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PHOSPHOLIPID ANALOGUE DISTRIBUTIONS OF IRANIAN ISOLATES OF CANDIDA

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ABSTRACT

The aim of this study was to analyse polar lipids of Candida species isolated from Ahwaz (Iran) by Fast Atom Bombardment Mass Spectrometry (FAB MS). Nine isolates of Candida Sp. were identified by growth at 45°C, production of chlamydoconidia on cornmeal agar, colonial colour on CHROMagar Candida, germ tube production and ID 32C kits. Then polar lipids were extracted from freeze-dried cultures and analysed using FAB MS. The most intense carboxylate and phospholipid molecular species anions were of *m/z* 281 ($C_{18:1}$) and *m/z* 515 (PA 23:2). However, the most intense carboxylate and phospholipid analogues in *Candida parapsilosis* were 292 (Un) and 555 (PA 26:3), which differed from other yeasts. Isolates were grouped by single linkage clustering based on correlation coefficient for strain pairs calculated with carboxylate and phospholipid molecular species distributions. FAB MS can differentiate the *C. albicans* based on analysis of polar lipid distributions. These findings support that differentiation between *C. albicans* and other species is possible based on polar lipids.
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Keywords: Fast Atom Bombardment Mass Spectrometry, Candida, Phospholipid, Fatty acid.

INTRODUCTION

FAB MS is a new and powerful ionisation method for the analysis of non-volatile compounds, such as complex lipids.[1] FAB MS has found a niche in the mass analysis of biological molecules and it has been extensively used for the analyses of a wide range of biological materials including peptides, carbohydrates, glycolipids and glycopeptides[2] in addition to phospholipids[3] and fatty acids.[4] It can also separate complex mixtures of these compounds. FAB MS has also been used to fingerprint phospholipids of bacteria,[5,6] protozoa,[7] yeasts[8-10] and moulds.[11] Also this technique was used for differentiation of *C. dubliniensis* from *C. albicans*[12] and for studying the effect of antifungals on polar lipids of Candida species.[13]

Most phospholipid classes, phosphatidylserine (PS), phosphatidylinositol (PI), PG, PE and PA (with the exception of phosphatidylcholine (PC) which is detected only in positive-ion FAB MS) are detected with much higher sensitivity in negative-ion FAB MS.[14] Also, FAB MS can provide data on individual phospholipid analogues more easily than other older methods.[15] The advantages of FAB MS are its ease of sample preparation, selective ionisation of polar compounds, rapidity of analysis and ability to analyse mixtures of analogues. These features yield data that could not be collected so simply by any other means.

The genus *Candida* contains approximately 200 species and seven species are major human opportunistic pathogens, namely, *C. albicans*, *C. tropicalis, C. parapsilosis, C. krusei, C. guilliermondii, C. kefyr* and *C. glabrata.* Phospholipids are important biomarkers in

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yeasts. The fatty acid composition of *Candida* species has been investigated previously by gas chromatography and thin layer chromatography[16,17] and by FAB MS.[8,9] PE, PG and PA have been described as major phospholipids in *Candida*.[8,9] Other phospholipids, such as PC, PI, and PS, have been reported.[16,18] In *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *C. famata* the most intense FAB MS peaks attributable to carboxylic anions have been found to be $C_{18:1}$.[8,9] whereas Abdi et al. reported *m/z* 253 ($C_{16:1}$) as major anions in *C. glabrata*.[9] The aim of this study was detection of phospholipid analogue distribution by FAB MS of *Candida* species isolated from Iran.

MATERIAL AND METHODS

Organisms

Nine isolates of Candida species were isolated from different patients (Mycology laboratory, Ahwaz University of Medical Sciences) and identified by CHROMagar Candida (CHROMagar Candida Company, Paris, France), germ-tube test in horse serum (Oxoid, England), Rapid ID 32 C kits (BioMerieux SA, France), growth at 45°C and typical microscopic morphology on cornmeal agar. The species of *Candida* isolated were as follows: *C. albicans* (4), *C. tropicalis* (2), *C. glabrata* (1), *C. lipolytica* (1) and *C. parapsilosis* (1).

Growth of the organisms and harvesting of cells

Each isolate was cultured on three plates of Sabouraud's Dextrose Agar (BBL, USA), after inoculation as a lawn followed by incubation aerobically at 37°C for 48 h. Cultures were harvested with a sterile cotton wool swab that had been moistened with sterile phosphate buffered saline (PBS) (pH=7.4, 0.01M) (Sigma, Poole, Dorset, UK). Harvested cells were washed twice with PBS then twice with distilled water at 3,000×g for 20 min. Wet cell suspensions were completely freeze-dried at -40°C and 10^{-2} Torr with a Modulyo freeze drier (Edwards, Crawley, UK).

