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Research Article

ELECTROPHORETIC PROTEIN BANDING PATTERNS AMONG PENICILLIUM STRAINS ISOLATED FROM SAUDI ARABIA

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Abstract

14 strains of *Penicillium* species were isolated from different localities and habitats from Jeddah, Saudi Arabia and cultivated on two different media: Czapek Dox's medium, in which $NaNO_3$ is the source of inorganic nitrogen, and Waksman's medium, in which pepton is the source of organic nitrogen.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique was used in this study to distinguish these isolates. The *Penicillium* isolates examined in this study consisted of six *Penicillium* species: *Penicillium corylophilum* (three isolates), *P. rubrum* (one isolate), *P. citrinum* (two isolates), *P. crustosum* (one isolate), *P. canesens* (six isolates) and *Penicillium* sp. (one isolate).

The electrophoretic protein patterns from *Penicillium* isolates grown on Czapek Dox's medium revealed the presence of 17 different bands (out of 14 polymorphic bands, there were three monomorphic bands and two unique bands). The electrophoretic protein pattern of the same isolates grown on Waksman's medium revealed the presence of 12 different bands (out of eight polymorphic bands, there were four monomorphic bands and one unique band).

Data were analysed by a clustering method and similarity coefficients using NTSYSpc version 2.02i. Two different phenograms were produced for the studied *Penicillium* species based on the analysis of the protein banding patterns. Data from the protein banding patterns produced from both media were combined and analysed to produce third phenogram, and the relationships between the species and isolates are discussed.

Keywords: *Penicillium* spp.; identification, proteins; SDS-PAGE; numerical analysis; relationships.

Introduction

Penicillium is a genus of ascomycetous fungi consisting of 304 species of major importance in the natural environment as well as in food and drug production. *Penicillium* species produces penicillin, a molecule used as an antibiotic, which kills or stops the growth of certain types of bacteria inside the body.

Species of *Penicillium* are ubiquitous soil fungi that prefer cool and moderate climates and, are commonly present wherever organic material is available. Many species produce highly toxic mycotoxins. The ability of these *Penicillium* species to grow on seeds and other stored foods depends on their ability to thrive in low humidity and to colonise rapidly by aerial dispersion, provided that the seeds are sufficiently moist. Some species have a blue colour, they are commonly found growing on old bread and giving it a blue fuzzy texture. *Penicillium* species are present in the air and dust of indoor environments, such as homes and public buildings. The fungus can be readily transported from the outdoors, and can grow indoors by using building material or accumulated soil to obtain nutrients for growth.

The taxonomy of *Penicillium* has always been complex due to its great number of species, which exhibit very few differences. This fact complicates the ability of researchers to understand their ecology and, diversity and consequently, the exploration of *Penicillium* by industry has been limited. Historically, the classification of organisms has been based on observable characteristics. The growth of isolates in the appropriate culture media, enabling their most characteristic features to be recognised, is still the most common procedure used to classify organism. Because many species classified in the sub-genus *Penicillium* are morphologically similar, species identification using traditional morphological techniques remains difficult.

The genus *Penicillium* is subdivided into four subgenera (Aspergilloides, Penicillium, Biverticillium and Furcatum) distinguished by the number of branch points between the phialide and the stipe down the main axis of the penicillus and other characteristics, such as the ratio of metula length

to phialide length, and the colony diameter on G25N, when the number of branch points is the same (Pitt and Hocking, 1997).

Morphological characteristics of microbes may be influenced by environmental factors, and genomic mutations cannot be investigated by morphological markers. Here, one additional step (the analysis of molecular markers) has been taken to overcome the complications associated with the morphological characterization of some *Penicillium* species.

In addition, recent trends in taxonomy stress the utmost importance of utilising other criteria that prove to be good phylogenetic or taxonomic markers. One of the methods most widely used for taxonomic classification at the species level has been sequencing and electrophoretic methods. Electrophoretic analysis of cellular proteins provides a measure of the number of protein products that are genetically programmed and thus can be a good taxonomic marker at the species level and even lower categories.

Electrophoresis is the migration of charged molecules through a solution in response to an electric field. Their rate of migration depends on the strength of the field, on the net charge, size and shape of the molecules and on the ionic strength, viscosity and temperature of the medium in which the molecules are moving. As an analytical tool, electrophoresis is simple, rapid and highly sensitive. It is used analytically to study the properties of a single charged species and as a separation technique (Maurer, 1971).

