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Morphological Identification of Foodborne Pathogens Colonizing Rice Grains in South Asia

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Abstract: The aim of this study was to identify the foodborne pathogens mainly, *Aspergillus* sp. colonizing rice grains using cultural and microscopic methods. Four differential media (Czapek Dox Agar (CZA), Czapek Yeast Agar (CYA), Malt Extract Agar (MEA) and Czapek yeast 20% sucrose agar (CYA20S)) were used for differentiation of five *Aspergillus* sp., colonizing rice grains comparing with standard cultures. We studied macroscopic (colony color and diameter, conidia color, exudates, sclerotia and colony texture) and microscopic (conidiophore color, length and breadth, conidia size, shape and surface texture, vesicle diameter and phialides length and breadth) characteristics for identification of 110 isolates of *Aspergillus* sp. isolated from 65 rice grain samples collected from various countries in South Asia (Cambodia, India, Indonesia, Malaysia and Thailand). According to morphological characters, all these isolates were belonging to *Aspergillus flavus* (45), *A. fumigatus* (8), *A. ochraceus* (7), *A. niger* (42) and *A. tamarii* (8). This is the first report on identification of large number of *Aspergillus* strains isolated from rice grains in South Asia.

Key words: Cereals, *Aspergillus* sp., morphology, aflatoxins, aspergillosis

INTRODUCTION

Rice is one of the important staple foods in the world and in the South Asia where high amounts of rice are consumed per capita per year. The main rice producing countries are Bangladesh, China, India, Indonesia, Myanmar, Thailand and Vietnam (Reiter *et al*., 2010). The environmental conditions in South Asian countries are characterized by high temperatures (26-39°C) coupled with high relative humidity (67-98%) and are hence conducive for the growth of mycotoxigenic fungi and the production of mycotoxins in nearly all agricultural crops throughout the year (Sales and Yoshizawa, 2005). Generally cereal crops usually are harvested during the rainy season resulting in high moisture content of the grains, sub-optimal conditions for processing and storage and potentially rapid accumulation of toxigenic fungi (Sales and Yoshizawa, 2005). Mycotoxigenic fungi are well-known to invade the rice grains under storage conditions and produce mycotoxins (Reddy *et al*., 2009, 2010). Several reports are available on colonization of rice grains with *Aspergillus* sp., from South Asia (Pitt *et al*., 1994; Sales and Yoshizawa, 2005; Park *et al*., 2005; Makun *et al*., 2007; Reddy *et al*., 2009; Lampak *et al*., 2009).

Mycotoxigenic fungal contamination not only causes deterioration of foods, but also causes food borne intoxicants in humans and animals as they may produce toxic secondary metabolites called mycotoxin (Murthy *et al*., 2009). Most human diseases caused by Aspergilli (aspergilloses) are associated with immunosupression. They are frequently fatal. As the number of immunosupressed people in the population has risen, so has the importance of infection by *Aspergillus*. *A. fumigatus* is involved in about 90% of human aspergilloses, followed by *A. flavus*, *A. terreus*, *A. niger*, *A. nidulans* and *A. ochraceus* (Bertout *et al*., 2001). Interest in *Aspergillus* sp., is increasing world wide due to the discovery of a growing number of naturally occurring *Aspergillus* toxins that have proved to be threat to the human and animal health (Bhat *et al*., 2010). *Aspergillus* sp. is known to produce aflatoxins, ochratoxins and gliotoxin (Reddy *et al*., 2009; Lanier *et al*., 2009). Aflatoxin B_1 (AFB1) has been classified as a class 1 human carcinogen and OTA as class 2B by the International Agency for Research on Cancer (IARC, 1993). Gliotoxin is one of the most abundantly produced epithiodioxopiperazine metabolites from *A. fumigatus*. Toxicological studies showed that gliotoxin can exacerbate the pathogenesis of aspergillosis (Sutton *et al*., 1996). A gardener developed a fatal aspergillosis and died after inhalation of decayed plant matter contaminated with *A. fumigatus* spores (Russel *et al*., 2008).

