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Intraspecific Variations Among Strains of *Penicillium aurantiogriseum* using Ubiquinone and Protein Profiles

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Ubiquinone and protein profiles of twenty nine *Penicillium aurantiogriseum* type strains were studied as chemotaxonomic markers. Both markers succeeded in classifying the investigated type strains into three (for ubiquinone) and four (for protein) main groups. The present study provides useful markers for the intraspecific discrimination of the investigated strains and concluded unique in profiling the ubiquinone and protein patterns using HPLC and SDS-PAGE, respectively, of twenty three *P. aurantiogriseum* type strains.

Key words: *Penicillium aurantiogriseum*; Protein; SDS-PAGE; Ubiquinone; HPLC; Intraspecific; Chemotaxonomy.

Classical fungal taxonomy based mainly on morphology which accepted as a universal approach for many groups. Identification of *Penicillium* species is still never to be easy; so many authors had more taxonomic efforts to help *Penicillium* classification. Recent attempts have been made to apply chemotaxonomic and molecular biological data to species recognition and delimitation, and even to determine phylogenetic relationships between *Penicillium* species and their related holomorphic fungi[1].

Penicillium aurantiogriseum was described as a plant pathogen[2], found principally on cereals produces a nephrotoxin causing renal tubular necrosis in rats and it is implicated in Balkan endemic nephropathy[3].

Only few information regarding the benefits of either of the *P.aurantiogriseum* as a microorganism or its extradites such as anicequol and quinazoline alkaloids, as wel as aurantiomides B and C[4] which were a novel inhibitors for anchorage-independent growth of human colon cancer tumor cells.

Three major problems still unsolved in *Penicillium* systematics. First, the species concepts of some species were not definite and there must be many cryptic species to be discovered. Second, the phylogenetic relationships among most of species have not been determined. Third, the evolutionary process of species is little known[5].

Ubiquinones are prenylated quinones

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which are present in nearly all organisms, in plants and animals including humans, and known since 1956. They are part of the inner membrane of mitochondria and bacterial membranes serving as transmitters of electrons and protons in the respiratory chain where they are reversibly transformed into corresponding hydroquinones (ubiquinols) via semiquinones. These compounds are important carriers in the electron transport chain of respiratory systems. The number of isoprene units attached to the quinone nucleus varies, and such differences in ubiquinone structure are excellent indicators in the classification of genera and sub-generic taxa in bacteria and yeasts. Although less common, these techniques have also being used in the taxonomy of black yeasts and filamentous fungi[6-7].

Ubiquinone, also known as coenzyme Q, belongs to the fat-soluble component in aerobic organisms and is widely distributed in nature with varying lengths of the side chain isoprene units[8-9].

Ubiquinones, especially the higher ones, CoQ8 to CoQ12, and particularly CoQ10, were widely used, e.g., in the treatment and prevention of various diseases such as heart and neurological diseases, in cosmetics and as food or dietary supplements. Ubiquinoles as part of the ubiquinone/ubiquinole redox-system are natural antioxidants. Its medical applications include the treatment of cardiovascular diseases[10], hypertension[11], and neurodegenerative disorders such as Alzheimer's, Huntington's, and Parkinson's diseases and Friedreich's ataxia[12-15].

A study of Kuraishi[16] revealed that the eight series of *Eupenicillium* were quite homogeneous in having the Q-9 system as a major ubiquinone. However, *Talaromyces* species, even those within the same series, were often heterogeneous in their ubiquinone systems. The discrepancy between the present *Talaromyces* systematics and the ubiquinone distribution represented a strong need for further assessment of the intrageneric assignment, grouping and classification.

Distribution of ubiquinones in *Penicillium* species and their teleomorphic genera including *Talaromyces* has been demonstrated by Kuraishi[17-19] for taxonomic assessment of the species. These chemotaxonomic approaches were useful to clarify the relationship between most series of *Penicillium*.

Electrophorietic protein profiles provide solutions to some taxonomical problems such as the insufficient morphological characters available to distinguish organisms that are genetically distinct as well as when the morphological diversity masks the genetic similarity, as illustrated by the phenomenon of alternation of generations and the occurrence of perfect and imperfect states. In addition, appropriate morphological structures may be rare, may require special conditions in order to develop, may be detectable only with highly specialized equipment, or may vary with environmental conditions[20].

Distinct and reproducible differences have been found between the protein patterns of related three studied *Penicillium* species; *P.chrysogenum P.frequentans* and. *P.griseofulvum* and of strains within species[21].

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of whole cell protein is widely used for fungal identification, since it offers the advantage to have a good level of taxonomic resolution at the species and subspecies levels[22-25]. SDS-PAGE of total seed proteins has found wide application in resolving systematic relationships and for inter and intra specific studies[26-27].

