ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Protein profile of the most common fungi at Sultan Idris education university, Malaysia

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Article History:

Received on: 14.11.2018
Revised on: 07.03.2019
Accepted on: 10.03.2019

Keywords:

Fungi allergy,
Fungi protein profile,
Sultan Idris Education University

ABSTRACT

Fungi are vital pathogens related to airway and deferent diseases. Exposure to fungi will have adverse effects on human health through severe immune responses. Allergic diseases like allergic rhinitis, asthma, airborne dermatitis, or allergic conjunctivitis. These diseases may well be aggravated by fungi allergens from the natural atmosphere or from the indoor environment in enclosed areas, workplaces and houses. Fungi allergy prevalence for the Malaysian population has yet to be reported. Therefore, information on allergens of fungi species in Sultan Idris Education University (UPSI), Sultan Azlan Shah Campus will be used to profile the nation prevalence on fungi allergy. Hence, the present study is conducted at UPSI to identify the ten most common airborne fungal species as a preliminary work prior to sensitization study on common fungal allergens. Dust was collected from 54 rooms situated within the 3 blocks at UPSI Sultan Azlan Shah Campus buildings using a vacuum cleaner. The sieved dust was cultured using PDA media and incubated at room temperature to propagate pure culture and sent to the Malaysian Agricultural Research and Development Institute for identification using PCR. In this study the 10 most common species were identified as, *Penicillium simplicissimum, Aspergillus aculeatus, Rhodosporidiobolus ruineniae, Ceriporia lacerate, Aspergillus caliodustus, Syncephalastrum* sp., Aspergillus sp., *Aspergillus fumigatus*, *Fusarium* sp., and *Penicillium canescens*. Fungi profile exhibited in this study will lead to a further study on fungi allergy with skin prick test and immunoblotting to be carried out.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v10i2.413

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INTRODUCTION

For decades, fungal spores and mycelial cells are identified to be a significant health risk. In contrast to airborne pollen, fungal spores aren't primarily related to IgE-mediated type I allergies but with a broad panel of different diseases too, e.g. life-threatening primary and secondary infections in immune-compromised patients (Simon-Nobbe *et al.*, 2008). Fungal sensitization for these people is generally thought to occur as a result of continual, transient inhalational exposures to a spread of fungal antigens. Results from recent epidemiological studies, however, recommend that fungal exposures can also somehow exacerbate allergic diseases, as well as a respiratory disorder (Goldman and Huffnagle, 2009). The presence of dampness (defined as adequate moisture to support fungal growth on obtainable substrates) tends to reinforce the assembly of fungal and different microorganism contaminants, that is why dampness has been related to adverse health effects(Seppänen and Kurnitski, 2009). Fungal sensitization is gener-

ally outlined as the presence of immediate cutaneous hyperreactivity or positive results for specific IgE antibodies to previously prepared fungal antigens(Ahluwalia and Matsui, 2018). The prevalence of respiratory hypersensitivity reaction to fungi isn't exactly known however is estimated at 20 to 30% of atopic individuals (allergy-predisposed) (O'Driscoll *et al.*, 2009), 6.17% or up to 6% of the general population (Horner *et al.*, 1995). General population estimates of the sensitization to fungal allergens vary from 8% to 14%. However, the estimates are significantly higher in atopic people. In one study, 50% of kids with respiratory illness demonstrated sensitization to a minimum of one fungal allergen whereas 76% of people with multiple hospitalizations for respiratory illness were noted to have sensitization to fungal allergens in another study(Goh *et al.*, 2005). This preliminary study used sodium dodecyl sulphate SDS-PAGE method to obtain protein patterns of these fungal species and to determine the feasibility of the fungi allergy study based on protein.