Extraction and analysis of lipids

Lipids were extracted by Drucker's[14] method. In brief, 10 mg of freeze-dried cells were extracted with 2 mL of freshly prepared methanol-chloroform (2:1 v/v) (BDH, Pool, England) for 4 h at 25°C and then the above process was repeated. All extracts (4 mL) were evaporated to dryness in a vacuum desiccator over dried silica gel for 5-6 h. Chloroform (1mL) aliquots were added to the dried extracts which were subsequently washed with 1 mL of distilled water then centrifuged at 3,000×g for 20 min. Finally extracts were vacuum dried and stored at -20°C prior to analysis. Extracts were suspended in m-nitrobenzyl alcohol (*m*-NBA) which was used as a matrix fluid then analysed in negative ion mode with a Concept IS mass spectrometer (Kratos, Manchester, UK) using xenon for fast atom bombardment.[4,8] All experiments were repeated so that analyses could be calculated. The FAB/MS spectra printed data collected for 800 peaks per sample with *m/z* values between 200-1000. A typical mass spectrum of *C. albicans* is shown in Fig. 1.

The 10 most abundant anion peaks per isolate observed in the mass spectra were selected for data analy-

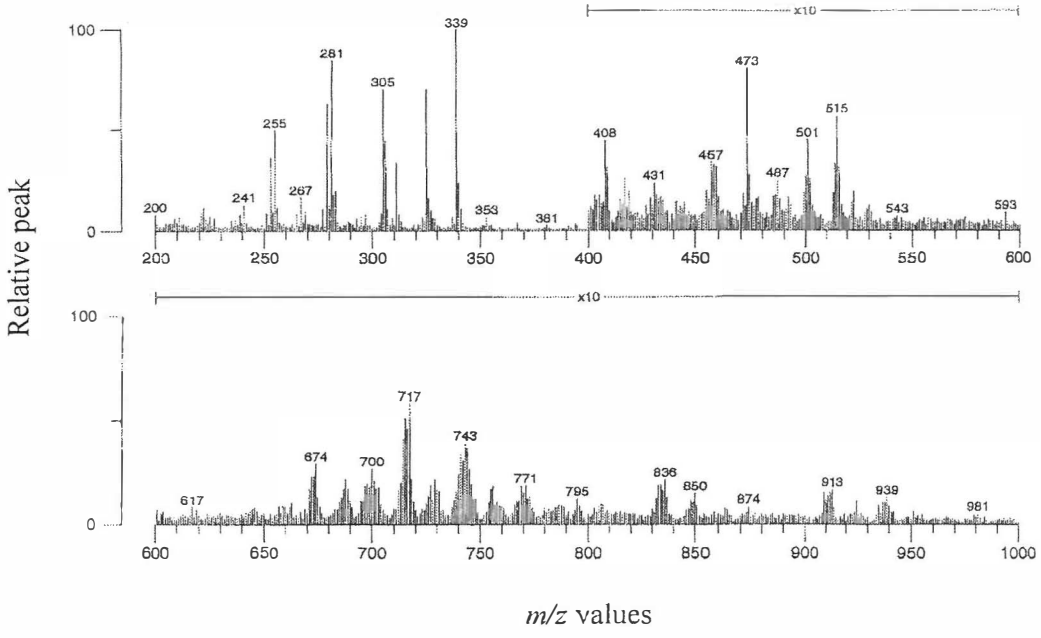

Fig. 1. Negative-ion FAB MS of *C. albicans* (IR16).

Table I. The ten most abundant anions in descending (m/z 200-300) carboxylic acid found in isolates of Candida.