Moreover proteins were expressed form of genome which can be biomarkers for identification of botanic drugs[28-29].

The analysis made by SDS-PAGE was the most efficient method for characterizing *Candida* strains from the oral cavity, because these species showed significant differences in protein electrophoretic patterns, and variation by gel showed good reproducibility. Moreover, this method could be used as a routine procedure for the identification of yeast strains from the oral cavity or other sites since a database of reference proteins has already been constructed[30].

The present study aims to investigate the taxonomic relationships among several type strains of *P. aurantiogresium* using SDS-PAGE of fungal whole cell protein as well as ubiquinone profiles on the level of intraspecific variations.

MATERIALS AND METHODS

Fungal strains

Twenty eight of *P.aurantiogriseum* type strains were purchased from the International Mycological Institute (IMI) culture collections;

(1)*P.aurantiogriseum* IMI 89372,
(2)*P.aurantiogriseum* IMI 92235,
(3)*P.aurantiogriseum* IMI 039761,
(4)*P.aurantiogriseum* IMI 40236,
(5)*P.aurantiogriseum* IMI 297908,
(6)*P.aurantiogriseum* IMI 243002,
(7)*P.aurantiogriseum* IMI 297971,
(8)*P.aurantiogriseum* IMI 29199,
(9)*P.aurantiogriseum* IMI 297909,
(10)*P.aurantiogriseum* IMI 284413,
(11)*P.aurantiogriseum* IMI 159109,
(12)*P.aurantiogriseum* IMI 89374,
(13)*P.aurantiogriseum* IMI 286084,
(14)*P.aurantiogriseum* IMI 44917,
(15)*P.aurantiogriseum* IMI 291195,
(16)*P.aurantiogriseum* IMI 291555,
(17)*P.aurantiogriseum* IMI 286094,
(18)*P.aurantiogriseum* IMI 280215,
(19)*P.aurantiogriseum* IMI 183170,
(20)*P.aurantiogriseum* IMI 291201,
(21)*P.aurantiogriseum* IMI 297910,
(22)*P.aurantiogriseum* IMI 297890,
(23)*P.aurantiogriseum* IMI 297902,
(24)*P.aurantiogriseum* IMI 204208,
(25)*P.aurantiogriseum* IMI 293185,
(26)*P.aurantiogriseum* IMI 297957,
(27)*P.aurantiogriseum* var. *neoechinulatum* IMI 296927
(28)*P.aurantiogriseum* var. *neoechinulatum* IMI 321491,

while the[29] *P.aurantiogriseum* isolate is an Egyptian local isolate that identified by the Regional Center for Mycology and Biotechnology (RCMB) culture identification and collection unit having the code of RCMB001002(i).

Media

Two media types were used; Malt Extract Agar (MEA) medium was used maintenance of the strains[31]. Ingredients in g/L distilled water were malt extract, 20.0; dextrose, 20.0; peptone, 1.0; and agar, 20. The other medium type was Yeast Extract Sucrose Broth (YES): Ingredients in g/L distilled water were yeast extract, 20.0; sucrose, 150.0. This semi-synthetic medium was used in liquid form for the cultivation of strains for the production of proteins and ubiquinones[3,32].

Protein-Sample extraction

Fungal proteins were extracted according the method of [33]. The freeze-dried mycelia were disrupted using high-speed homogenizer and grounded with an approximately equal weight of clean cold sand and with Tris-HCl buffer (pH8; adjusted by 5M NaOH) (containing 100mM Tris-HCl, 20mM Na_2 EDTA, 0.5mNaCl and 1%SDS) in cold mortar. The obtained slurry was filtered through Whatman No.1 filter paper and then the supernatant was centrifuged at 3000 rpm for 10 min. The supernatant collected and re-extracted with absolute ethanol then decanted under -20°C overnight. Total protein content of mycelial extract was determined by the method of Lowry[34], with bovine serum albumin as the standard.

Protein profiling using SDS PAGE

Total mycelial extract was separated on a 12% SDS-PAGE using a discontinuous buffer system 3ml stacking (5%) and 10 ml resolving (12%) gel. A Bio Rad Mini-Protean II cell was used. A page ruler unstained protein ladder (Fermentas co.) was also run along with protein samples as standard (Molecular weight 10-200 KDa). SDS PAGE was performed at constant current of 30mAmp and voltage of 60 V. Gels were stained using Coomassie brilliant blue dye R-250[35] photographed and documented.