MATERIALS AND METHODS

Isolation and Identification: Dust was collected from 54 rooms situated within the 3 blocks at UPSI Sultan Azlan Shah Campus buildings where people spend approximately 5–6 hours per working day. Dust samples were collected between July and September 2017, rooms occupants were informed of dust vacuuming by placing a letter was circulated to all the occupants at block 1, 2, 3 on the notice board before few days, the dust was collected by using a hand-held vacuum cleaner (vo. temm 2-way power, 650 w, China) operated for 5-10 min The dust was collected from the floor, carpet, chairs, corners, computers and all furniture inside the room. The vacuum filter cab was decontaminated with a 95% ethanol wipe between each room sampling and allowed to dry. All dust samples were put in a zipped plastic bag; the dust was sieved in 400 µm in the laboratory and kept at - 20°C. The dust then cultured by using PDA as a culture media. Ten most common species were identified by molecular methods using PCR carried out by the Malaysian Agricultural Research and Development Institute.

Mass Cultivation and Cell Fractionation: In this study, potato dextrose agar used to grow isolates in completely sterile conditions, and incubated at room temperature for 48-72 hours, and after appropriate growth of fungi examined in terms of morphological colony and microscopic, then some fragments were transferred to erlens with 250 ml of sabouraud 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 12-14 days. Fungus colonies were separated from the medium by using Wattman paper, number 1 and Bookhner funnelled in a condition completely sterile and were washed with sterile PBS, three stages (Rath, 2001). Cells Disruptions was performed by using Three Methods;

1- Freeze and Tow.
2- Glass beads: disruption by using glass beads (diameter, 1mm) on a vortex mixer for 1 min.
3- Sonication the samples using (UP50H, Hielscher, Germany) for 1 to 2 min in ice

Crude Extracts Preparation and Measuring Protein Value: The crude extracts were separated from intact cells and cell walls remaining after cell disruption, by centrifuged at 14,000 rpm at 4ºC for 30 min. Then, the supernatants were filtered by using sterile syringe filters 0.2 µm. Then, the extracts were lyophilised using a freeze dryer for one night. After that lyophilised extracts were stored at -20°C until use. The total protein content in each prepared extract was determined by using the Bio-Rad Protein Assay Kit (BioRad, USA), following the manufacturer's instruction (Bradford, 1976) (Latge and Paris, 1991).

Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoresis (SDS-PAGE): SDS-PAGE was done by using a gel containing 11% polyacrylamide separating gel and stacking gel of 4%. The samples were then treated in a Laemmli sample buffer (BioRad, USA) with 5% 2-mercaptoethanol and heated at 97ºC for 5 minutes prior to electrophoresis (Laemmli, 1970). Precision plus protein standards (BioRad, USA) were run as a reference, along with samples the molecular weights were determined using densitometer (BioRad) and scanner G800.

RESULTS AND DISCUSSIONS

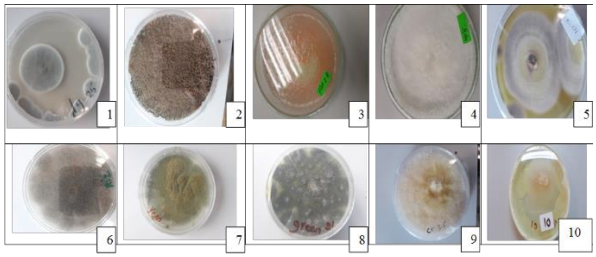

Figure 1: 1. ***Penicillium simplicissimum 2. Aspergillus aculeatus 3. Rhodosporidiobolus ruineniae 4. Ceriporia lacerata 5. Aspergillus caliodustus 6. Syncephalastrum sp. 7. Aspergillus sp. 8. Aspergillus fumigatus 9. Fusarium sp. 10. Penicillium canescen***

The fungal sampling from the rooms revealed the occurrence of 13 fungal, but only 10 most prevalent with the frequency between 51 (94%) to 31 (57%) (Table 1) were identified. 6 genera were identified

Table 1: Occurrence of the most prevalent fungi sampled at UPSI

Species	1	2	3	4	5	6	7	8	9	10
Frequency	51	46	40	50	31	34	39	42	42	45
Percentage	94	85	74	92	57	62	72	77	77	83

