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Determining Protein Patterns for Three Fungus Species *Aspergillus fumigatus*, Asp. *Flavus* and Asp. *Niger*, Obtained from Outdoor Air in Iran

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Abstract: *Aspergillus* species are saprophytic fungi widely distributed in nature and are associated with a number of human disease. The aim of this study was to compare electrophoretic protein patterns *Aspergillus fumigatus*, *Asp. flavus* and *Asp.niger* and identify the differences of three protein patterns. In this study, three species of *Aspergillus fumigatus*, *Asp.flavus* and *Asp.niger* which were separated from outdoor air in Iran were used to compare electrophoretic protein patterns antigens isolated of *Aspergillus*. First, these fungi were grown in saboraud glucose agar and preserved at 27°C for 48-72 hours and then they were cultured in saboraud glucose broth to provide protein extract of above mentioned fungi and Bradford method was used in order to measure the level of fungi extracts protein. Protein was dissociated by means of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) with 11% separating gel. The gel was stained with coomassie brilliant blue G250 and after stabilizing, gel staining and disstaining, different protein bands were appeared. Results revealed, 69 protein bands with molecular weights between 11/5 and 178KD. Among these bands, protein bands with molecular weights of 15, 23/5, 27, 33/5 and 61KD are in two species of *Asp.fumigatus* and *Asp.flavus*, protein bands 28/5, 40 and 47KD are in two species of *Asp.flavus* and *Asp.niger*, the band with 120 KD is in *Asp.fumigatus* and *Asp.niger* and protein bands 23, 35 and 36 KD are among these bands which were presented in every three species of *Aspergillus*. In addition, a number of strong and weak protein bands were recognized. It was concluded that there was no meaningful relation between protein bands, which were obtained from isolated fungal sp. under study and these three isolates did not follow the same electrophoretic protein patterns.

Key words: *Aspergillus* · Protein · SDS-PAGE.

INTRODUCTION

Aspergillus species are common group of filamentous fungi, which have universal dissemination and are readily recovered from soil; decaying vegetation, air and many other environments, their conidia turns into aerosol and are largely distributed in the environment and are inhaled by humans and animals [1-3].

Some members of this genus (*Asp. fumigatus*, *Asp. flavus* and *Asp. niger*) can operate as an opportunistic aggressor and cause a group of diseases known as Aspergillosis, especially in individuals with weaken immune systems [4-8].

This study used SDS-PAGE method to obtain electrophoretic protein patterns of these three species and to dissociate them from one another based on protein bands.

MATERIALS AND METHODS

Isolates: *Aspergillus fumigatus* strain A41, *Aspergillus flavus* strain A6 and *Aspergillus niger* strain A10, dissociated from outdoor air in Iran, which were preserved in the collection of Mycology Department, Veterinary Faculty, Tehran University were used to accomplish this research. Table 1 shows the list of these afore mentioned fungi.

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Table 1: List of fungi used in electrophoresis

Strain	Fungus
A41	*Aspergillus fumigatus*
A6	*Aspergillus flavus*
A10	*Aspergillus niger*

Mass Cultivation: In this study, isolates preserved in distilled water were grown in saboraud glucose agar in sterile conditions, and placed in incubator at 27°C for 48-72 hours, and examined after appropriate growth of fungi in terms of morphological colony and microscopic, then some fragments were transferred to erlens with 500 ml of saboraud glucose broth containing chloramphenicol in order to obtain protein and mass-produce in condition completely sterile of any fungus species and agitated in the shaker (150 rpm) at 25°C for 10-12 days.

Separating Fungus Colonies: Fungus colonies were separated from medium by using Wattman paper, number 1 and Bookhner funnel in condition completely sterile and were washed with sterile PBS, three stages [9].

Cell Fractionation

Cells Disruptions Were Performed Using Two Methods: (i) Freeze and Tow was used to break up the fungus piles, (ii) Glass beads: disruption was performed using glass beads (diameter, 1mm) on a vortex mixture for 1 min until about 80-90% cells were disrupted. The membrane of *Aspergillus niger* was more solid and rigid than that of others, so liquid nitrogen was used to break it.