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Generally identification of the *Aspergillus* sp. is based on the morphological characteristics of the colony and microscopic examinations (McClenny, 2005; Diba *et al*., 2007). Though molecular methods continue to improve and become more rapidly available, microscopy and cultural methods remain commonly used and essential tools for identification of *Aspergillus* sp. (Diba *et al*., 2007). However, the aim of this study was to identify large number of *Aspergillus* isolates through macroscopical and microscopical characters isolated from 65 rice grain samples collected from South Asian countries. As far as we know this is the first study of its kind for identification of *Aspergillus* species in South Asia through morphological characters.

MATERIALS AND METHODS

Collection of rice grain samples and isolation of *Aspergillus* sp.: A total of 65 rice grain samples were randomly collected from sundry markets and supermarkets in South Asia during the year 2009. The samples were mainly from white rice, basmati, black glutinous rice, brown rice, fragrant rice and white glutinous rice originating from Cambodia, India, Indonesia, Malaysia and Thailand. All samples were analyzed for *Aspergillus* sp. using the agar-plate method according to Reddy *et al*. (2009). Hundred seeds were placed on one-half strength Potato Dextrose Agar (PDA) added with rose bengal at a concentration of 50 ppm. The plates were incubated at 25±2°C for 7 days and then the *Aspergillus* colonies emerging out from rice grains were transferred onto fresh potato dextrose agar plates for further studies.

Purification of cultures through single spore isolation: All *Aspergillus* sp. strains were purified through single spore isolation technique (Samapundo *et al*., 2007). The single conidial isolates were maintained on low nutrient medium for further studies.

Identification of *Aspergillus* sp. through morphological characters: All *Aspergillus* sp. isolates were identified to the species level using taxonomic systems of *Aspergillus* by Klich (2002). All *Aspergillus* sp. were cultured on Czapeks yeast agar (CYA; Czapek concentration 10.0 mL, K_2HPO_4 1.0 g, powdered yeast extract 5 g, sucrose 30 g, agar 15 g, distilled water 1 L), Czapeks yeast agar with 20% sucrose (CYA20S; Czapek concentration 10.0 mL, K_2HPO_4 1.0 g, powdered yeast extract 5 g, sucrose 200 g, agar 15 g, distilled water 1 L), Malt extract agar (MEA; powdered malt extract 20 g, peptone 10 g, glucose 20 g, agar 20 g, distilled water 1 L) and Czapeks Dox agar (CZA; Czapek concentration 10 mL, K_2HPO_4 1 g, sucrose 30 g, agar 17.5 g, distilled water 1 L) at 25±2°C for 7 days. Some of the CYA plates were also incubated at 37°C for 7 days. Macroscopical characters included colony color and diameter, conidia color, exudates, sclerotia, colony texture and shape. Microscopic characteristics for the identification were color and length of conidial heads, stipes, vesicles shape and seriation, conidia size, shape and roughness and phialides length and breadth (Diba *et al*., 2007). To confirm our identification, we compared the morphological characteristics of tested *Aspergillus* isolates with those of the standard species obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India.

RESULTS

A total of 110 isolates of *Aspergillus* spp. which included *A. flavus* (45), *A. fumigatus* (8), *A. ochraceus* (7), *A. niger* (42) and *A. tamari* (8) were obtained from 65 rice samples collected from South Asian countries. Morphological characters were studied for identification of all these isolates along with standard cultures using four differential culture media.

Morphological characters of *A. flavus* isolates: Conidia of all isolates were light sparse grey green to pale blue green or parrot green, mycelium fluffy creamy white to dull white color and exudates were present on surface, reverse uncolored to yellowish or orange and wrinkled mycelial growth; soluble pigments were absent; very few sclerotia were present in wheat brown color (Fig. 1a-h). On reverse side of MEA plates no wrinkles were observed. Conidia very sparse in dull blue green color reverse yellowish orange to light peach on CZA (Table 1). Macroscopical and microscopical characters of *A. flavus* are presented in Table 1.

Morphological characters of *A. fumigatus* isolates: Conidial colors on CYA25 grayish, mycelium white, inconspicuous to florescence; exudates were absent; reverse uncolored to yellowish, red brown or green, soluble pigments were absent; sclerotia were absent in all media (Table 2). On MEA, conidia colored as on CYA25, mycelium white, reverse uncolored to dull yellow or grey, soluble pigments were absent. Macroscopical and microscopical characters of *A. fumigatus* are presented in Table 2.