Ubiquinone Extraction and identification

Fungal ubiquinones were extracted according the method of [36]. The freeze-dried mycelia were disrupted using high-speed homogenizer and grounded with an approximately equal weight of clean cold sand and with a sufficient amount of chloroform: methanol (2:1(v/v)) using enough quantity of glass beads was added. Then freeze dried until complete extraction. The dried mixture was thawed and mixed with 3 ml methanol: n-hexane (3:2; v/v) in a universal bottle. The mixture was vortexed for 1 min at least; then, 8 ml of n-hexane was added, mixed and centrifuged at a low speed for 10 min. The top n-hexane layer was removed without disturbing the methanol: n-hexane interface. The combined hexane extracts were dried in foil covered bottles to avoid photodegradation. The dried extracts were dissolved in a sufficient amount of ethyl acetate just prior to chromatographic analysis and leaved for

about two hours allowing the ubiquinone oxidation occur.

The extracted ubiquinones were separated by high performance liquid chromatography (Kromasil C8 (Eka Chemicals, Sweden) column 5C18-P (4.6x250mmx5µm), while the mobile phase was acetonitrile: isopropanol (84:16, v/v), flow rate: 1.0 ml/min at 50C°.

The chromatograms were recorded at 210nm UV detector and identified by using the standard of ubiquinones: Q9 Coenzyme, Q10 Coenzyme and Q10 (H_2) Coenzyme (Sigma Chemical Company - USA)[37-38].

Statistical Cluster Analysis of the Phylogenetic Relationships

The role of the ubiquinone and protein profiles as useful criteria for studying phylogenetic relationships among the investigated penicillia were evaluated by using statistical cluster analysis with joining (tree clustering) being the clustering method using the Statistica software for Windows release (4.5 F, State Soft. Inc.1993).

RESULTS

The obtained results concerning ubiquinone analysis of the investigated penicillia species revealed that the Q-9, was the major ubiquinone formed, since this type of ubiquinone molecule concentration constituted more than 90% of those found in all species, while both Q-10 and Q-10 (H_2) were also found but in minor concentrations. Ubiquinone systems produced by the investigated isolates as well as their concentrations were summarized in (Table 1).

Ubiquinone (Co-Q) profiles of the twenty nine *P. aurantiogriseum* type strains revealed the presence of three different types of ubiquinone; Q-9 was found in all the investigated strains.While, each of Q-10 and Q-10(H_2) were restricted distribution among the investigated organisms (Table 1).

A phylogenetic tree based on ubiquinone revealed the grouping of the investigated isolates into three clades. (Fig. 1)

The first clade embraced *P.aurantiogriseum* strain numbers 29, 28, 26, 25, 24, 23, 27, 21, 20, 18, 15, 5, 12, 1, 8, 7, 6, 4 and 3 joining them together at a linkage distance zero assuming that all of them are numerous replica of the same strain.

The second clade included *P. aurantiogriseum* strain numbers 14, 11, 19, 16 and 2 joining them together at a linkage distance of 0.5. While, the third clade gathered *P.aurantiogriseum* strain numbers 17, 22, 9, 13 and 10 at a linkage distance of 1.0.

It should be noted that the first two groups were spaced out from each other by the linkage distance of 1.1, while they were both apart from the third clade by a linkage distance of 1.7. The ubiquinone profile revealed a valuable chemo-categorization indicator for the studied strains. But, it wouldn't be possible to substantiate such relationships without a supplementary assessment using other markers.

The obtained results concerning protein analysis of the investigated penicillia species revealed that twenty eight discrete types of protein and the approximate molecular weights of the polypeptide ranged from 7 kDa to 180 kDa ,also several proteins were common among most of the investigated organisms (115, 90, 83, 75, 63, 60, 55, 49, 23, 17, 13 and 10 KDa) while, he peptide band of approx 7, 15, 22 and 150 KDa could be advantageously accepted as chemotaxonomic markers for those species owing to their minor and restricted spreading throughout the investigated strains that ranged from 9-10 strains (Fig. 2).

DISCUSSION

Penicillium is one of the most economically important genera among filamentous fungi. Much of their economic impact is deleterious, with food spoilage, mycotoxin production and bio-deterioration heading the list, but in fact their potential for economic utility is equally important[39]. Some authors have published the usage of a compilation of the physiological techniques used in the identification of filamentous fungi. They list a variety of biochemical methods which range from simple agar-based tests to more sophisticate chromatographic and electrophorietic methods. Numerous alternative approaches have been developed, including nutritional and physiological studies, serologic tests, secondary metabolites, ubiquinone systems, protein and fatty acids profiling. Although some of these were very useful for identifying poorly differentiated fungi