The genus *Penicillium* is one of the largest and most widely distributed fungal genera described to date. As a result, its taxonomic classification has become complicated and species discrimination within this genus is difficult (Redondo *et al*., 2009).

Many studies have been conducted using electrophoresis as a tool to differentiate between *Penicillium* ssp. isolates, such as: Grassin & Fauquembergue (1996); Banke *et al*., 1997; Dupont *et al*., 1999; Boysen *et al*. 2000; Chávez *et al*., 2002; Samson *et al*.,2004; Cho *et al*.. 2005; Bakri *et al*., 2007; Cardoso *et al*., 2007; Xi *et al*., 2007; Abulhamd, 2009; Redondo *et al*., *2009;* Inkha & Boonyakiat, 2010; Roslan *et al*., 2010; Elhariry *et al*., 2011; Francis *et al*., 2011; Mohammad *et al*., 2011 and Tiwari *et al*., 2011.

In this study, biochemical assay was performed on 14 isolates of *Penicillium* species that had been previously collected from different localities and habitats in Jeddah, Saudi Arabia and cultivated on two different media: Czapek Dox's medium in which $NaNO_3$ is the source of inorganic nitrogen and Waksman's medium in which pepton is the source of organic nitrogen.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique was used in this study to distinguish these isolates. The *Penicillium* isolates included six *Penicillium* species, *Penicillium corylophilum* (three isolates), *P. rubrum* (one isolate), *P. citrinum* (two isolates), *P. crustosum* (one isolate), *P. canesens* (six isolates) and *Penicillium* sp. (one isolate).

The data were analysed by a clustering method and similarity coefficients using NTSYSpc version 2.02i. The similarities between the studied isolates are represented as phenograms and will be discussed.

Materials and Methods

Collection of Penicillium Samples and Isolates

In this study, 14 isolates of *Penicillium* made up of six different species were examined. These species included the following: *Penicillium corylophilum* Dierckx. (three isolates), *P. rubrum* Stoll. (one isolate), *P. citrinum* Thom. (two isolates), *P. canescens* Sopp. (six isolates), *P. crustosum* Thom. (one isolate) and *Penicillium* sp. (from an oily sewage dump). Fungal isolates were sampled from five different substrate sources and different ecosystems in both terrestrial and marine environments in Jeddah, Saudi Arabia (agricultural soil, marine fauna, a sewage dump, an oily sewage dump and wheat grains).

Culture, Identification and Cultivation of Penicillium Isolates

Samples were cultured over Czapek (CZ), Potato Dextrose Agar (PDA) and malt extract agar media to isolate the fungal species that were present in these samples. The colours of the isolates on the various media and their morphological features under a light microscope were used to identify the different species using the references of Ainsworth (1971) and Pitt (1979). The studied isolates and their original sources are presented in Table 1.

Fungal Protein Electrophoresis Technique (SDS-PAGE)

The isolated fungi were cultivated on two types of media to determine their protein banding patterns: Czapek Dox's medium, in which $NaNO_3$ is the source of inorganic nitrogen and Waksman's medium, in which pepton is the source of organic nitrogen. For each fungus, a triplicate set of 250 ml Erlenmeyer conical flasks, containing 50 ml of medium was prepared, sterilised at 121 °C for 15 minutes under 1.5x atmospheric pressure, cooled and inoculated with the experimental fungus. Then, the cultures were incubated at 25 °C for seven days. After that, the contents of each set of flasks (the fungal mats) were collected and subjected to SDS-PAGE as described by Laemmli (1970) and modified by Studier (1973). Then 1 g of each fungal mat was mixed with 0.025 ml of extraction buffer (Tris-HCl pH 8.8) with gentle agitation and refrigerated for 24 hours. The slurry was centrifuged at 12.000 rpm for 10 minutes. The supernatant was kept at 0 °C until use. The gel and banding profile of each isolate was photographed, scanned and analysed using the Gel Doc 2000 Bio-Rad system. The molecular weight standard used for the gel analysis was the

Bio-Rad protein marker Mid-Low range (116.000 -14.200 kDa.).

Table 1: Names and sources (Habitats) of studied isolates of *Penicillium*.