Morphological characters of *A. niger* isolates: Conidia are black and densely packed on CYA; hyphae inconspicuous, white to dull yellow; exudates were absent; reverse uncolored to florescent yellow and

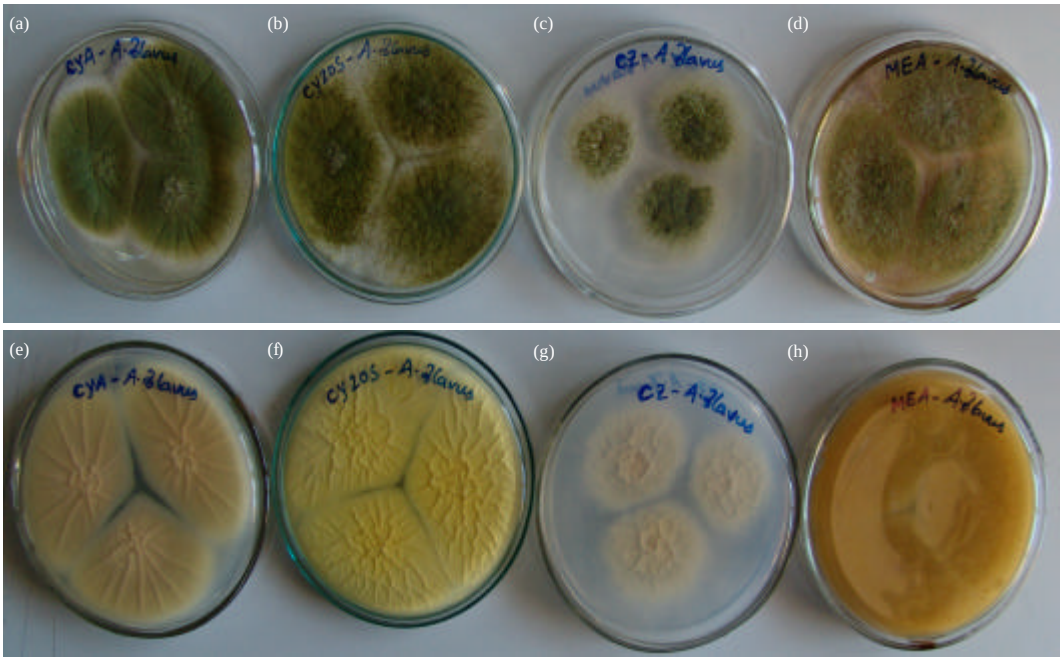

Fig. 1: Macroscopical characters of *A. flavus* on different agar media. (a-d) Front view and (e-h) Reverse view. (a, e) Mycelia growth on CYA; (b, f) Growth on CYA20S; (c, g) Growth on CZA; (d, h) Growth on MEA

Table 1: Macroscopic and microscopic characters of *A. flavus* isolates

Characters	CYA 25°C	MEA	CYA 37°C	CYA20S	CZA
Macroscopic characters					
Colony diameter (mm)	73±4.0	70±3.2	70±2.5	71±3.5	68±2.0
Colony color	Parrot green	Parrot green	Parrot to deep green	Light parrot to deep parrot green	Light parrot to parrot green
Conidia color	Parrot green	Parrot green	Parrot green	Parrot green	Parrot green
Mycelium	Fluffy white to parrot green	White to parrot green	Fluffy white to parrot green	Fluffy parrot green	Normal parrot green
Exudates	Present	Nil	Nil	Nil	Nil
Reverse	Pale yellow to orange and wrinkled mycelial growth	Pale yellow to orange and normal mycelial growth	Pale yellow wrinkled mycelial growth	Yellow to orange wrinkled mycelial growth	Yellowish orange to light peach wrinkled mycelial growth
Sclerotia	Very few	A few	Many	A few	Nil
Sclerotia shape	Globose	Globose	Globose	Globose	-
Sclerotia color	Wheat brown	Wheat brown	Dark brown to black shiny	Light brown	-
Microscopic characters					
Conidiophore length (µm)	550-678	428-522	Not tested	608-738	651-785
Conidiophore breadth (µm)	11.6-12.6	9.1-10.3	Not tested	10.6-11.0	10.0-14.0
Conidiophore color	Un color to pale brown	Un color to pale brown	Not tested	Un color to pale brown	Un color to pale brown
Conidiospore size (µm)	3.3-4.3	2.8-3.4	Not tested	3.4-4.0	3.4-4.6
Conidiospore	Globose	Globose	Not tested	Globose	Globose
Surface texture	Smooth to finely roughened	Smooth to finely roughened	Not tested	Smooth to finely roughened	Smooth to finely roughened
Vesicle diameter (µm)	25.6-28.0	24.7-33.5	Not tested	27.3-29.1	24.7-33.5
Phialides	Biseriate	Uniseriate	Not tested	Biseriate	Biseriate
Length (µm)	6.7-8.5	5.6-6.0	Not tested	7.2-8.6	5.6-7.8
Breadth (µm)	3.3-3.9	2.4-2.6	Not tested	3.3-3.9	2.6-3.6