Table 1. Ubiquinone profile of the investigated *P.aurantiogriseum* type strains

P. aurantiogriseum type-strains			Co Q 9		Co Q10		Co Q10 (H_2)	
NO.	RCMB Code	Culture-Collection Code	R. T. (min)	Concentration (mg/ml)	R. T. (min)	Concentration (mg/ml)	R. T. (min)	Concentration (mg/ml)
1.	001 002 (1)	IMI 89372	9.6	0.27	-	-	-	-
2.	001 002 (2)	IMI 92235	9.8	0.28	10.8	0.17	11.3	0.08
3.	001 002 (3)	IMI 039761	9.2	0.24	-	-	-	-
4.	001 002 (4)	IMI 40236	8.9	0.39	-	-	-	-
5.	001 002 (5)	IMI 297908	8.3	0.23	9.8	0.13	10.2	0.07
6.	001 002 (6)	IMI 243002	9.4	0.23	-	-	10.3	0.16
7.	001 002 (7)	IMI 297971	9	0.21	-	-	-	-
8.	001 002 (8)	IMI 29199	8.3	0.23	-	-	-	-
9.	001 002 (9)	IMI 297909	9.8	0.24	9.2	0.13	10.4	0.05
10.	001 002 (10)	IMI 284413	8.8	0.23	-	-	-	-
11.	001 002 (11)	IMI 159109	9.5	0.31	8.3	0.2		
12.	001 002 (12)	IMI 89374	9	0.2	-	-	-	-
13.	001 002 (13)	IMI 286084	8.9	0.24	-	-	-	-
14.	001 002 (14)	IMI 44917	8.2	0.29	9.3	0.13	-	-
15.	001 002 (15)	IMI 291195	9.3	0.38	-	-	9.7	0.23
16.	001 002 (16)	IMI 291555	8	0.24	-	-	-	-
17.	001 002 (18)	IMI 286094	8.6	0.22	8.9	0.19	9.5	0.07
18.	001 002 (20)	IMI 280215	8.5	0.25	-	-	-	-
19.	001 002 (21)	IMI 183170	8.1	0.31	-	-	-	-
20.	001 002 (22)	IMI 291201	9.7	0.35	-	-	9.2	0.29
21.	001 002 (23)	IMI 297910	9.8	0.3	9	0.15	-	-
22.	001 002 (24)	IMI 297890	8.1	0.24	-	-	9.9	0.13
23.	001 002 (25)	IMI 297902	8.2	0.24	-	-	-	-
24.	001 002 (26)	IMI 204208	8.7	0.35	9.4	0.15	-	-
25.	001 002 (27)	IMI 293185	8.2	0.31	9.1	0.17	-	-
26.	001 002 (28)	IMI 297957	9.5	0.24	-	-	9.5	0.19
27.	001 003 (1)	IMI 296927	8.8	0.37	-	-	-	-
28.	001 003 (2)	IMI 321491	9.5	0.21	9.4	0.15	-	-
29.	001002(*i*)	RCMB	8.8	0.2	9.8	0.13	-	-

such as yeasts and black yeasts, they were only complementary tools of morphological data in most cases. Moreover, it was a very difficult to depend only on the morphological data to distinguish among the subspecies[36].

Many authors described the usage of ubiquinone systems in a generic and intrageneric level in fungi[26,40]. However, some obtained satisfactory results at the infrageneric level[18, 41-43].

According to the available literature on ubiquinone chemotaxonomy over intraspecific level was found, hence it was encouraging to investigate its potential ability to discriminate among strains of the same species.The phylogenetic tree based on the current ubiquinone revealed the grouping of the investigated strains into three clades. These results are consistent with that published by[44] which succeeded to group the same strains, based on RAPD-PCR and volatile oils as well as secondary metabolites, into different clades indicating differences among those strains and hence reflecting the use of ubiquinone as a useful marker on the level of intraspecific.

Additionally, the study of [39] examined 335 strains and 118 species of *Penicillium sensu lato* and determined that the Q9 ubiqinone type was predominant in the species of *Penicillium sensu stricto*.

Also, several studies reported the importance of ubiquinone in yeast taxonomy[45], and to characterize *Malbranchea* and *Coccidioides*[46]. Moreover, a reliable ubiquinone profile within several strains of *Cunninghamella* was found out[38].