Sample number	Sources (Habitats)	Identification
1	Agricultural soil	*Penicillium corylophilum* Dierckx.
2	Agricultural soil	*Penicillium corylophilum* Dierckx.
3	Marine fauna	*Penicillium rubrum* Stoll.
4	Sewage dump	*Penicillium citrinum* Thom.
5	Sewage dump	*Penicillium corylophilum* Dierckx.
6	Sewage dump	*Penicillium citrinum* Thom.
7	Oily sewage dump	*Penicillium* sp.
8	Agricultural soil	*Penicillium canescens* Sopp.
9	Agricultural soil	*Penicillium crustosum* Thom.
10	Agricultural soil	*Penicillium canescens* Sopp.
11	Agricultural soil	*Penicillium canescens* Sopp.
12	Agricultural soil	*Penicillium canescens* Sopp.
13	Agricultural soil	*Penicillium canescens* Sopp.
14	Wheat grains	*Penicillium canescens* Sopp.

Table 2: Survey of polymorphic and monomorphic SDS-PAGE genotype and bands of total bulk protein samples from the studied isolates of *Penicillium* grown on Czapek Dox's medium.
[+ = Present and - = Absent]

Band No.	Molecular weight (kDa)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	91.700	-	-	-	-	-	-	-	-	-	+	+	+	-	+
2	78.790	-	-	-	+	-	-	-	-	-	-	-	-	-	-
3	74.600	-	-	-	-	-	-	-	-	-	-	+	-	-	-
4	71.900	-	-	-	-	-	-	-	-	+	+	+	-	+	-
5	65.600	-	-	-	-	-	-	-	-	-	+	+	+	+	-
6	59.300	-	-	-	-	-	-	-	-	-	+	+	+	+	+
7	56.230	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	51.080		+	-	-	+	+	+	-	+	+	+	-	-	-
9	43.500	-	-	-	-	-	+	-	-	-	-	-	-	+	+
10	40.500	-	-	-	-	-	-	-	-	+	+	+	+	+	+
11	35.000	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	29.660	+	+	+	+	-	-	+	+	-	-	-	+	+	+
13	25.700	-	-	-	-	-	-	-	-	+	+	+	+	+	+
14	21.700	-	-	-	-	-	-	-	-	-	-	-	+	+	+
15	19.300	+	+	+	+	+	+	+	+	+	+	+	+	+	+
16	13.880	+	-	+	+	-	-	-	+	-	-	+	-	-	-
17	12.860	+	+	+	+	+	+	+	+	-	-	-	+	+	+
Total number of bands		6	6	6	7	5	6	6	6	7	10	12	12	12	11

Numerical Analysis

The SDS-PAGE data obtained for each isolate were pooled together and coded to create the data matrix used for computation, where the absence of a band was scored as "0" and the presence of a band was scored as "1" for each species. The relationships between the studied species, expressed by a similarity coefficient, have been represented using a phenogram based on the analysis of the recorded characters using NTSYSpc version 2.02i (1998). The similarity index was estimated using the Dice coefficient of similarity (Nei and Li, 1979). The average of the similarity matrices was used to generate a tree by the Unweighted Pair-Group Method Arithmetic Average (UPGMA). The similarity matrix was developed by the SPSS computer package system ver.16.

Two different phenograms were produced for the studied *Penicillium* species based on analysis of their protein banding patterns and third phenogram was produced based on combined data obtained from both media.

Results and Discussion

SDS-PAGE Protein Banding Pattern Analysis

In this study, SDS-PAGE was performed to examine the protein banding patterns of 14 isolates representing six *Penicillium* species isolated from different sources and grown on two media (Czapek Dox's medium and Waksman's medium).

The electrophoretic protein banding patterns of Penicillium isolates grown on Czapek Dox's medium

The electrophoretic protein patterns of *Penicillium* isolates grown on Czapek Dox's medium revealed the presence of 17 different bands. The electropherograms of those isolates are shown in Fig. 1(A) and the distribution of the bands is described in Table 2.

The highest number of bands recorded in a single isolate was 12 bands in isolates number11, 12 and 13, while the lowest number of bands found was five bands in one isolate (no. 5). The molecular weight of the products ranged from 91.700 to 12.860 kDa. The highest molecular weight band recorded was 91.700 kDa., and this band was found in four isolates (10, 11, 12 and 14), while the lowest molecular weight band recorded was 12.860 kDa. This band was recorded in all samples, except for the isolates numbered 9, 10 and 11 (Table 2).