Isolates	Major carboxylate anions mass/charge (m/z)
C. albicans (IR12)	281>255>279>253>282>222>221>280>257>283
C. albicans (IR14)	281>279>255>253>222>200>283,297>267>282
C. albicans (IR15)	281>279>255>253>282,283>267>222>277,280
C. albicans (IR16)	281>279>255>253>283>282>267>222>280>277
C. tropicalis (IR2)	281>279>255>253>222>267>211>239>221>200,297
C. tropicalis (IR7)	281>279>255>253>222>297>211>239>282>280
C. lipolytica (IR10)	281>279>255>277>253>283>222>221>282>297
C. glabrata (IR5)	281>253>283>255>282>280>221,222>252>254
C. parapsilosis (IR13)	292>281>255>265>290>279>293>282>294>263

Keys: 200: Un, 207: Un, 211: ($C_{13:1}$), 221: ($C_{14:3}$), 222: Un, 239: ($C_{15:1}$), 252: Un, 253: ($C_{16:1}$), 254: ($C_{16:1}$)*, 255: ($C_{16:0}$), 257: OH-$C_{15:0}$, 263: ($C_{17:3}$), 265: ($C_{17:2}$), 267: ($C_{17:1}$), 277: ($C_{18:3}$), 279 ($C_{18:2}$), 280: ($C_{18:2}$)*, 281: ($C_{18:1}$), 282: ($C_{18:1}$)*, 283: ($C_{18:0}$), 290: Un, 291: ($C_{19:3}$), 292: Un, 293: ($C_{19:2}$), 294: Un, 297: ($C_{19:0}$), Un: Unidentified peaks, *: First isotope peak.

Table II. The ten most abundant anions in descending (m/z 500-1000) phospholipid analogue anions found in isolates of Candida.

Isolates	Major phospholipid analogue anions mass/charge (m/z)
C. albicans (IR12)	515>715,716>501>717>714>743>742>745>514
C. albicans (IR14)	515>501>514>673>743>516>742,716>714>717
C. albicans (IR15)	515>715>501,717>716>714>743>742>745>741
C. albicans (IR16)	515>501>717>715>716>743>714>514>619>744
C. tropicalis (IR2)	515>501>514>516>500>688>502>513>517>674
C. tropicalis (IR7)	515>501>514>500>743>742>771,516>769,741
C. lipolytica (IR10)	515>501>514>500>516>502>619>513,714,716,739
C. glabrata (IR5)	515>514>742>516>714>500>741>513>671
C. parapsilosis (IR13)	555>535>585>726>800,586>584>583,773>742,769

Keys: 500, Un; 501, PA 22:2; 502, Un; 513, Un; 514, Un; 515, PA 23:2; 516, Un; 517, Un; 535, Un; 555, PA 26:3; 583, PA (28:4); 584, PE 25:4; 585, PA 28:2; 586, PE 25:3; 619, PE (27:1); 671, PA (34:2); 673, PA (34:1); 674, PE 31:1; 688, PE 32:1; 714, PE (34:2); 715, *PE (34:2); 716, PE (34:1); 717, PG 32:2; 726, Un; 739, PI 27:0; 741, PE (34:4); 742, PE (36:2); 743, PG (34:3); 744, PE (36:1); 745, PG 34:2; 769, PG 36:4; 771, PG 36:3; 800, Un; PA, phosphatidic acid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; *First isotope peak; Un, unidentified peaks.

sis. Fatty acids were present as low mass ions (*m/z* 200-300) while phospholipid anions occurred as high mass anions (*m/z* 500-1000). Data were entered into a spreadsheet (Excel, v. 4.0 for windows) normalised (% peaks=100) and than copied and pasted into SPSS (v. 8.0 for windows) in order to perform statistical analyses.

RESULTS

In the present study 9 Candida strains from Iran (IR2, IR5, IR7, IR10, IR12, IR13, IR14, IR15, IR16) were analysed by FAB MS. Fig. 1. shows the negative-ion FAB MS spectrum for *C. albicans* (IR16).