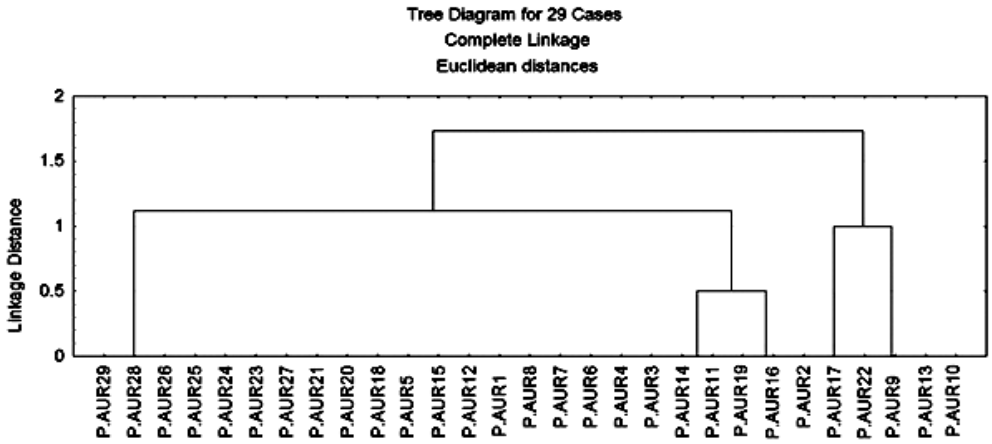

Fig. 1. Phylogenetic tree for ubiquinone marker *of P.aurantiogriseum* strains (1-29) based on complete linkage of Euclidean distances values

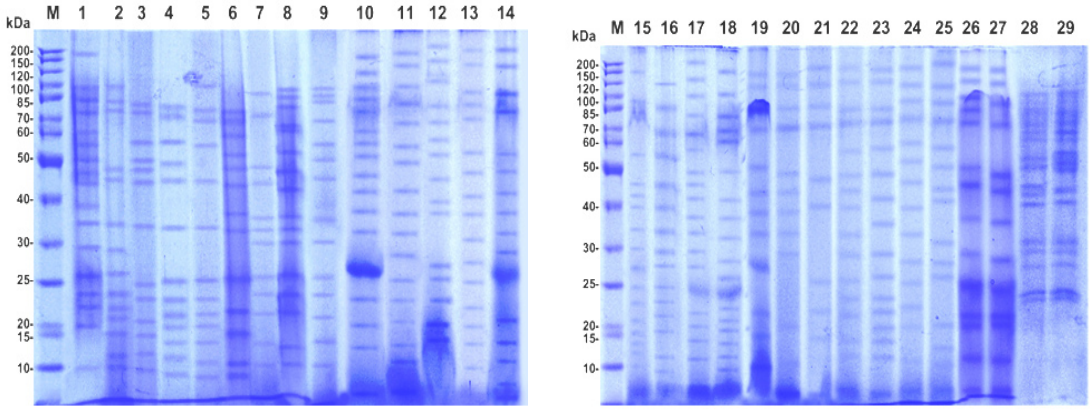

Fig. 2. SDS-PAGE of Protein Profiling of *P.aurantiogriseum* strains (1-29) Showing Banding Pattern of Inter and Intra Specific Relationships. M= Marker.

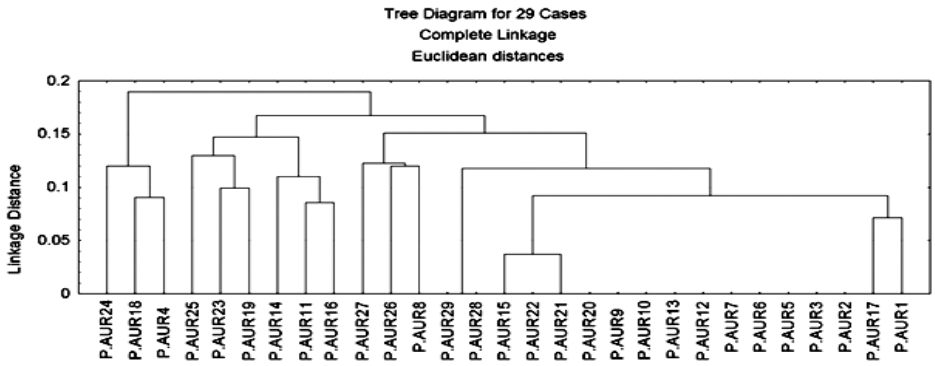

Fig. 3. Phylogenetic tree for Protein marker of *P.aurantiogriseum* strains (1-29) based on complete linkage of Euclidean distances.

It materialized more supportive considering the use of SDS-PAGE marker outline in penicillia chemotaxonomy, since this marker confirmed the former results obtained from ubiquinone marker, with some variations in ordering the tested strains.

The obtained results of SDS- PAGE marker confirmed the results acquired by the study of [44] who studied the intraspecific variations among the same strains of *P. aurantiogriseum* using the profiles of RAPD-PCR, fatty acid; secondary and volatile metabolites as taxonomic markers.