A total of 14 polymorphic bands were observed in the protein profiles of the studied isolates (Table 6). Of the polymorphic bands, two were identified unique band has the molecular weight of 78.790 kDa. and is present in characterised isolate no. 4 and the other band has a molecular weight of 74.600 kDa. and is present in characterised isolate no. 11.

With respect to the monomorphic bands, three bands were detected (at approximately 56.230, 35.000 and 19.300 kDa.). These bands allow better discrimination between the studied *Penicillium* isolates.

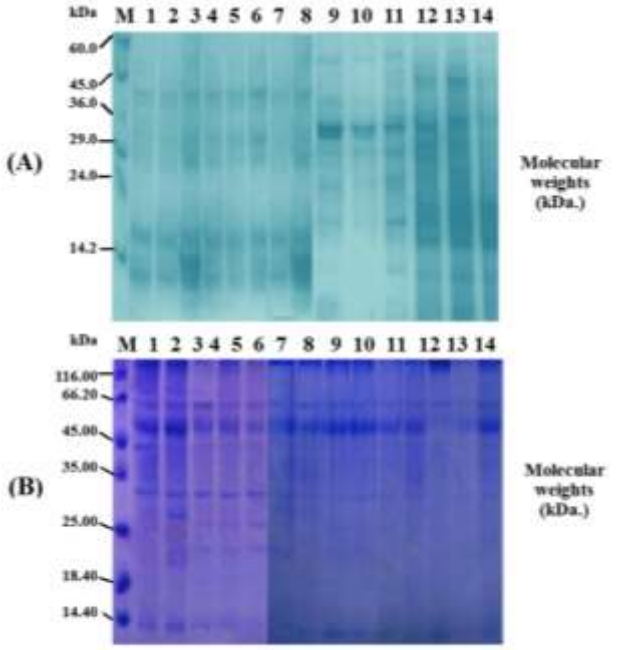

Fig. 1: Electrophoretic banding profiles of fungal proteins extracted in Tris-HCl buffer of the studied isolates of *Penicillium* species grown on Czapek Dox's medium (A) and grown on Waksman's medium (B) .

Similarity matrix between the studied isolates of Penicillium species grown on Czapek Dox's medium based on SDS-PAGE analysis

The similarity matrix for the isolates of *Penicillium* species that were grown on Czapek Dox's medium was developed using SPSS computer package and, based on the SDS-PAGE analysis (Table 3). The highest similarity value was 0.893, between *Penicillium* isolates *P. canescens* (12) and (13), and the lowest similarity value was 0.185, between isolates *P. corylophilum* (2) and *P. citrinum* (6).

The electrophoretic protein banding patterns of *Penicillium* isolates grown on Waksman's medium

The electrophoretic protein pattern of the same isolates of *Penicillium* grown on Waksman's medium revealed the presence of 12 different bands. The electropherograms of those isolates are shown in Fig. 1(B) and the distribution of the bands recorded described in Table 4

The highest number of bands recorded in one isolate was 11 bands in *Penicillium* isolates numbers 2 and 6, while the lowest number of bands was five bands, which was recorded in *Penicillium* sp. (no. 7). The molecular weight of the products ranged from 61.230 to 13.210 kDa. The highest molecular weight band was recorded in all of the *Penicillium* isolates (monomorphic band), while the lowest one appeared in all isolates, except sample number 6 (Table 4)

Table 3. Similarity matrix between the studied isolates of *Penicillium* species grown on Czapek Dox's medium based on SDS-PAGE analysis.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	1	0.288	0.288	0.288	0.418	0.207	0.288	0.288	0.288	0.318	0.232	0.237	0.742	0.624
2		1	0.257	0.307	0.245	0.185	0.333	0.507	0.207	0.427	0.278	0.247	0.633	0.604
3			1	0.288	0.288	0.288	0.418	0.217	0.230	0.288	0.288	0.518	0.383	0.464
4				1	0.364	0.267	0.564	0.247	0.433	0.264	0.364	0.564	0.542	0.772
5					1	0.532	0.318	0.539	0.271	0.278	0.518	0.417	0.532	0.711
6						1	0.432	0.383	0.132	0.429	0.519	0.383	0.383	0.632
7							1	0.811	0.742	0.853	0.843	0.604	0.485	0.742
8								1	0.742	0.812	0.742	0.633	0.874	0.742
9									1	0.485	0.742	0.485	0.383	0.874
10										1	0.634	0.874	0.604	0.509
11											1	0.883	0.633	0.883
12												1	0.893	0.742
13													1	0.742
14														1

Table 4: Survey of polymorphic and monomorphic SDS-PAGE genotype and bands of total bulk protein samples from the studied isolates of *Penicillium* grown on Waksman's medium.