± = Standard deviation

wrinkled mycelial growth; soluble pigments were absent; sclerotia were absent in all media. On MEA, reverse uncolored to light brown and no wrinkles were present. On CZA, reverse florescent yellow to light white, wrinkled. Macroscopical and microscopical characters of *A. niger* are presented in Table 3.

Morphological characters of *A. ochraceus* isolates: Conidial color on CYA25 near wheat to light yellow; fluffy mycelial growth, white or creamy white, inconspicuous to florescence; exudates were absent; reverse dull yellow to dark yellow or some times brown and wrinkled mycelial growth; soluble pigments were absent; sclerotia were

Table 2: Macroscopic and microscopic characters of *A. fumigatus* isolates

Characters	CYA 25°C	MEA	CYA 37°C	CYA20S	CZA
Macroscopic characters					
Colony diameter (mm)	62±2.2	58±2.0	60±3.5	64±4.0	55±1.5
Colony color	Grayish	Grayish	Grayish	Grayish	Grayish
Conidia color	Dark green	Dark green	Grayish brown	Dark green	Grayish turquoise
Mycelium	White	White	White	White	White
Exudates	Nil	Nil	Nil	Nil	Nil
Reverse	Uncolored to green	Yellowish	Uncolored to green	Uncolored to green	Uncolored to red brown
Sclerotia	Nil	Nil	Nil	Nil	Nil
Microscopic characters					
Conidiophore length (μm)	265-365	285-326	Not tested	315-386	225-345
Conidiophore breadth (μm)	5.6-10.6	4.1-10.3	Not tested	9.5-11.0	8.5-10.0
Conidiophore color	Uncolored to grayish	Uncolored to grayish	Not tested	Uncolored to grayish	Uncolored to grayish
Conidiospore size (μm)	2.3-3.0	2.8-3.4	Not tested	2.6-3.0	2.4-3.5
Conidiospore	Globose	Globose	Not tested	Globose	Globose
Surface texture	Smooth to finely roughened	Smooth to finely roughened	Not tested	Smooth to finely roughened	Smooth to finely roughened
Vesicle diameter (μm)	22.4-28.0	24.7-33.5	Not tested	27.3-29.8	24.5-33.5
Phialides	Uniseriate	Uniseriate	Not tested	Uniseriate	Uniseriate
Length (μm)	6.5-8.0	5.6-6.9	Not tested	7.2-8.8	5.0-7.8
Breadth (μm)	2.3-2.5	2.2-2.6	Not tested	2.0-3.1	2.6-3.2