These results are in coherence with the conclusion of [47] who stated that the SDS-PAGE protein profiling character was found effective for phylogenetic studies in *Crotalaria* species. It had proved its advantage over morphological identification. The intraspecific variations between the two accessions of *C.pallida* they investigated could be resolved only by DNA based techniques like RAPD and ISSR markers as they mentioned[48].

Also, the current results are in resemblance with the study on four species of *Crotalaria* based on EST SSR markers[13]. Protein markers are simple and effective in revealing inter specific relationships. The interesting fact revealed by protein markers is that it had clustered *C.grahamiana*; a foliate species, along with the simple leafed species whereas the classical taxonomic key clusters *C.grahamiana* with the compound leafed species which is not supported by the protein data. Since seed storage proteins are the products of gene expression with genetic stability and not been affected much by environment, the protein banding patterns poses a much validated phylogenetic relationship than relying on few morphological characters[49, 50].

The study of [51] used SDS-PAGE and silver staining technique to identify yeast presence in the oral cavities. Another report described the characterization of *Candida* species from oral cavities using meticulous enzyme electrophoresis[52].

CONCLUSION

The current study is unique in profiling the ubiquinone as well as SDS-PAGE pattern of 23 type strains of *P.aurantiogriseum* and proofed to be successful chemotaxonomical markers over the intraspecific level, a level difficult to discriminate using only morphology.

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REFERENCES

1. Pitt, J.I., Cruickshank, R.H. Speciation and synonymy in *Penicillium* subgenus *Penicillium* – towards a definitive taxonomy. In: *Modern concepts in Penicillium and Aspergillus classification* (Samson RA, Pitt, JI, Eds.) Plenum Press, New York, USA, 1991; pp 103-119.
2. Dierckx, R.P. Un essai de revision du genre *Penicillium*. *Ann. Soc. Sci. Bruxelles,* 1901*;* 25(1): 88.
3. Frisvad, J.C., Samson, R.A. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. In*: Penicillium* subgenus *Penicillium*: *new taxonomic schemes, mycotoxins and other extrolites*. (Samson, R.A.; Eds.) *Studies in Mycology,* Centraalbureau voor Schimmelcultures. Netherlands, 2004*;* **49***:* pp.1-174.
4. Xin, Z.H., Fang, Y., D.L., Zhu, T., Duan, L., Chen, J., Gu, Q.Q., Zhu, W.M. Aurantiomides A-C, quinazoline alkaloids from the sponge-derived fungus *Penicillium aurantiogriseum* SP0-19. *J. Nat. Prod.*, 2007*;* **70**(5):853-5.
5. Long, W., Wen-Ying, Z. Phylogenetic analyses of penicillia based on partial calmodulin gene sequences. *BioSystems*, 2007; **88**: 113–126.
6. Yamada, Y., Sugihara, K., van Eijk, G.W., Roeijmans, H.J. de Hoog, G.S. Coenzyme Q systems in Ascomycetous black yeasts. *Antonie Leeuwenhoek Int. J. Genet*. 1989; **56**:349–356.
7. Yaguchi, T., Someya, A., Udagawa, S., A reappraisal of intrageneric classification of *Talaromyces* based on the ubiquinone systems. Mycoscience, 1996; **37**: 55–60.
8. Lester, R.L., Crane, F. L. The natural occurrence of coenzyme Q and related compounds. *J. Biol. Chem*., 1959; **234**: 2169–2175.