Table 2: Profile of fungal protein bands and molecular weight

Species	Molecular weight (kD)																
	< 10	11	12	13	14	15	16	17	18	19	20-23	24	25	26	27	28	29
1	✓	✓			✓			✓		✓	✓		✓				
2	✓																
3	✓	✓					✓						✓	✓			
4			✓				✓				✓			✓			✓
5	✓	✓			✓	✓				✓	✓		✓			✓	
6			✓	✓	✓		✓	✓			✓		✓				✓
7	✓		✓	✓		✓		✓		✓				✓		✓	
8		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓		✓	
9			✓	✓	✓	✓	✓	✓					✓	✓			✓
10		✓	✓		✓		✓	✓			✓	✓	✓		✓		✓
Total	5	5	6	4	6	4	6	6	1	4	6	1	7	5	1	3	4
*%	50	50	60	40	60	40	60	60	10	40	60	10	70	50	10	30	40

Table 2: Profile of fungal protein bands and molecular weight (Contd…)

Species	Molecular weight (kD)																
	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
1				✓	✓		✓				✓						✓
2					✓		✓			✓							
3	✓		✓		✓	✓				✓			✓				
4				✓			✓				✓			✓	✓		
5	✓			✓					✓				✓		✓	✓	
6	✓			✓		✓						✓			✓		
7			✓		✓		✓			✓			✓	✓			
8	✓	✓		✓	✓			✓		✓		✓			✓	✓	
9				✓			✓									✓	
10		✓		✓		✓	✓		✓			✓					
Total	4	2	2	7	5	3	6	1	2	4	2	3	3	2	4	3	1
*%	40	20	20	70	50	30	60	10	20	40	20	30	30	20	40	30	10

Table 2: Profile of fungal protein bands and molecular weight (Contd…)

Species	Molecular weight (kD)															
	48	49	50-60	61	62	63	67	68	69	70	71	73	75	78	82	83
1			✓	✓				✓								
2	✓				✓											
3		✓	✓					✓								
4	✓		✓			✓			✓				✓		✓	
5			✓	✓			✓			✓		✓				✓
6		✓	✓									✓				✓
7			✓				✓							✓		
8			✓		✓			✓				✓		✓		
9			✓								✓					
10		✓	✓								✓					
Total	2	3	9	2	1	1	2	3	1	1	2	3	1	2	1	2
*%	20	30	90	20	10	10	20	30	10	10	20	30	10	20	10	20

that made up the ten species. Aspergillus gave rise to four species, namely Asp. *aculeatus*, Asp. *caliodustus*, Asp. *fumigatus* and an unidentified Aspergillus sp. followed by Penicillium genus with P. *simplicissimum* and P. *canescens*. Remaining species were *Rhodosporidiobolus ruineniae, Ceriporia lacerata*, *Syncephalastrum* sp. And *Fusarium* sp. *Penicillium simplicissimum* was found with the highest occurrence while *Aspergillus* caliodustus as the least. The three rooms, which no fungal were found, were considered the clean rooms at UPSIcampus.to date, *Rhodosporidiobolus ruineniae, Ceriporia lacerata,* and *Syncephalastrum* sp. have

not been reported to elicit allergy. Protein and molecular weights of components of 10 species extract demonstrated between 9 to 30 protein bands with molecular weights from 5 to 217 kD were observed [Table 2, Figure 2]. Asp. *Fumigatus* contained the highest number of protein bands; 31 protein bands with a molecular weight from 11 to212 kD while Asp. *aculeatus* has the least with 9 protein bands ranged between 9 to 112 kD. Among these bands, major protein bands (with more than 60% frequency) were 12 kD (60%), 14 kD (60%), 16 kD (60%), 17(60%), 25 kD (70%), 33 kD (70%), 36 kD (60%), 50-60 kD (90%) and 114-250 kD (80%) molecular weight found in most species [Table 2, Figure 2].