± = Standard deviation

Table 3: Macroscopic and microscopic characters of *A. niger* isolates

Characters	CYA 25°C	MEA	CYA 37°C	CYA20S	CZA
Macroscopic characters					
Colony diameter (mm)	90±0.0	80±2.5	80±4.0	90±0.0	70±2.5
Colony color	Dark black	Black	Dark black	Dark black	Dark black
Conidia	Black	Black	Dark black	Dark black	Dark black
Mycelium	Dull white	Dull white	Dull white	Dull white	Dull white
Exudates	Nil	Nil	Nil	Nil	Nil
Reverse	Florescent yellow and wrinkled mycelial growth	Pale brown normal mycelial growth	Pale yellow wrinkled mycelial growth	Pale yellow	Florescent yellow to light white wrinkled mycelial growth
Sclerotia	Nil	Nil	Nil	Nil	Nil
Microscopic characters					
Conidiophore length (μm)	855-957	1002-1168	Not tested	927-1119	1309-1561
Conidiophore breadth (μm)	11.4-15.0	14.4-15.6	Not tested	14.1-15.1	15.0-16.0
Conidiophore color	Hyaline to yellowish or slightly brown	Hyaline to yellowish or slightly brown	Not tested	Hyaline to yellowish or slightly brown	Hyaline to yellowish or slightly brown
Surface texture	Smooth	Smooth	Not tested	Smooth	Smooth
Conidiospore size (μm)	2.9-3.3	3.3-4.0	Not tested	2.8-3.2	3.6-4.0
Conidiospore	Globose	Globose	Not tested	Globose	Globose
Surface texture	Rough walled	Rough walled	Not tested	Rough walled	Rough walled
Vesicle diameter (μm)	26.6-29.4	29.0-31.0	Not tested	27.3-30.7	30.0-32.3
Phialides	Biseriate	Biseriate	Not tested	Biseriate	Biseriate
Length (μm)	5.8-6.8	6.7-8.0	Not tested	5.7-6.3	7.0-8.2
Breadth (μm)	2.9-3.3	2.7-3.1	Not tested	2.9-3.3	2.8-3.4

± = Standard deviation

absent in all media. On MEA, conidia not dense usually pale to light yellow; reverse light yellow, pale orange to grayish gold shades; and no wrinkles were present; colonies not densely sporulating, variable in appearance (Table 4). Macroscopical and microscopical characters of *A. ochraceus* are presented in Table 4.

Morphological characters of *A. tamarii* isolates: Colony and conidia color on CYA25 dark olive green; mycelium white to dull white, usually inconspicuous, reverse uncolored to red tinge with chocolate brown or brown and straight wrinkled mycelial growth; the outer surface of the colony was circular; colonies usually quite low, velutinous; sclerotia, exudates and soluble pigments were absent in all media (Table 5). On MEA, mycelium usually inconspicuous, reverse brown color with normal, sparse, loose, not dense and floccose growth and in all other media wrinkled growth; on CY20S generally puffy growth with slightly more yellow green than on CYA25; reverse reddish brown with wrinkled mycelial growth. On CZA, conidia very sparse in dark bluish green or olive green colors reverse reddish tinge to dark brown shade; on CYA37 dark blackish brown and concentric rings were observed on reverse (Table 5). Macroscopical and microscopical characters of *A. tamarii* are presented in Table 5.

Table 4: Macroscopic and microscopic characters of *A. ochraceus* isolates

Characters	CYA 25°C	MEA	CYA 37°C	CYA20S	CZA
Macroscopic characters					
Colony diameter (mm)	45±2.5	47±3.0	50±2.0	55±1.5	33±1.0
Colony color	Creamy white to yellow	Creamy white to yellow	Creamy white to yellow	Creamy white to yellow	Light yellow
Conidia	Wheat to yellow	Yellow	Yellow	Yellow	Yellow
Mycelium	Dull white with cream mat	Fluffy white to dull wheat	White	Dull white to creamy white	Dull white
Exudates	Nil	Nil	Nil	Nil	Nil
Reverse	Dark yellow wrinkled mycelial growth	Pale yellow normal mycelial growth	Turmeric yellow and normal mycelial growth	Dark yellow less wrinkled mycelial growth	Dark florescent yellow normal mycelial growth
Sclerotia	Nil	Nil	Nil	Nil	Nil
Microscopic characters					
Conidiophore length (μm)	843-1059	403-521	Not tested	846-974	403-521
Conidiophore breadth (μm)	10.0-11.2	7.0-8.4	Not tested	10.0-12.0	7.3-8.7
Conidiophore color	Uncolored to yellowish or pale brown	Uncolored to yellowish or pale brown	Not tested	Uncolored to yellowish or pale brown	Uncolored to yellowish or pale brown
Surface texture	Coarsely roughened	Coarsely roughened		Coarsely roughened	Coarsely roughened
Conidiospore size (μm)	2.8-3.2	2.3-2.7	Not tested	3.0	2.5-2.7
Conidiospore	Ellipsoid	Ellipsoid	Not tested	Ellipsoid	Ellipsoid
Surface texture	Smooth to finely roughened	Smooth to finely roughened	Not tested	Smooth to finely roughened	Smooth to finely roughened
Vesicle diameter (μm)	32.0-34.0	21.5-24.5	Not tested	32.0-34.0	20.0-24.0
Phialides	Biseriate	Biseriate	Not tested	Biseriate	Biseriate
Length (μm)	7.8-8.2	6.4-7.6	Not tested	7.8-8.2	7.0-8.0
Breadth (μm)	2.2-2.6	2.3-2.5	Not tested	2.3-2.7	2.2-2.4