9. Hatefi, Y.CoenzymeQ (ubiquinone). *Adv. Enzymol*., 1963; **25**:275–328.
10. Sarter, B. Coenzyme Q10 and cardiovascular disease: A review. *J Cardiovasc Nurs,* 2002; 16:9–20.
11. Wilburn, A.J., King, D.S., Glisson, J., Rockhold, R.W., Wofford, M.R. The natural treatment of hypertension. *J. Clin. Hypertens,* 2004; **6**:242–248.
12. Cooper, J.M., Schapira, A.H. Friedreich's Ataxia: disease mechanisms, antioxidant and coenzyme Q10 therapy. *Biofactors*, 2003; **18**: 163–171
13. Shults, C.W. Coenzyme Q10 in neurodegenerative diseases. Curr. Med. Chem., 2003; **10**: 1917–1921.
14. Beal, M.F. Mitochondrial dysfunction and oxidative damage in Alzheimer's and Parkinson's diseases and coenzyme Q10 as a potential treatment. *J. Bioenerg. Biomembranes*, 2004; **36**: 381–386.
15. Turunen, M., Olsson, J., Dallner, G. Metabolism and function of coenzyme Q. *Biochim. Biophys. Acta*, 2004; **1660**: 171–199.
16. Kuraishi, H., Katayama-Fujimura, Y., Sugiyama, J., Yokoyama, T. Ubiquinone systems in fungi I. Distribution of ubiquinones in the major families of ascomycetes, basidiomycetes, and deuteromycetes, and their taxonomic implications. *Trans. Mycol. Soc.,* 1985; **26**: 383–395.
17. Kuraishi, H., Aoki, M., Itoh, M., Katayama, Y., Sugiyama, J., Pitt, J.I. Distribution of ubiquinones in *Penicillium* and related genera. *Mycol. Res*., 1991a; **95**: 705–711.
18. Kuraishi, H., Sugiyama, J., Yamada, Y. Distribution of ubiquinone systems in fungi. Bull. *JFCC*, 1991b; **7**: 111–133.
19. Kuraishi, H., Itoh, M., Tsuzaki, N., Katayama, Y., Yokoyama, T., Sugiyama, J. The ubiquinone system as a taxonomic aid in *Aspergillus* and its teleomorphs. In: *Modern Concepts in Penicillium and Aspergillus Classification* (Samson, R.A., & Pitt, J.I. Eds.) Plenum Press, New York. 1990; pp 407–421.
20. Robert, H. Electrophoretic Protein Profiles as Criteria in the Taxonomy of Fungi and Algae. *Bulletin of the Torrey Botanical Club,* 1973; **100**(5): 253-259.
21. Bent K, J. Electrophoresis of Proteins of 3 *Penicillium* Species on Acrylamide Gels. *J. gen. Microbiol.,* 1967; **49**:195-200
22. Samelis, J., Tsakalidou, E., Metaxopoulos, J., Kalantzopoulos, G., Differenciation of *Sterptococcus sake* and *Lactobacillus curvatus* isolated from naturally fermented Greek dry salami by SDS-PAGE of whole cell proteins, *J. Appl. Bacteriol*., 1995; **78**: 157-163.
23. Sacilik, S.C., Osmanagaoglo, O., Gunduz, U., Cokmus, C.,. Availibility of use of total extracellular proteins in SDS-PAGE for characterization of gram positive cocci. *Turk. J. Biol*. 2000; **24**: 817-823.
24. Sanchez, I., Sesena, S., Palop, L., Identification of lactic acid bacteria from spontaneous fermentation of 'Almagro' eggplant by SDS-PAGE whole cell protein fingerprinting. *Int. J. Food Microbiol*., 2003; **2555**(24): 181-189..
25. Joelri, L., Michael, R., John, S., Britto, P., Senthilkumar, S.R. Phylogenetic relationships of *Crotalaria* species based on seed protein polymorphism revealed by SDS-PAGE. *International Research Journal of Plant Science*, 2011; **2**(5): 119-128.
26. Karihaloo, J.M., Kaur, M., Singh, S. Seed protein diversity in *Solanum melongena* L. and its wild and weedy relatives. *Genetic Resources and Crop Evolution,* 2002; **49**(6): 533-539.
27. Wang, M.L., Mosjidis, J.A., Morris, J.B., Dean, R.E., Jenkins, T.M., Pederson, G.A. Genetic diversity of *Crotalaria germplasm* assessed through phylogenetic analysis of EST-SSR markers. *Genome,* 2006; **49**:707-715.
28. Muhammad, I.C., Abdul, S.M., Wang, Y., Cheng, Y., Tariq, M., Muhammad, A. Proteins as Biomarkers for taxonomic Identification of Traditional Chinese Medicines (TCMs) from Subsection *Rectae* Genus *Clematis* from China. *World Appl. Sci.,* 2010; **8**: 62-70.
29. Sher, A.K., Habib, A., Shah, M.K., Ayub, K., Sardar, A., Muhammad, S. Confirmation of sunflower F1 hybrids using SDS-PAGE analysis. *African Journal of Biotechnology,* 2010; **9**(29): 4516-4520.
30. Rodrigues, J.A.O., Höfling, J.F., Tavares, F.C.A., Duarte, K.M.R., Gonçalves, R.B., Azevedo, R.A. Braz, A., Evaluation of Biochemical And Serological Methods To Identify And Clustering Yeast Cells Of Oral *Candida* Species By Chromagar Test, SDS-PAGE and ELISA. *J. Biol*., 2004; **64**(2): 317-326.
31. Smith, D., Onions A.H.S. The Preservation and Maintenance of Living Fungi. *J. Kew., Commonwealth, Mycological Institute, Australia*, 1983; 214-219.
32. Peter, D.S., Michael, J.K. Characterization and differentiation of filamentous fungi based on fatty acid composition. *Appl. Environ. Microbiol*., 1996; **62**: 4182-4191.
33. David, G. James, L. Biochemical Changes during the Growth of Fungi. 1-nitogen compounds and carbohydrate changes in *Penicillium atroventum*.

