

## Outer Membrane Proteins of Filamentous Fungi Isolated from Wheat (*Triticum* spp.) In Lagos State, Nigeria

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**Abstract:** This study determined the outer membrane proteins of filamentous fungi isolated from wheat in Lagos State, Nigeria using SDS-PAGE. The results obtained revealed 22 protein bands with molecular weights ranging from 8-16KDA. *Aspergillus fumigatus* and *Aspergillus niger* contains 5 and 3 protein bands ranging from 10 to 15 KDA and 10 to 14 KDA respectively. *Aspergillus flavus*, *Alternaria*, *Rhizopus*, *Penicillium* and *Fusarium* spp contains 2 protein bands ranging from 11 to 14 KDA. *Trichoderma* however revealed 4 protein bands that ranges from 8-16KDA. Among these bands, protein band with molecular weight of 13KDA were found in *Asp. fumigatus*, *Asp.niger*, *Fusarium* species, *Rhizopus* and *Penicillium* spp. It can thus be inferred that the different filamentous fungi studied have different antigenic determinants as revealed by difference in their electrophoretic patterns.

**Key words:** Outer membrane protein • Filamentous fungi • Wheat

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### INTRODUCTION

The mycotoxigenic fungi involved with the human food chain belong mainly to three genera: *Aspergillus*, *Fusarium* and *Penicillium* while *Fusarium* species are destructive plant pathogens producing mycotoxins before or immediately after harvesting. *Penicillium* and *Aspergillus* species are more commonly found as contaminants of commodities and foods during drying and subsequent storage [1]. Some of these fungi 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 while some are inhaled by humans and animals [2]. The potential for spoilage and mycotoxin production depend upon the types of fungi present, the composition of the food, the conditions of handling and storage. For example, dried foods are susceptible to spoilage and toxin production if storage temperature is suitable for fungal growth [3]. Moreover, wheat are collected in tropical areas by simple

methods and are commonly exposed to many contaminants before being dry enough to prevent microbial growth. They are also stored in conditions favouring contamination by insects, rodents and other vermin [4]. The mycological quality of wheat in the Lagos market is quite poor, bearing many genera and species of fungi [5]. Most fungi are present on wheat of post-harvest and storage type, which develop after harvest if relative humidity is not controlled during storage [3]. Fungi are the predominant contaminants of wheat, but most such microbial populations are probably regarded as commensal residents on the plant that survived drying and storage. Soil and air is the main inoculum source for causing contamination in crude wheat [6]. Due to the recognition of the increasing importance of filamentous fungi as a ubiquitous contaminants of wheat circulating in Lagos, Nigeria, there is need to devise an effective method to type the organism. Analysis of the outer membrane proteins of filamentous fungi can be used as a sensitive method of characterizing the fungi on the basis of their protein band profile. Keeping these facts in mind,

the present study was undertaken to characterize the outer membrane proteins using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

### MATERIALS AND METHODS

**Fungal Strain:** The Fungal strain used in this study were isolated in our previous study [5] and preserved in silica gel until needed.

**Culturing and Sub Culturing:** In this study, isolates preserved in silica gel were grown in Sabouraud Dextrose Agar in sterile conditions at 27°C for 48-72 hours. The isolates were then subcultured into 500 ml bottle of sabouraud dextrose broth containing chloramphenicol. This was aimed to obtain pure fungal isolates. The isolates were agitated in the shaker (150 rpm) at 25°C for 10-12 days to obtain the proteins.

**Separating Fungus Colonies:** Fungus colonies were separated from medium by using Whatman number 1 filter paper through a funnel under a sterile condition using sterile PBS in a three stages manner for washing [8].

### Cell Fractionation

**Cells Disruptions:** Disruption was performed first by grinding in liquid nitrogen and subsequently using glass beads (diameter, 1mm) on a vortex mixture for 1 min until about 80-90% cells were disrupted.

### 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 protein content of these solutions were determined according to the method of Bradford [9]. The supernatants were kept in micro tubes at -20°C until used [8, 10].

### 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 [11]. 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 (Staining with coomassie brilliant blue (G250)). Staining was done by using coomassie brilliant blue G250 (sigma) (Burnie,1989)

### RESULTS

The protein pieces and molecular weights of components of the isolated filamentous fungi under study was distinguished using SDS-PAGE method (Plate 1).

Accordingly, there were observed 22 protein bands with molecular weights ranging from 8-16KDA. *Aspergillus fumigatus* and *Aspergillus niger* contains 5 and 3 protein bands ranging from 10 to 15 KDA and 10 to 14 KDA respectively. *Aspergillus flavus*, *Alternaria*, *Rhizopus*, *Penicillium* and *Fusarium* spp contains 2 protein bands ranging from 11 to 14 KDA. *Trichoderma* however revealed 4 protein bands that ranges from 8-16KDA. Among these bands, protein bands with molecular weights of 13KDA were found in *Asp. fumigatus*, *Asp.niger*, *Fusarium* species, *Rhizopus* spp and *Penicillium* spp.