± = Standard deviation

Table 5: Macroscopic and microscopic characters of *A. tamarii* isolates

Characters	CYA 25°C	MEA	CYA 37°C	CYA20S	CZA
Macroscopic characters					
Colony diameter (mm)	60±1.0	65±2.5	46±4.0	68±2.0	55±3.5
Colony color	Olive green	Olive green	Olive green	Olive green	Olive green
Conidia	Dark olive green	Dark olive green	Dark olive green	Dark olive green or more yellow green	Dark olive green
Mycelium	Dull white	Dull white	Dull white	Fluffy growth at centre and dull white	Dull white
Exudates	Nil	Nil	Nil	Nil	Nil
Reverse	Red tinge with chocolate brown and wrinkled	Brown and normal	Dark blackish brown and wrinkled	Reddish brown with wrinkled growth	Reddish tinge to dark brown and wrinkled
Wrinkle	10-15	Nil	15-20	25-35	5-8
Wrinkle length (mm)	0.5-25	Nil	0.1-15	0.5-35	0.5-15
Sclerotia	Nil	Nil	Nil	Nil	Nil
Microscopic characters					
Conidiophore length (μm)	466 -576	368-448	Not tested	491-585	356-442
Conidiophore breadth (μm)	8.5 -9.9	8.2-9.0	Not tested	8.5-9.3	7.9-8.8
Conidiophore color	Light brown	Light brown	Not tested	Light brown	Light brown
Surface texture	Slightly roughened	Slightly roughened	Not tested	Slightly roughened	Slightly roughened
Conidiospore size (μm)	5.7-6.2	4.9-5.3	Not tested	5.2-5.4	4.5-4.7
Conidiospore surface texture	Thick walled highly roughened	Thick walled highly roughened	Not tested Not tested	Thick walled highly roughened	Thick walled highly roughened
Vesicle diameter (μm)	24.2-28.6	24.3-27.9	Not tested	26.1-29.9	24.4-27.2
Phialides	Uniseriate	Biseriate	Biseriate	Uniseriate	Uniseriate
Length (μm)	8.5-9.1	7.7-8.1	Not tested	8.6-8.8	7.8-8.0
Breadth (μm)	4.2-5.0	3.7-4.1	Not tested	4.2-4.6	3.6-3.8

± = Standard deviation

DISCUSSION

When rice grains with moisture content higher than the desired level enter the storage system, invasion of both field and storage fungi take place. The harmful effects of such fungal invasion are glume or grain discoloration, loss in viability, quality and toxin contamination (Reddy *et al*., 2008). *A. flavus* is of ubiquitous occurrence in nature. Since the discovery of aflatoxins, it has become the most widely reported food-borne fungus, reflecting its economic and medical importance and ease of recognition, as well as its

universal occurrence. *A. parasiticus* is less common, but the extent of its occurrence is obscured by the tendency for *A. flavus* and *A. parasiticus* to be reported only as *A. flavus* (Reddy *et al*., 2009). In this study we observed low frequency of *A. fumigatus* in some of the rice grains. *A. fumigatus* is an airborne fungus and infection occurs by inhaling conidia which may colonize airways prior to invasion. It is an opportunistic fungal pathogen responsible for most cases of Invasive Aspergillosis (IA), the most common systemic filamentous fungal infection worldwide (Bertout *et al*., 2001).