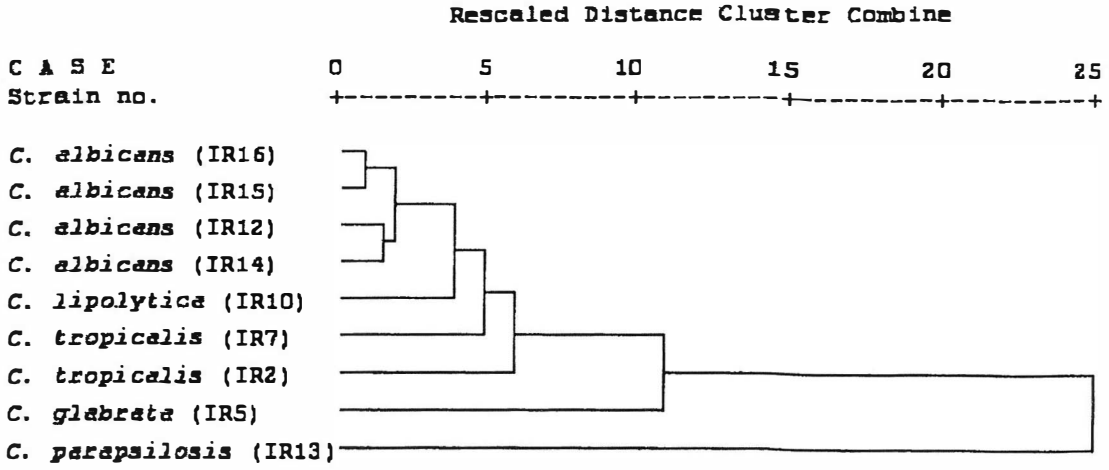

Fig. 2. Dendrogram showing relationships of *Candida* species based on single-linkage of r-values of *Candida* pairs calculated using fatty acid distributions.

Carboxylate anions

Table I shows the ten most abundant intensities (*m/z* values) for carboxylate anions of *Candida* isolates. A peak of *m/z* 281 corresponding to expected presence of oleic acid, ($C_{18:1}$) was the major lower mass anion in 8 *Candida* strains whereas in *C. parapsilosis* (IR13) *m/z* 292 (un) was the major carboxylate anion. Other major carboxylate anions observed were at *m/z* 255 ($C_{16:0}$), 253 ($C_{16:1}$) and 281 ($C_{18:1}$) in *C. albicans* (IR12), *C. glabrata* (IR5) and *C. parapsilosis* (IR13), respectively. The most similar isolates, *C. albicans* (IR15) and *C. albicans* (IR16) had a 'r' value of 0.992, while the least similar isolates *C. parapsilosis* (IR13) and *C. tropicalis* (IR2) revealed a 'r' value of 0.424.

Phospholipid analogue anions

The ten most intense phospholipid analogue anions of isolates are shown in Table II. All isolates revealed a major phospholipid analogue peak at *m/z* 515, tentatively identified as PA (23:2) with the exception of *C. parapsilosis* (IR13). Overall, in 3 isolates (IR12, IR16, IR15) PG was the major class of phospholipid whereas PA was the major phospholipid family in 2 isolates (IR14, IR7). In 4 isolates (IR13, IR5 IR2, IR10) unidentified peaks were the major higher mass polar lipid anions. PI was found in only one (IR7) isolate.

Clustering

Figs. 2 and 3 show the relationship of isolates of *Candida* strains by single linkage clustering of coefficients based on carboxylate and phospholipid analogue anions distribution, respectively. The similarity of the fatty acid compositions of the 4 isolates of *C. albicans* (IR16, IR15, IR14, IR12) separates them from other isolates (Fig. 2). The dendrogram shows that *C. parapsilosis* (IR13) is quite divergent from other isolates. The dendrogram 3 (Fig. 3) prepared based on phospholipid analogue anions shows that 3 Iranian isolates of *C. albicans* (IR12, IR15, IR16) are clearly clustered together. The next cluster contained 2 isolates of *C. tropicalis* (IR7, IR2), *C. lipolytica* (IR10) and *C. albicans* (IR14). The dendrogram shows that *C. parapsilosis* (IR13) is quite divergent from the other isolates.

DISCUSSION

The aim of the study was to analyse phospholipid profiles of *Candida* strains and introduce this technique for taxonomic yeasts in Iran. FAB MS was used for characterization of polar lipids of *Candida* species in the UK.[8-10] Some of the *Candida* species, such as *C. albicans*, *C. parapsilosis, C. tropicalis* and *C. glabrata*, were analysed, however negative-ion FAB MS analysis of *C. lipolytica* has not been undertaken previously.

Carboxylate anions

Overall, major fatty acid profiles displayed in *Candida* isolates in the range of *m/z* 200-3000 were 281 ($C_{18:1}$), 279($C_{18:2}$) and 255 ($C_{16:0}$). This general finding agrees with data obtained by others using FAB MS for analysis of carboxylic acid of *Candida*. As a result, there were no major differences in the proportion of individual carboxylate anions in different yeasts from different geographical locations. However, the proportions of carboxy-