Plate 1:

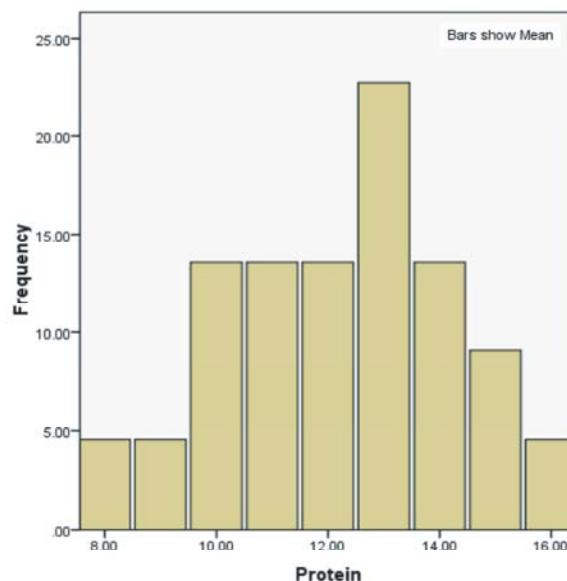


Fig. 1: Frequency of occurrence of protein bands in the studied fungal isolates

Table 1: Occurrence of Outer Membrane Protein in the Studied Fungi

Organisms	Molecular weight (KDA)								
	8	9	10	11	12	13	14	15	16
Aspfu	-	-	+	+	+	+	-	+	-
Aspni	-	-	+	-	-	+	+	-	-
Altspp	-	-	-	-	+	-	+	-	-
Aspfl	-	-	+	+	-	-	-	-	-
Fussol	-	-	-	+	-	+	-	-	-
Rhispp	-	-	-	-	+	+	-	-	-
Pensp	-	-	-	-	-	+	+	-	-
Trispp	+	+	-	-	-	-	+	+	+
Total	1	1	3	3	3	5	4	2	1

Aspfu = *Aspergillus fumigatus*, Aspni = *Aspergillus niger*, Altspp = *Alternaria spp*, Aspfl = *Aspergillus flavus*, Fussol = *Fusarium solani*, Rhispp = *Rhizopus species*, Penspp = *Penicillium spp*, Trispp = *Trichoderma spp*

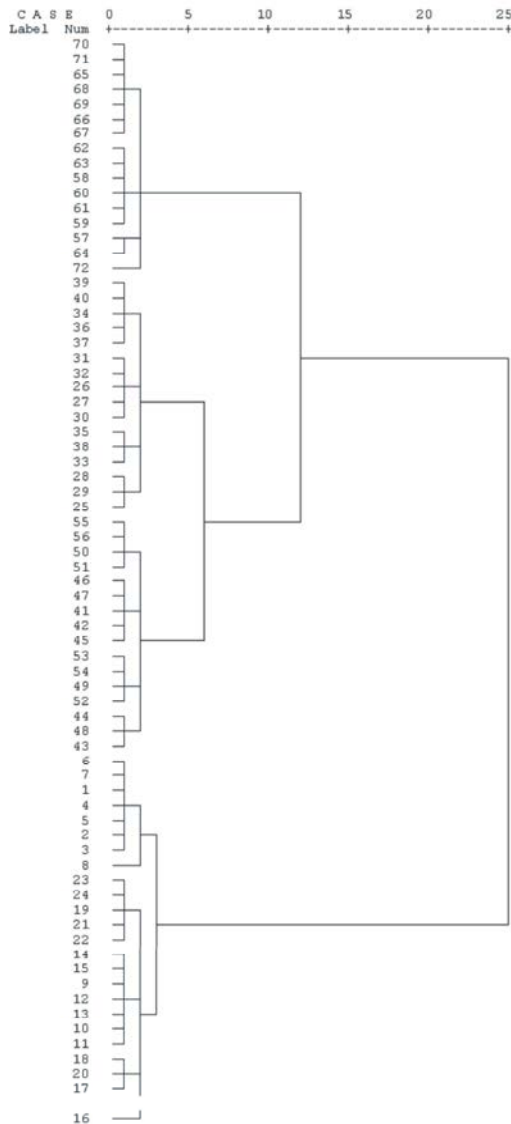


Fig. 2: Dendrogram showing the relationship between the outer membrane proteins of the isolated filamentous fungi

The above figure represents the frequency of occurrence of specific protein bands. As shown in this figure, protein bands with molecular weight of 8 and 9KDA have percentage occurrence of 4.55% while protein bands 10, 11 and 12KDA shows 13.6% frequency of occurrence. On the other hand, protein bands 14, 15 and 16 represents percentage occurrence of 13.6, 9.1 and 4.55% respectively.

The relationship between the outer membrane proteins of the studied filamentous fungi are depicted in the above dendrogram. As shown above, when the dendrogram relating the various outer membrane proteins was truncated at 56%, all the observed proteins could be classified into two major clusters. At 40% level of truncation, the dendrogram was demarcated into into three clusters while at 20% level of clusters, the dendrogram was divided into four major clusters. The molecular weight 8,9,10,11,12,13,14,15 and 16 were designated by the following values respectively 1-8, 9-16,17-24,25-32,33-48,49-56,57-64, 65 -72 and 73-78.

**DISCUSSION AND CONCLUSION**

Fungal infection and contamination of stored foods has been well documented [13-16]. The main impact of these isolates on agriculture is in saprophytic degradation of products before and after harvesting and in the production of mycotoxins [17]. Regarding the results of the electrophoretic bands and according to the statistical analysis, no meaningful relation was observed between protein bands obtained from the studied isolates. Therefore, the isolates under study differ from one another in terms of antigenic variety and do not follow the same electrophoretic pattern. This observation disclosed the possibility of using polyacrylamide electrophoresis for characterizing whole cell proteins of the studied filamentous fungi [18]. However, the use of a probe to

check for a possible cross reaction within and between isolates is suggested to see which of them share similar antigenic determinants. The profile generated from SDS PAGE is relatively simple and materials and equipment required are generally less costly than those needed for genomic studies. In addition the profiles are stable and reproducible. Methodological differences between laboratories have little effect in identification of outer membrane proteins. It can thus be concluded that SDS-PAGE is a valuable tool for rapid identification of OMP. However, excellent results will be obtained when combined with genotyping as a confirmatory procedure.

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