Several reports are available on contamination of rice grains with *Aspergillus* sp. A high incidence of *A. flavus* was found in the seed mycoflora of rice (Reddy *et al*., 2008). The rice crop exposed to frequent and heavy rainfall and flood is subjected to infection by *Aspergillus* sp. (Reddy *et al*., 2009). Begum and Samajpati (2000) isolated mycotoxin-producing fungi from contaminated grains of rice sold in the local markets of Calcutta, India. Recently, Jayaraman and Kalyanasundaram (2009) reported *Aspergillus* sp. contamination of rice bran oils. Sales and Yoshizawa (2005) described the incidence of *A. flavus* and *A. parasiticus* in rice bran (14%) and rough rice (78%). Abdullah *et al.* (1998) reported the incidence of aflatoxingenic fungi in rice grains from Malaysia. Recently, Lampak *et al.* (2009) reported various fungal mycoflora (*Acremonium*, *Aspergillus*, *Bipolaris*, *Colletotrichum*, *Curvularia*, *Drechslera*, *Fusarium*, *Geotrichum*, *Nigrospora* and *Penicillium*) in brown rice from Thailand. A study by Purwoko *et al.* (1991) revealed that *A. flavus* was the dominant fungi in broken rice and rice bran samples in Indonesia. Still today there are no reports on occurrence of *Aspergillus* sp. in rice from Cambodia. In this we made attempts to isolate and identify *Aspergillus* sp. in rice samples collected from Cambodia.

Many *Aspergillus* strains are very close in their morphological characters and chances are very high to misidentify them. Therefore, accurate identification of *Aspergillus* sp. is important to develop proper management practices to control these toxigenic fungi and their mycotoxins in food grains. Recently, Kim *et al.* (2009) and Diba *et al.* (2007) studied the morphological characters for identification of clinical *Aspergillus* sp. isolates. Alwakeel (2007) identified *Aspergillus* sp. isolated from kitchen samples in Riyadh, Saudi Arabia using morphological methods. Morya *et al.* (2009) used morphology based methods for identification of *Aspergillus* sp. isolated from soil of teak forest. But none of them studied morphological characters for *Aspergillus* isolates from rice grains. This is the first report on identification of huge number of *Aspergillus* sp. isolates obtained from rice grain samples collected from various countries in South Asia through morphological characters.

Askun (2006) used three (CZ, MEA and PDA) differential media for identification of *Aspergillus* sp. using morphological characteristics isolated from maize kernels. Similarly, Khosravi *et al.* (2007) used only two differential media (PDA and CZ) for identification of Aspergilli isolated from nut products in Iran. In this study we used morphological method with four differential culture media for identification of five important *Aspergillus* species isolated from rice grains. Using this method, all standard strains were identified successfully. For the identification of Aspergilli based on morphological methods requires adequate growth for evaluation of colony characteristics and microscopic features. Diba *et al.* (2007) reported that use of potato dextrose, potato flake, malt extract, inhibitory mould agar, or similar sporulation agars as primary isolation media for *Aspergillus* may accelerate growth rate and the production of conidia (Diba *et al*., 2007). In our study, using four differential media including CZA, CYA, CYA20S and MEA with macroscopic and microscopic characteristics of fungal growth on this culture media enabled us to discriminate five *Aspergillus* species isolated from rice grains. We preserved all 110 isolates of *Aspergillus* sp. at Universiti Sains Malaysia, Malaysia culture collection centre for future studies.

CONCLUSION

This study concludes that *Aspergillus* sp. can contaminate rice grains under storage conditions. Though molecular methods are well developed for identification of *Aspergillus* sp., the developing countries are still highly depending on morphological identification. In this study we have identified five *Aspergillus* sp. isolated from rice grains based on their morphological characters. Most important human pathogen, *A. fumigatus* were also observed in few rice grain samples destined for human consumption. In our view, morphological method using the differential media is the most reliable and sensitive assay to identify important *Aspergillus* sp. isolated from rice grains or some other sources. This study may be a basis for those who are interested to study morphological characters for *Aspergillus* sp. in developing countries. Mycotoxin profiles produced by these *Aspergillus* sp. isolates are under progress.

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