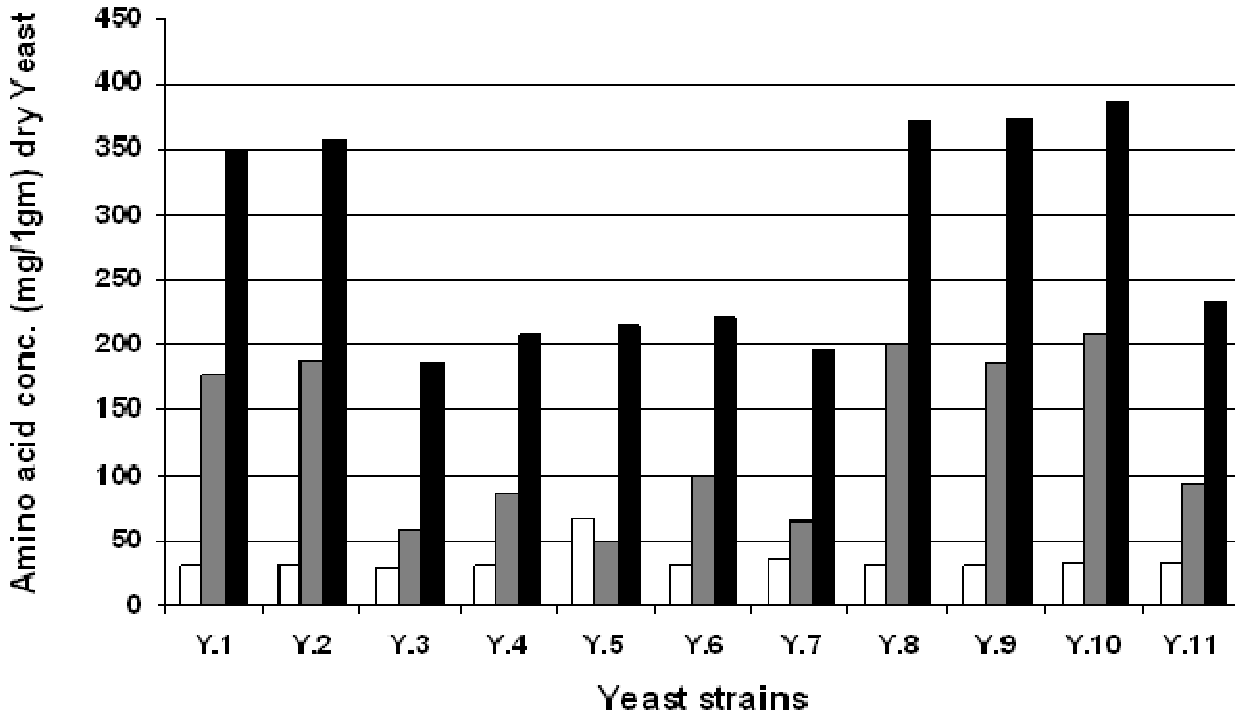

Figure 5. Amino acid contents analysis using HPLC technique of eleven Yeast strains. (Y.1), *R. glutinis* (EMCC 175); (Y.2), *S. occidentalis* (EMCC 201); (Y.3), *S. cervisiae* (EMCC 69); (Y.4), *S. cervisiae* (Taif 1); (Y.5), *S. cervisiae* (Taif 2); (Y.6), *S. cervisiae* (Taif 3); (Y.7), *S. cervisiae* (Taif 4); (Y.8) *D. hansenii* (EMCC 30); (Y.9), *K. lactis* (EMCC 8); (Y.10), *P. jadinii* (EMCC 102); (Y.11), *K. marxianus* (EMCC 76).Black columns represent the total amount of amino acids; gray columns represent the essential amino acids and white columns represent the aromatic amino acids.

amino acids will be correlated with the expression of high amount of total protein in the yeast strains.

Total protein analysis

The total protein banding patterns revealed in a 12.5% of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for 11 yeast strains is shown in Figure 6. Although, there are some differences between yeast strains in protein banding patterns, the total protein profile does not confirm the differences between the 11 yeast strains at total amino acid contents level. Most bands ranging from 28 to 208 KDa and few lower molecular weight protein bands less than 28 KDa was also produced and some yeast samples showed differences in protein patterns. The most intensive observed bands were between 20 and 50 KDa and 98 to 208 KDa. Significantly, there are several changes in total protein banding patterns in the high molecular weight proteins ranging from 99 to 208 KDa. All samples contain band in 99 KDa, while this band is absent in samples No.1 (Y1) and 8 (Y8), (White arrow heads). Also, sample No. 8 (Y8) was missing a protein fragment in the region below 119 KDa. (Gray arrow head), while its present in all yeast samples. Finally, there is a difference among the eleven strains in patterns size in the region directly below 37 KDa, where samples No. 3 (Y3) and 7 (Y7) are different from the rest of yeast strains (Black arrow heads).