Journal of Bacteriology. 1964; **88**(1): 114-121.

34. Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* 1951; **193**:265-275.
35. Hames, B.O., Rickwood, D. "Gel Electrophoresis of Proteins, a Practical Approach" (2nd Ed.) Oxford University Press, USA, 1990; pp. 1-147
36. Paterson, J.M., Bridge, C. Biochemical techniques for filamentous fungi. *CAB International Press, London, United Kingdom.* 1994; pp. 64-69.
37. Yamada, Y., Kondo, K. Coenzyme Q System in the classification of the yeast-like genera *Sporobolomyces* and *Rhodosporidinne*. *J Gen Appl Microbiol.,* 1973; **19**: 59-77.
38. Ricardo, K., Shiosaki, K., Okada, N., Buarque, de G., Pragati, N., Peter, S. Falcão, N., Henrique, da S., Kazutaka, F., Makoto, M., Galba, M., de C. Biochemical markers in taxonomy of the genus *Cunninghamella. Rev. Iberoam. Micol.,* 2001; **18**: 123-127.
39. Smedsgaard, J. Nielsen, K.F., Larsen, T.O., Lund, F., Thrane, U., Frisvad, J.C. Chemical identification of fungi - metabolite profiling and metabolomics. In: *Fungal Biotechnology in Agricultural, Food and Environmental Applications*. (Arora, D.K., ed.): Marcel Dekker, New York, USA, 2004; pp.19-35.
40. Takizawa, K., Okada, K., Maebayashi, Y., Nishimura, K., Miyaji, M., Fukushima, K. Ubiquinone system of the form-genus *Chrysosporium*. *Mycoscience*, 1994; **35**: 327-330.
41. Schubert, M., Kreisel, H. Ubiquinones in selected species of *Penicillium* and related teleomorph genera. *Persoonia,* 1991; **14**: 341-346.
42. Kuraishi, H., Aoki, M., Itoh, M., Katayama, Y., Sugiyama, J., Pitt, J. Distribution of ubiquinones in *Penicillium* and related genera. *Mycol. Res*. 1991; **95**:705-711.
43. Kuraishi, H., Mutumi, Itoh., Yoko, K., Tadayoshi, I., Atsuhiko, H., Junta, S. Ubiquinone systems in fungi. V. Distribution and taxonomic implications of ubiquinones in Eurotiales, Onygenales and the related plectomycete genera, except for *Aspergillus, Paecilomyces, Penicillium*, and their related teleomorphs. *Antonie van Leeuwenhoek,* 2000; **77**: 179–186.
44. El-meleigy, M.A., Mokhtar, M.M. and Helmy E.A.M. Biochemical Revision of *Penicillium hordei*. *Annals Agric. Sci.,* 2009; 54(1), 3-17.
45. Billon-Grand, G. Minor ubiquinones of the yeast coenzyme Q system: Importance in the taxonomy of the yeasts. *J. Gen. Appl. Microbiol.*, 1987; **33**: 381-390.
46. Okada, K., Takizawa, K., Maebayashi, Y.L., Ubiquinone systems of the genus *Cladosporium* and morphologically similar taxa. FEMS Immunol. Med. Microbiol., 1996; **16**: 39-43.
47. Raj, L.J.M., Britto, S.J., Prabhu, S., Senthilkumar, S.R. Phylogenetic relationships of *Crotalaria* species based on seed protein polymorphism revealed by SDS-PAGE. *International Research Journal of Plant Science,* 2011; **2**(5): 119-128.
48. Samson, R. A. Problems caused by new approaches in fungal taxonomy. *Mycopathologia*, 1991; **116**: 149–150.)
49. Ghafoor, A., Ahmad, Z., Qureshi, A.S., Muhammad, B. Genetic relationship in *Vigna mungo* (L.) Hepper and *V. radiata* (L.) R. Wilczek based on morphological traits and SDS-PAGE. *Euphytica,* 2002; **123**(3): 367-378.
50. Panigrahi, J., Kumar, D.R., Mishra, M., Mishra, R.P., Jena, P. Genomic relationships among 11 species in the genus *Cajanus* as revealed by seed protein (albumin and globulin) polymorphisms. *Plant Biotechnol. Reports,* 2007; **1**(2): 109-116.
51. Maiden, M.F.J. Tanner, A. Identification of oral yeasts by polyacrylamide gel electrophoresis. *Oral Microbiol. Immunol*., 1991; **6** : 187-190.
52. Rosa, E.A.R., Pereira, C.V., Rosa, R.T., Höfling, J. F., , Grouping oral *Candida* species by multilocus enzyme electrophoresis. *Internat. J. Syst. Evolut. Microbiol*., 2000; **50** : 1343-1349.