Band No.	Molecular weight (kDa)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	61.230	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	52.400	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	44.300	+	-	-	-	+	-	-	-	-	-	-	-	-	-
4	41.000	+	-	-	-	+	-	-	-	-	-	-	-	-	-
5	40.150	+	+	-	-	+	-	-	-	-	-	-	-	-	-
6	38.660	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	36.790	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	30.910	+	-	-	-	-	-	-	-	-	-	-	-	-	-
9	27.000	+	+	+	+	-	-	+	+	+	+	+	+	+	+
10	25.560	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	22.320	-	-	+	+	-	-	-	-	+	+	+	-	-	-
12	13.210	+	+	+	+	-	-	+	+	+	+	+	+	+	+
Total number of bands		6	11	8	8	8	11	5	7	7	8	8	8	7	7

[+ = Present and - = Absent]

Eight of the recorded bands were found to be a polymorphic type band within the protein profile of the studied isolate (Table 6). Of the polymorphic bands, one was identified to be unique, and this band has a molecular weight of 30.913 kDa. (characterised isolate no. 8). With respect to the monomorphic bands, four monomorphic bands were detected (at approximately 61.230, 52.400, 38.660 and 36.790 kDa.). These bands allow further discrimination between the studied *Penicillium* isolates. Bands with the molecular weights of 27.000 and 13.210 kDa. were present in all studied isolates except, sample no. 6 (*P. citrinum*).

Similarity matrix between the studied isolates of Penicillium species grown on Waksman's medium based on SDS-PAGE analysis

The similarity matrix created for the studied isolates of *Penicillium* species grown on Waksman's medium based on the SDS-PAGE analysis is shown in Table 5. The highest similarity value was 0.934, between *Penicillium* isolates between *Penicillium* isolates *P. canescens* (12) and (13), and the lowest similarity value was 0.091, between isolates *P. citrinum* (4) and *P. canescens* Wide variations in the protein patterns were observed between *Penicillium* isolates grown on Czapek Dox's medium and those grown on Waksman's medium. This phenomenon has previously

observed in other fungi. Electrophoretic data obtained by Hofling *et al*. (2001) showed that yeast species exhibit different protein patterns when grown in different culture media. In their opinion, some possible explanation for this event were that the precursors required for the synthesis of some proteins may be present in one medium and absent in another or that some enzymes are formed in the presence of their specific substrate, yielding distinct protein profiles. The same results were obtained by Al-Hazmi and Kamel (2012) using different isolates of *Aspergillus* ssp.

Numerical Analysis of SDS-PAGE Data

The data obtained from the SDS-PAGE analysis of each isolate were pooled together and coded to create the data matrix for computation, where "0" represented the absence of a band, and "1" represented for the presence of a band in each species. The relationships between the studied isolates and species, expressed using the similarity coefficient, presented as a phenogram, based on the analysis of the recorded attributes using the NTSYSpc version 2.02i, as described by Rohlf (1998). For the data analysis, the total number of recorded attributes (29) in each isolate were scored, combined together in one set of data and coded to create the data matrix for computation (Table 7).

The constructed phenogram is based on the estimated SDS-PAGE proteins of 14 fungal isolates belonging to the genus *Penicillium* grown on both media Czapek Dox's and Waksman's (Fig. 2-C) and revealed a very close relationship between *Penicillium rubrum* Stoll. (3) from marine fauna and *Penicillium canescens* Sopp. (8) from agricultural soil, both isolates clustered at 0.42 due to the presence of 13 common bands. In addition, a very close relationship was observed between *Penicillium corylophilum* Dierckx. (2) from agricultural soil and *Penicillium* sp. (7) from the oily sewage dump, both isolates clustered at 0.49. The same observations were scored between isolates (12 & 14), (9 & 10) and (1 & 5), at the levels of 0.68, 0.80 and 1.20, respectively.