Preparing Crude Extracts and Measuring Protein Value: After cell disruption, the crude extracts were separated from intact cells and cell walls remaining by centrifugation at 25000 rpm for 30 min through three stages. The clear supernatants obtained. The protein content of these solutions were determined according to the method of Bradford [10]. The supernatants were kept in micro tubes at-20°C until used [9, 11].

Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoresis: The extracts of fungi were analysed by making use of SDS-PAGE method with %11 separating gel and %4 stacking gel in a discontinuous buffer system according to the method of Laemmli [12].The extracts were boiled for 5 min with a reducing sample buffer (containing 2-mercaptoethanol) and 35microlitre of each sample was loaded on a gel. Along with the samples, standard marker (Fermentase) was also electrophoresis, which is a mixture of-fourteen pieces of pure neoformed proteins in different sizes, thus, these protein pieces are distinguished as fourteen bands from 10 to 200KD, which respectively are 200, 150, 120, 110, 85, 70, 60, 50, 40, 30, 25, 15 and 10KD. Staining was done by using coomassi brilliant blue G250 (sigma) [13].

RESULTS

Way of analyzing protein pieces and molecular weights of components of *Aspergillus* extract under this study was distinguished through SDS-PAGE method (Figure1).

Standard curve was drawn based on the manner of standard protein movement in gel and defining their RF and using this curve, molecular weights of fungi extracts components were stimated (Figure 2).

Accordingly there were observed 69 protein bands with molecular weights from 11/5 to 178KD.

Aspergillus fumigatus contains 31 protein bands with the maximum band from 15 to 120 KD, *Aspergillus niger* contains 26 protein bands with the minimum band from 11/5 to 178 KD and *Aspergillus flavus* contains 27 protein bands from 12 to 91 KD (Table 2).

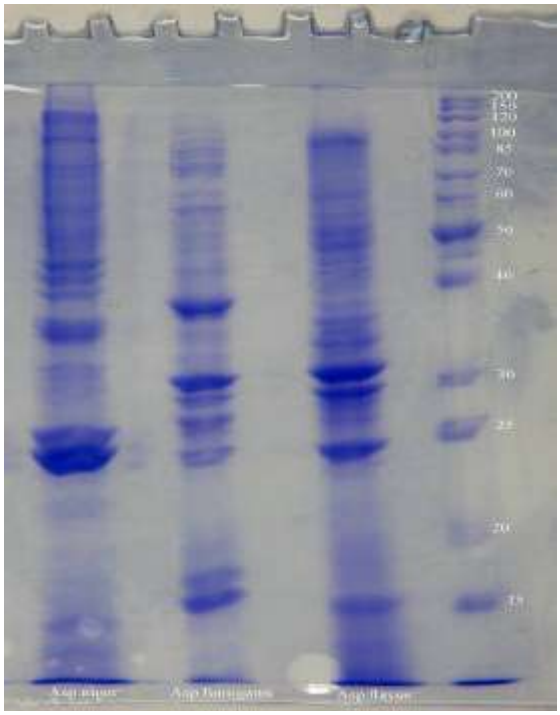

Fig. 1: Electrophoretic protein patterns of the *ASP.fumigatus*, *ASP.flavus* and *ASP.niger* extracts by SDS-PAGE method with 11% separating gel. (Staining with coomassie brilliant blue (G250))

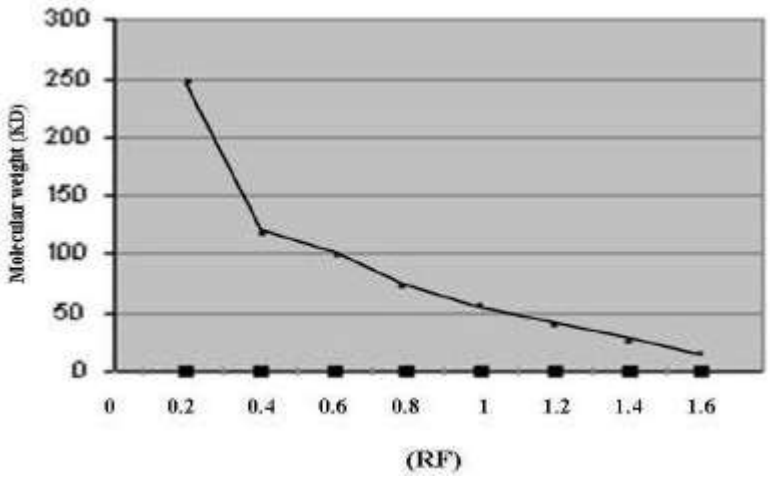

Fig. 2: Standard diagram of molecular weight by counting separated protein of molecular weight by means of SDS-PAGE with %11 separating gel