Table 2: Profile of fungal protein bands and molecular weight (Contd)

Species	Molecular weight (kD)			
	85-105	112	114-250	No. of bands
1	✓			17
2	✓	✓		9
3			✓	16
4			✓	18
5			✓	22
6			✓	18
7	✓		✓	20
8	✓		✓	30
9		✓	✓	19
10	✓		✓	28
Total	5	2	8	
*%	50	20	80	

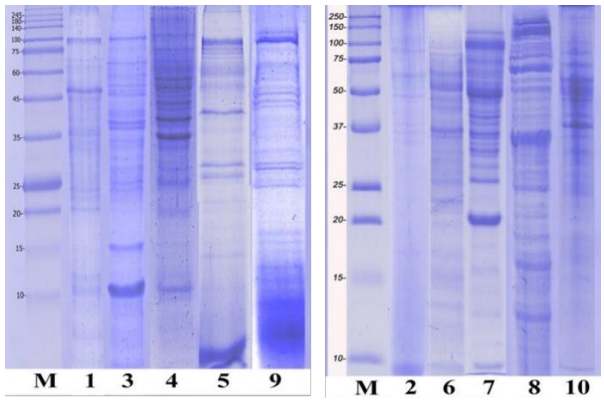

Figure 2: SDS-PAGE analysis of 10 fungi species *(1), Penicillium simplicissimum (2), Aspergillus aculeatus (3), Rhodosporidiobolus ruineniae (4), Ceriporia lacerata (5), Aspergillus caliodustus (6), Syncephalastrum sp. (7) Aspergillus sp. (8) Aspergillus fumigatus (9), Fusarium sp. (10), Penicillium canescens*. (M) Is molecular weight markers in kilo-Dalton (kDa)

The present study shows 94% of the rooms except for the clean rooms within the three blocks at UPSI campus are exacerbated with fungi growth. Thus, the indication of high fungal colonization which increases the fungal exposition and then leads into a fungal allergy to the occupants. Aspergillus spp which dominated the fungal profile at UPSI also reported by Saeednejad et al. (Azar *et al.*, 2010). Furthermore, *Aspergillus fumigatus* is also listed as one of the species that can operate as an opportunistic aggressor and cause a group of the illness called as Aspergillosis, particularly in people with the weakened immune system (Çetinkaya *et al.*, 2007)(Kurup and Banerjee, 2000). In the present study, Penicillium spp. was found in most of the rooms was also reported by Goh et al. (Biomedicine, 2005). They found that among 76.1% of workers with a fungal allergy has a risk of 3.658 when they occupied space with high indoor mould concentration. One-dimension SDS-PAGE runs in this study reveals 118 protein bands with a molecular weight between 5 and 217 kD in all the ten species. There were 62 protein bands identified for all the four Aspergillus spp. (Asp*. fumigatus*, Asp. *aculeatus*, Asp. *caliodustus* and unidentified Asp. compared to 69 bands as reported by Saeednejad et al. (Azar *et al.*, 2010) for the three species (Asp. *niger*, Asp. *fumigatus*, and Asp. *flavus*) they studied. Comparison of the same species in both studies, which is the Asp. *Fumigatus* showed that there were only 27 protein bands in their study compared to 32 bands in the present study. Thus, there is only 11protein bands or 34.48% electrophoretic protein pattern similarities between the studies. P. *simplicissimum* contains 19 protein bands with a molecular weight from 5 to 100 kD and P. *canescens* contain 28 protein bands with a molecular weight from 11 to 212 kD. In another study on *Penicillium echinulatum* which conducted by Schneider revealed protein bands in the range of 50 to 75 kD and the higher molecular weight band was 120 kD (Schneider *et al.*, 2016). While in this study the higher molecular band is in *Penicillium canescens* 212 kD. *Aspergillus fumigatus* contains 31 protein bands with a molecular weight from 11 to 199 kD as the higher molecular band. The band found in this study is heavier than reported by Saeednejad with the highest molecular band was 120 kD (Azar *et al.*, 2010). *Fusarium* sp. contains 19 protein bands with the molecular weights from 12 to 137 kD while in the previous study by Khosravi and his colleagues on *Fusarium solanii*, they identified protein band with a molecular weight from 24 kD to 112 kD (Khosravi *et al.*, 2012). The results vary between these studies may be due to the methodology employed including the allergens extraction and protein separation method in this study to identify the protein bands.

CONCLUSION

This preliminary study has successfully profiled the fungi proteins and eventually evident the viability of fungi allergy study. Subsequently, this will

lead to skin prick test and immunoblotting procedures to be carried out later.

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