The phenogram demonstrates that the examined isolates (OTU's) have a similarity coefficient of approximately 1.67, at this leve,l *Penicillium citrinum* Thom. (6) from the sewage dump is split from the other isolates. Then, at a level of approximately 1.51, the remaining isolates are divided into two groups, GROUP I comprises six isolates, and II comprises seven isolates. GROUP I included five isolates of *Penicillium canescens* (5/6) (10, 11, 12, 13 & 14) and the only isolate of *Penicillium crustosum*, while GROUP II (at the similarity coefficient of 1.32) included two isolates of *Penicillium corylophilum* (2/3) (1 & 5) which distinguished from each other at the level of 1.20, the isolates of *Penicillium citrinum* (4), the isolates of *Penicillium corylophilum* (2), the isolates of *Penicillium rubrum* (3), and the isolates of *Penicillium canescens* (8) and *Penicillium* sp. (7).

Based on these results demonstrating common bands, and according to the statistical analysis, a very close relationship was observed between the protein bands obtained from isolates of *Penicillium canescens* (10, 11, 12, 13 & 14). Therefore, the cellular proteins from isolates of this species follow the same electrophoretic pattern. This result does not reflect the differences in the geographic origin and source of the same fungal species that had been previously reported by Houseny (2005), Afifi *et al*. (2006) and Al-Hazmi & Kamel (2012). SDS-PAGE of fungal cellular proteins can be a useful tool to distinguish between these species or isolates of the genus *Penicillium* (as shown by the close relation between protein bands obtained from isolates of *Penicillium canescens* (10, 11, 12, 13 & 14) (Fig. 2).

Table 5. Similarity matrix between the studied isolates of *Penicillium* species grown on Waksman's medium based on SDS-PAGE analysis.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1	0.851	0.823	0.811	0.910	0.920	0.683	0.826	0.714	0.255	0.426	0.756	0.625	0.426
2		1	0.847	0.737	0.847	0.690	0.737	0.847	0.714	0.357	0.598	0.426	0.666	0.285
3			1	0.818	0.863	0.737	0.831	0.831	0.598	0.357	0.821	0.598	0.426	0.201
4				1	0.821	0.814	0.702	0.637	0.598	0.426	0.883	0.817	0.598	0.091
5					1	0.437	0.680	0.447	0.598	0.426	0.747	0.834	0.837	0.255
6						1	0.756	0.547	0.714	0.426	0.647	0.812	0.837	0.357
7							1	0.647	0.714	0.357	0.852	0.647	0.625	0.357
8								1	0.714	0.357	0.807	0.441	0.695	0.213
9									1	0.357	0.637	0.731	0.653	0.418
10										1	0.631	0.810	0.877	0.353
11											1	0.738	0.812	0.357
12												1	0.934	0.341
13													1	0.321
14														1

Table 6: Number and types of protein bands as well as the percentage of the total polymorphisms, generated for *Penicillium* isolates.

Medium	Monomorphic bands	Polymorphic		Total bands	Polymorphic %
		Non unique	Unique		
Czapek Dox's	3	12	2	17	82.35%
Waksman's	4	7	1	12	66.67%
Total	7	19	3	29	-------