Table 2: Frequency of protein bands, obtained of *Aspergillus* species understudy based on molecular weigh (kilodalton)

Molecular weight								
Species	11/5	12	13/5	15	18	22/5	23	23/5
ASP.fumigatus	-	-	-	+	+	-	+	+
ASP.flavus	-	+	-	+	-	+	+	+
ASP.niger	+	-	+	-	-	-	+	-
total	1	1	1	2	1	1	3	2
*%	33/3	33/3	33/3	66/6	33/3	33/3	100	66/6
Molecular weight								
Species	24/5	25	25/5	26	27	28	28/5	29/5
ASP.fumigatus	-	+	+	-	+	+	-	+
ASP.flavus	-	-	-	+	+	-	+	-
ASP.niger	+	-	-	-	-	-	+	-
total	1	1	1	1	2	1	2	1
*%	33/3	33/3	33/3	33/3	66/6	33/3	66/6	33/3
Molecular weight								
Species	30	30/5	31	31/5	32	33	33/5	34
ASP.fumigatus	-	-	-	+	+	-	+	+
ASP.flavus	-	+	-	-	-	+	+	-
ASP.niger	+	-	+	-	-	-	-	-
total	1	1	1	1	1	1	2	1
*%	33/3	33/3	33/3	33/3	33/3	33/3	66/6	33/3
Molecular weight								
Species	34/5	35	36	37	37/5	38/5	39	40
ASP.fumigatus	-	+	+	+	-	-	-	-
ASP.flavus	-	+	+	-	+	-	+	+
ASP.niger	+	+	+	-	-	+	-	+
total	1	3	3	1	1	1	1	2
*%	33/3	100	100	33/3	33/3	33/3	33/3	66/6
Molecular weight								
Species	41	42	42/5	43	46	47	48/5	49/5
ASP.fumigatus	+	-	-	-	+	-	-	+
ASP.flavus	-	+	-	+	-	+	-	-
ASP.niger	-	-	+	-	-	+	+	-
total	1	1	1	1	1	2	1	1
*%	33/3	33/3	33/3	33/3	33/3	66/6	33/3	33/3

Table 2: Continued

Molecular weight Species	50	50/5	52/5	55	56/5	57	58	61
ASP.fumigatus	-	-	+	-	-	+	-	+
ASP.flavus	+	-	-	+	-	-	+	+
ASP.niger	-	+	-	-	+	-	-	-
total	1	1	1	1	1	1	1	2
*%	33/3	33/3	33/3	33/3	33/3	33/3	33/3	66/6
Molecular weight Species	63	69	70	73/5	74	74/5	76/5	78/5
ASP.fumigatus	-	-	+	-	+	-	+	-
ASP.flavus	-	-	-	+	-	-	-	-
ASP.niger	+	+	-	-	-	+	-	+
total	1	1	1	1	1	1	1	1
*%	33/3	33/3	33/3	33/3	33/3	33/3	33/3	33/3
Molecular weight Species	80	81	83	84/5	86	91	93	95
ASP.fumigatus	-	+	-	+	-	-	+	-
ASP.flavus	+	-	-	-	+	+	-	-
ASP.niger	-	-	+	-	-	-	-	+
total	1	1	1	1	1	1	1	1
*%	33/3	33/3	33/3	33/3	33/3	33/3	33/3	33/3
Molecular weight Species	101	103	111	120	178	total		
ASP.fumigatus	-	+	+	+	-	31		
ASP.flavus	-	-	-	-	-	27		
ASP.niger	+	-	-	+	+	26		
total	1	1	1	2	1			
*%	33/3	33/3	33/3	66/6	33/3			

*Frequency in three species of *Aspergillus*, mentioned.