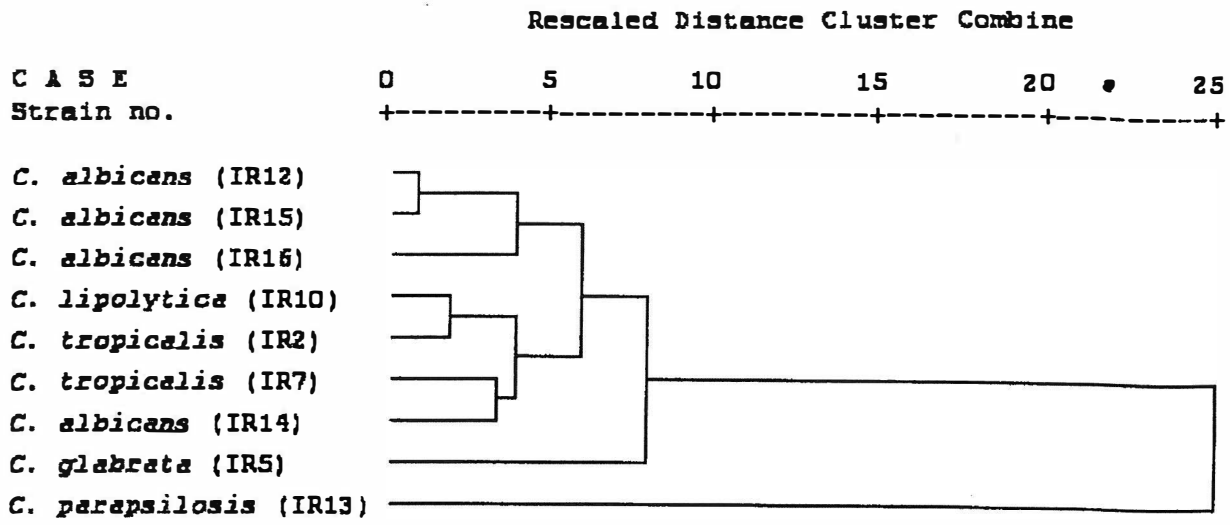

Fig. 3. Dendrogram showing relationships of *Candida* species based on single-linkage of r-values of *Candida* pairs calculated using phospholipid analogue distributions.

late anions of *C. albicans* differ from other species. The finding shows that separation of this species from other species on the basis of fatty acid profiles is possible (dendrogram 2). Both *C. tropicalis* isolates examined (IR2, IR7) have the same carboxylate anion profiles, for example, *m/z* 281> 279> 255> 253. The Pearson Correlation Coefficient calculation also confirmed these finding (r=0.904). The present findings confirmed those of Abdi and Drucker who observed the same carboxylate anions in the two strains of *C. tropicalis* tested.[8] The profile of carboxylate anion of *C. glabrata* (IR5) is more similar to profiles reported by Mahmoudabadi *et al.*[10] The isolate of *C. parapsilosis* (IR13) has different carboxylate anion profiles. For example m/z 292, 297 and 281 were as major carboxylate anion in C. parapsilosis (IR13) and dendrogram 2 also confirms this finding. *C. lipolytica* was analysed for the first time by FAB MS in this study. The most intense carboxylate anion (*m/z* 281) is identical in this species, so that there are no microbiological studies with which to compare the present findings for phospholipid analogues.

Phospholipid analogue anions

The major phospholipid analogue anion found in all isolates was 5155(PA 23:2), with the exception of *C. parapsilosis* that 555 (PA 26:3) was the major phospholipid analogue. Abdi and Drucker have reported PG as the major phospholipid in 3 isolates of *C. albicans* isolated from the UK.[8] However Abdi et al. and Mahmoudabadi et al. have reported PE and PA as the major class of phospholipid in 5 UK isolates of *C. albicans*, respectively.[9] The findings show that there is a considerable diversity in the global population of *C. albicans*. The source and geographical factors probably affect phospholipid distribution, so that Korachi *et al.* separated different Porphyromonas gingivalis from different countries and sources based on polar lipids by FAB MS.[19] The major phospholipid analogue anions were *m/z* 515, 501 and 514 in both strains of *C. tropicalis* and the 'r' value for this strain-pair was 0.876. The most abundant phospholipid class was PG in both strains of *C. tropicalis* whereas Abdi and Drucker have reported PE as the major phospholipid in other strains of *C. tropicalis*.[8] The most intense phospholipid analogue anion was of m/z 515 in *C. glabrata* (IR5), whereas the most abundant phospholipid analogue anions were *m/z* 555> 535>585 in *C. parapsilosis*. The profile of phospholipid analogue anions of *C. parapsilosis* differs from other tested yeasts and dendrogram 3 also confirms this finding. There are no published data on polar lipids of Iranian isolates of yeasts for comparison.