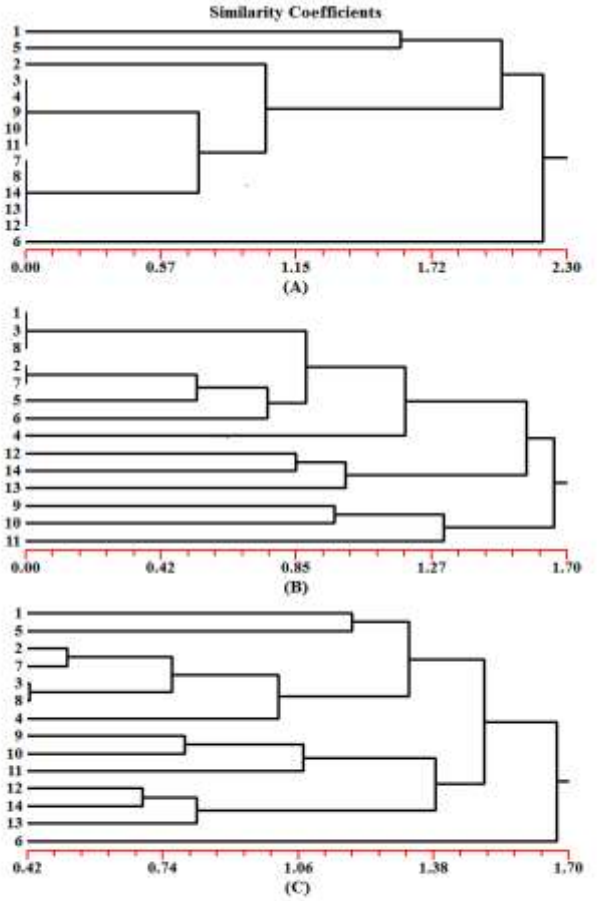

Fig. 2: UPGMA-phenogram constructed from the 14 isolates of *Penicillium* species grown on the following: (A) Czapek Dox's medium, (B) Waksman's medium and (C) both media, based on protein attributes after extraction with HCl buffer.

The use of isozymes electrophoresis as a tool to differentiate between *Penicillium* isolates was demonstrated by Banke *et al*. (1997). In their work, eighty-four isolates of *Penicillium chrysogenum* and related species were examined by isozyme analysis. They concluded that four main groups could be defined by cluster analysis: *P. chrysogenum* var. *chrysogenum*, *P. flavigenum* sp. *nov*., *P. chrysogenum* var. *dipodomyis* and *P. nalgiovens*e. *P. flavigenum* was described as a new species and *P. chrysogenum* var. *dipodomyis* was raised to the species level as *P. dipodomyis*. Additionally, the genetic distances and cluster analysis suggested that the groups should be considered to be four separate species.

Bent (1967) stated that soluble proteins extracted from mycelium of *Penicillium griseofulvum* were varied greatly with age of culture in the pattern and overall intensity of the protein bands. While, Afifi *et al.* (2006) and Al-Hazmi & Kamel (2012), stated the effect of localities upon cellular proteins of *Penicillium* and *Aspergillus* species isolates, respectively.

Finally, each of *Penicillium* species could be distinguished by the protein pattern, which was reproducible and characteristic of the fungus at any particular locality. In addition, the results indicate the need to determine the effects of localities and growth conditions when gel electrophoresis of mycelial proteins is used for taxonomic purposes.

References

Abulhamd AT (2009) Molecular and Secondary Metabolite Profiles Interrelationships among *Penicillium expansum* Strains. *J. Appl. Sci. Res.* **5**: 1335.

Afifi AF, Kamel EA, Fawzi EM, Houseny MM (2006) Protein patterns (SDS-PAGE) as a mean in classification and identification of *Penicillium* spp. *New Egyptian J. Micro.* **14**: 280.

Ainsworth GC (1971) *A Dictionary of fungi*. Commonwealth Mycological Institute, Kew, Surrey.

Al-Hazmi NA and Kamel EA (2012) Ecosystem Impact on Fungal Identification Using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Technique. *African J. Microbiol. Res.* **6**: 3492.

Bakri Y, Arabi MI and Jawhar M (2007) RAPD Technique is a Useful Tool to Distinguish *Penicillium* Species. *Polish J. Micro.* **56**: 273.

Banke, S., Frisvad, J.C., Rosendahl, S. (1997) Taxonomy of *Penicillium chrysogenum* and related xerophilic species, based on isozyme analysis. *Mycol. Res.* **101**: 617. DOI: 10.1017/S0953756296003048

Bent KJ (1967) Electrophoresis of Proteins of 3 *Penicillium* Species on Acrylamide Gels. *J. Gen. Microbiol*. **49**: 195.

Boysen ME, Jacobsson KG, Schnürer J (2000) Molecular Identification of Species from the Penicillium roqueforti Group Associated with Spoiled Animal Feed. *Appl. Environ. Microbiol*. 66: 1523. DOI: 10.1128/AEM.66.4.1523-1526.2000

Cardoso PG, De Queiroz MV, Pereira OL, De Araújo EF (2007) Morphological and Molecular Differentiation of the *Penicillium expansum* and *Penicillium Griseoroseum*. *Brazilian J. Microbiol.* **38**: 71. DOI: 10.1590/S1517-83822007000100015

Chávez R, Fierro F, Gordillo F, Martín JF, Eyzaguirre J (2002) Electrophoretic karyotype of the filamentous fungus *Penicillium purpurogenum* and chromosomal location of several xylanolytic genes. *FEMS Microb. Letts.* **205**: 379. DOI: 10.1111/j.1574-6968.2001.tb10976.x