Among these bands, protein bands with molecular weights of 15, 23/5, 27, 33/5 and 61 KD were shown in two species of *Asp. fumigatus* and *Asp. flavus*, protein bands 28/5, 40 and 47 KD are in two species of *Asp. flavus* and *Asp. niger*, the band with 120 KD is in *Asp. fumigatus* and *Asp. niger* and protein bands 23, 35 and 36 KD are among these bands which were presented in every three species of *Aspergillus*. Thus, common protein bands 23, 35 and 36 KD were observed with frequency 100% in three species of *Aspergillus*, and other mentioned common bands with frequency 66/6% were observed in various species of *Aspergillus* in this study [Table 2].

A number of strong protein bands were observed among the protein bands obtained from *Aspergillus* species in the study, which are from 15 to 95 KD. There were seen 6 bands with 15, 23/5, 25/5, 28, 29/5 and 37 KD in molecular weights in *Asp.fumigatus*, 9 bands with 15, 23/5, 28/5, 30/5, 33/5, 36, 47, 50 and 91 KD in molecular weights in *Asp.flavus* and 11 strong protein bands with 23, 24/5, 34/5, 35, 36, 38/5, 40, 42/5, 56/5, 74/5 and 95 KD in molecular weights in *Asp.niger* [Figure 1].

A number of weak protein bands were observed from 22/5 to 178 KD as well, some of them are important at times in analyzing the species.

11 bands with 31/5, 32, 33/5, 34, 35, 41, 46, 49/5, 52/5, 111 and 120KD in molecular weights in *Asp.fumigatus*, 5 bands with 22/5, 23, 73/5, 80 and 86KD in molecular weights in *Asp.flavus* and 4 bands with 28/5, 30, 31 and 178KD in molecular weights in *Asp.niger* were observed [Figure1].

According to the results from SDS-PAGE and statistical analysis, a meaningful relation was not achieved between the protein bands obtained from three isolates of the study; as a result, there could be seen 5 common bands between *Asp.fumigatus* and *Asp.flavus*, 3 common bands between *Asp.flavus* and *Asp.niger*, 1 common band between *Asp.fumigatus* and *Asp. niger* and 3 common bands were also observed between these three species.

DISCUSSION

Species of *Aspergillus* are saprophytic fungi with universal dissemination and dissociated from different environmntal sources, including food stuffs, soli, plants, air and even nonconductor and fireproof materials. The genus *Aspergillus* includes 185 species; around 20 species have so far been reported as causative

agents of opportunistic infections in people [2, 14, 15]. *Aspergillus fumigatus* is the most common causing disease followed by *Aspergillus flavus* and *Aspergillus niger*. *Aspergillus Spp*. are well-know to play a role in three different clinical setting in man including opportunistic infection, allergic states, toxicoses. Immunosuppression is the major factor predisposing to development of opportunistic infections [7, 8, 14].

A number of *Aspergillus* species antigens were reported as fungus allergen which causes disease like ABPA in atopic patients. In addition, *Aspergillus flavus* produces a substance called aflatoxin which is both toxin and carcinogen that can contaminate materials such as nuts [1, 8, 16]. In this research, three prevalent species of *Aspergillus*, *Asp.fumigatus*, *Asp. flavus* and *Asp. niger* obtained from outdoor air in Iran, were used to achieve electrophoretic protein patterns of these fungi. The results from SDS-PAGE method indicate that extracts obtained from these fungi have maximum 69 protein bands which range from 11/5 to 178 KD, among this, *Asp. fumigatus* had the maximum bands (31 bands) and *Asp. niger* had the minimum bands (26 bands).

In this study, the bands with molecular weights of 15, 23/5, 27, 33/5 and 61KD were found in two species of *Asp.fumigatus* and *Asp.flavus*, protein bands 28/5, 40 and 47KD are in two species of *Asp.flavus* and *Asp.niger*, band 120 KD is in *Asp.fumigatus* and *Asp.niger* and protein bands with molecular weights of 23, 35 and 36 KD are among these bands which were presented in every three species of *Aspergillus*.

Regarding the results of common bands and according to the statistical analysis, no meaningful relation was observed between protein bands, obtained from three isolates under the study.

Therefore, the isolates under this study differ from one another in terms of antigenic variety and do not follow the same electrophoretic pattern.

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