In conclusion, novel data are presented on carboxylate anions and phospholipid analogues anions of *C. albicans, C. parapsilosis, C. lipolytica, C. glabrata* and *C. tropicalis* for chemotaxonomy of yeasts.

REFERENCES

1. Matsubara T, Hayashi A: FAB/mass spectrometry of lipids. Prog in Lipid Res 30: 301-322, 1991.
2. Boone CM, Pergantis SA, Heerma W: Investigation of beam-induced reactions occurring under fast-atom bombardment conditions between triethanolamine and various phospholipids. Rapid Commun Mass Spectrometry 11: 1205-1211,

1997.

3. Drucker DB, Wardle HM, Boote V: Phospholipid profiles of *Clostridium difficile*. J Bacteriol 178: 5844-5846, 1996.
4. Sadek F, Drucker DB, Boote V, Bennett KW, Eley A: Phospholipids of Fusobacterium spp. J Appl Microbiol 85: 302-308, 1998.
5. Drucker DB, Megson G, Harty DWS, Riba I, Gaskell SJ: Phospholipids of *Lactobacillus* spp. J Bacteriol 177: 6304-6308, 1995.
6. Tavana AM, Korachi M, Boote V, Hull PS, Love DN, Drucker DB: Phospholipid analogues of *Porphyromonas gingivalis*. J Appl Microbiol 88: 791-799, 2000.
7. Villas Boas MHS, Lara LS, Wait R, Bergter EB: Identification of plasmenylethanolamine as a major component of the phospholipids of strain DM 28C of *Trypanosoma cruzi*. Molec Biochem Parasitol 98: 175-186, 1999.
8. Abdi M, Drucker DB: Phospholipid profiles in the oral yeast *Candida*. Arc Oral Biol 41: 517-522, 1996.
9. Abdi M, Drucker DB, Boote V, Korachi M, Theaker ED: Phospholipid molecular species distribution of some medically important *Candida* species analysed by fast atom bombardment mass spectroscopy. J Appl Microbiol 87: 332-338, 1999.
10. Mahmoudabadi AZ, Boote V, Drucker DB: Characterisation of polar lipids of oral isolates of *Candida*, *Pichia* and *Saccharomyces* by fast atom bombardment mass spectrometry. J Appl Microbiol 90: 668-675, 2001.
11. Birch M, Drucker DB, Riba I, Gaskell SJ, Denning DW: Polar lipids of *Aspergillus fumigatus*, *A. niger*, *A. nidulans*, *A. flavus* and *A. terreus*. Med Mycol 36: 127-134, 1998.
12. Mahmoudabadi AZ, Coleman D, Drucker DB: Differentiation of *Candida dubliniensis* from *C. albicans* based on polar lipids. IADR, San Diego, USA, 2002.
13. Mahmoudabadi AZ, Drucker DB, Boote V: Effect of nystatin on the phospholipid analogues of *Candida albicans*, 8th Iranian Students Seminar in Europe (ISS), Manchester, UK, 2001.
14. Drucke DB: Fast atom bombardment mass spectrometry of phospholipids for the bacterial chemotaxonomy. In Mass Spectrometry for the Characterization of Microorganisms. A.C.S. Symposium series. 541. Edited by Fenselau, C. Washington D.C. USA. pp. 18-34, 1994.
15. Tavana AM, Drucker DB, Hull PS, Boote V: Phospholipid molecular species distribution of oral *Prevotella corporis* clinical isolates. FEMS Immunol and Med Microbiol 21: 57-64, 1998.
16. Segal E: Vaccines for the management of dermatophyte and superficial yeast infection. In: McGinnis MR, Borgers M, (eds), Current Topics in Medical Mycology. New York: Springer-Verlag, pp. 36-49, 1989.
17. Kaneko H, Hosohara M, Tanaka M, Itoh T: Lipid composition of 30 species of yeast. Lipids 1: 837-844, 1976.
18. Viljoen BC, Kock JLF, Britz TJ: The significance of long-chain fatty acid composition and other phenotypic characteristics in determining relationships among some *Pichia* and *Candida* species. J Gen Microbiol 134: 1893-1899, 1988.
19. Korachi M, Love D, Goldstein EJ, Citron DM, Blinkhorn AS, Boote V, Drucker DB: Comparative phospholipid analogue distributions of *Porphyromonas gingivalis* isolated from cats in Australia and the USA. Veter Microbiol 26: 153-163, 2001.