Cho HS, Hong SB and Go SJ (2005) First Report of *Penicillium brasilianum* and *P. daleae* Isolated from Soil in Korea. *Mycobiol*. **33**: 113. DOI: 10.4489/MYCO.2005.33.2.113

Dupont J, Magnin S, Marti A and Brousse M (1999) Molecular tools for identification of *Penicillium* starter cultures used in the food industry. *Int. J. Food. Microbiol.* **49**: 109. DOI: 10.1016/S0168-1605(99)00055-0

Elhariry H, Bahobial AA, Gherbawy Y (2011) Genotypic identification of *Penicillium expansum* and the role of processing on patulin presence in juice. *F. Chem. Toxic.* **49**: 941.

Francis F, Jaber K, Colinet F, Portetelle D and Haubruge E (2011) Purification of a new fungal mannose-specific lectin from *Penicillium chrysogenum* and its aphicidal properties. *Fungal Biol*. **115**: 1093.

Grassin C and P Fauquembergue (1996) Fruit juices. In: Godfrey T, West S. (Eds.) *Industrial Enzymology*. MacMillan, London.

Hofling JF, Rosa EAR, Pereira CV, Boriollo MFG and Rodrigues JAO (2001) Differentiation and numerical analysis of oral yeasts based on SDS-PAGE profiles. Influence of the culture media on the whole cell-protein extracts. *Brazilian J. Biol.* **61**: 507.

Houseny MM (2005) *Protein patterns (differences and similarities) among some taxa of filamentous fungi as a base for classification of fungi*. M. Sc. Thesis, Faculty of Education, Ain Shams University, Cairo, Egypt.

Inkha S and Boonyakiat D (2010) Induction of resistance to *Penicillium digitatum* in tangerine fruit cv. Sai Num Phung flavedo by hot water treatment. Songklanakarin. *J. Sci. Technol.* **32:** 445.

Laemmli UK (1970) Cleavage of structural proteins during assembly of head bacteriophage T4. *Nature* **227**: 680.

Maurer HR (1971) Disc Electrophoresis, p. 44, Walter de Gruyter Verlag, Berlin,.

Mohammad S, Mhaindarkar VP, Kumar S, Khan MI and Bhosale SH (2011) Isolation and Phylogenetic Analysis of Marine Fungus *Penicillium* sp. Sdb1 and Partial Characterization of its Cysteine Protease Inhibitor. *Int. J. Adv. Biotech. Res.* **2**: 135.

Nei M, Li WH (1979) Mathematical model for studing genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*, 76: 5269.

Pitt JI (1979) *The genus Penicillium and its teleomorphic states Eupenicillium and Talaromyces*. New York, New York: Academic Press.

Pitt JI and Hocking AD (1997) *Fungi and Food Spoilage*. Maryland, Aspen Publishers Inc., Maryland. DOI: 10.1007/978-1-4615-6391-4

Redondo C, Cubero J, Melgarejo (2009) P Characterization of *Penicillium* Species by Ribosomal DNA Sequencing and BOX, ERIC and REP-PCR Analysis. *Mycopathologia* **168**: 11. DOI: 10.1007/s11046-009-9191-y

Rohlf FJ (1998) NTSYSpc numerical taxonomy and multivariate analysis system user guide. *Exeter Software*, New York, USA.

Roslan HA, Ngo CS and Muid S (2010) Genetic diversity of *Penicillium* species isolated from various sources in Sarawaka, Malaysia. *J. Cell Mol. Biol*. **7&8**:13.

Samson RA, Seifert KA, Angelina FA, Kuijpers AFA, Houbraken JAMP and Frisvad JC (2004) Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial β-tubulin sequences. *Sts. Myco*. **49**: 175.

Studier FW (1973) Analysis of bacteriophage T7 early RNAs and proteins of slab gels. *J. Mol. Biol.* **79**: 237.

Tiwari KL, Jadhav SK, Kumar A (2011) Morphological and Molecular Study of Different *Penicillium* Species. *Middle-East J. Sci. Res.* **7**: 203.

Xi L, Xu X, Liu W, Li X, Liu Y, Li M, Zhang J and Li M (2007) Differentially expressed proteins of pathogenic *Penicillium marneffei* in yeast and mycelial phases. *J. Med. Micro*. **56**: 298. DOI: 10.1099/jmm.0.46808-0