Conclusion

In this study, several parameters were measured among the 11 yeast strains. These parameters include molecular markers (RAPD, ISSR, and SSR) and biochemical analysis (total protein profile and total amino acids estimation using high performance liquid chromatography (HPLC)). The different molecular markers have confirmed each other and together supported the biochemical analysis data, as the clustering analysis indicated that five yeast strains, *R. glutinis* (EMCC 175), *S. occidentalis* (EMCC 201), *D. hansenii* (EMCC 30), *K. lactis* (EMCC 8) and *P. Jadinii* (EMCC 102) were in one sub cluster. Also, they had almost same amount of total and essential amino acids.

The long term objective is to correlate a specific PCR product with very important traits. In this aspect, the results of RAPD, ISSR, SSR and the HPLC analysis were very promising because they have shown a special product that can be used in animal feeding programs. The total protein profile does not confirm the differences among the 11 yeast strains at the level of the amino acid contents.

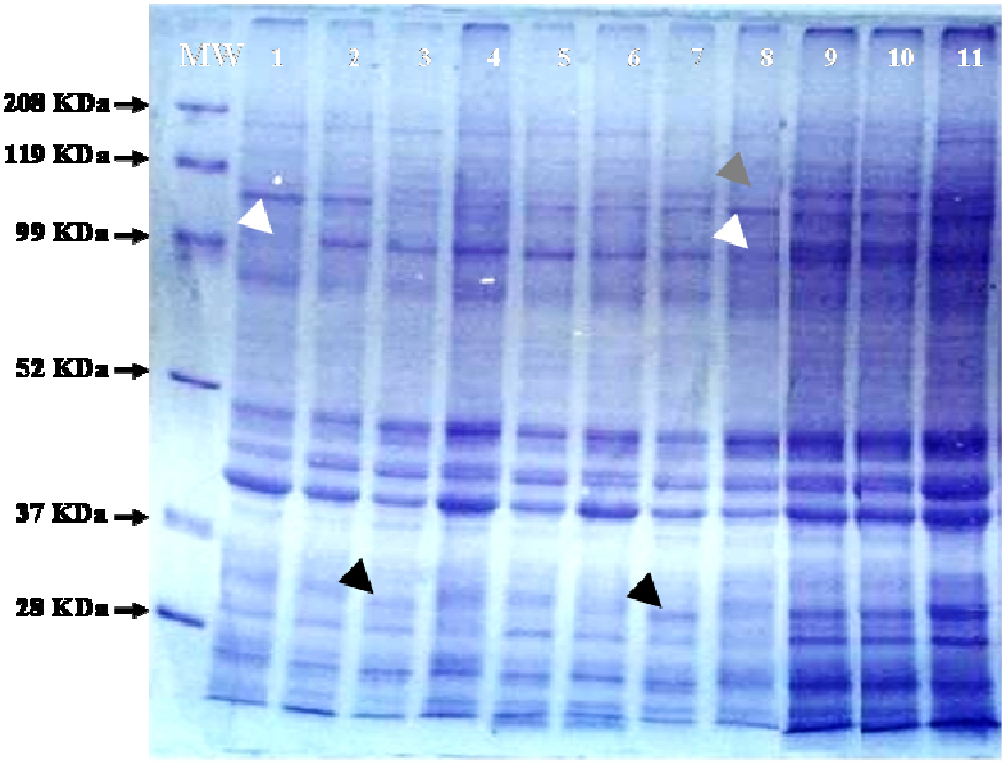

Figure 6. Total protein profile of 11 Saudi and Egyptian Yeast strains. **(**1) (Y1) *R. glutinis* (EMCC 175); (2) (Y2) *S occidentalis* (EMCC 201); (3) (Y3) *S. cervisiae* (EMCC 69); (4) (Y4) *S. cervisiae* (Taif 1); (5) (Y5) *S. cervisiae* (Taif 2); (6) (Y6) *S. cervisiae* (Taif 3); (7) (Y7) *S. cervisiae* (Taif 4); (8) (Y8) *D. hansenii* (EMCC 30); (9) (Y9) *K. lactis* (EMCC 8); (10) (Y10) *P. jadinii*(EMCC 102); (11) (Y11) *K. marxianus* (EMCC 76). MW is Bio Rad product.

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