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RESEARCH ARTICLE

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ASPERGILLUS FLAVUS ISOLATES FROM GROUNDNUT (ARACHIS HYPOGAEA L.)

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ABSTRACT

In the present study four different culture media, Czapek's-Dox agar (CZA), Potato dextrose agar (PDA), Yeast extract sucrose agar (YESA) and *Aspergillus flavus* and *parasitic us* agar (AFPA) were used to know the efficacy for the growth of *A. flavus*. A total of 38 strains of *A. flavus* including aflatoxigenic and non-aflatoxigenic strains were analyzed for their growth response on PDA at different range of pH and temperatures. The results indicated that pH 8 and temperature 30 °C were found to be optimum for the growth of *A. flavus* isolates in PDA medium. Further extracellular enzyme activities indicated that there was slight significant difference among tested isolates of *A. flavus* appeared in plate screening assay carried out for amylase, protease and lipase activity. However, the pectinase activity was same in all the tested isolates. In conclusion both aflatoxigenic and non-aflatoxigenic isolates of *A. flavus* did not show significant variation in extracellular enzyme activities.

INTRODUCTION

Aspergillus flavus is a ubiquitous, saprophytic fungus of *Aspergillus* section *Flavi.* It has worldwide distribution in the environment and very competitive with high temperature and low water activity, may become the dominant fungal species in host crops (Payne, 1998). It is a weak opportunistic plant pathogen and secretes aflatoxins into many agricultural crops. The United Nation's Food and Agriculture Organization (FAO) has estimated that as much as 25% of the World's food is significantly contaminated with mycotoxins (CAST, 1989), much of this contamination is by aflatoxin.

Growth, sporulation of *A. flavus* and production of toxic metabolites mainly aflatoxin under *in vitro* conditions is majorly affected by physical factors such as composition of the media, incubation period, temperature, pH etc. The radial growth rate of *Aspergillus* sp. colonies reached the highest at 30-40 °C (Malama *et al*., 1987).

High nutritive value of seeds makes them to attack by various microorganisms, of which fungi play a dominant role in infecting quality and longevity of seeds during storage (Christensen and Kaufman, 1969). Fungi associated with seed in ill storage condition uses the seed contents and release the extracellular hydrolytic enzymes and cause bio-deterioration of seeds by degrading host barriers seems to be a common strategy used by pathogenic fungi (Sieber *et al*., 2000; Roncero *et al*., 2003).

Naturally all the fungi produce a large number of enzymes to breakdown complex materials for their growth. Production of extracellular hydrolytic enzymes by seed borne fungi has a role during the process of seed deterioration and has been considered helpful to their tissue maceration, invasion and subsequent colonization in host plants (Chenglin *et al*., 1996). Extracellular hydrolytic enzymes such as α-amylases, cellulases, cutinases, pectinases and proteases from phytopathogenic fungi during infection of plant tissues is well documented (Dean and Timberlake, 1989; Cotty *et al*., 1990; Gupta *et al*., 1993; Woloshuk *et al*., 1997). These enzymes and metabolites allow fungi to either increase their own fitness or decrease surrounding organism's fitness, ensuring survival and reproduction further.

Aspergillus species are natural ''factories'' for the production of enzymes such as cellulase, pectinase, xylanase, amylase, protease and lipase (Betts and Dart, 1989; Cotty *et al.*, 1990; Long *et al*., 1998). Important characteristic of *Aspergillus* spp. is their ability to produce hydrolytic enzymes and secondary metabolites in response to environmental parameters, allowing them to adapt to complex and changing environments. *Aspergillus flavus* is most successful in infection because it has the ability to utilize enormous variety of organic material present in seeds as food by breaking down into simpler form (Ogundero, 1981; Woloshuk *et al.*, 1997). The conversion of starch to sugar during postharvest deterioration of stored products and the increase in the concentration of glucose after infection by pathogenic fungi have been attributed to the hydrolysis of starch, disaccharides and oligosaccharides by amylases into simple sugars (Akpan *et al*., 1999; Damien *et al*., 2010). Various species of *Aspergillus*, *Penicillium*, *Mucor*, *Rhizopus*, *Trichothecium* and *Gleotrichum* are well known producers of lipases (Long *et al*., 1996; Sipahioglu and Heperkan, 2000). Proteases of *A. flavus* and *A. parasiticus* have been shown to be important components in the fungal infection (Monod *et al.*, 2002; Reed and Kita, 2004). *Aspergillus parasiticus* extracellular proteases facilitate groundnut seed colonization (Asis *et al*., 2009).

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Hence, in the present study an attempt has been made for the identification of suitable culture medium, temperature and pH for growth of *A. flavus*. Further, the variation in the production of extracellular enzymes by *A. flavus* isolates was studied in relation to their toxigenic nature.

MATERIALS AND METHODS

Aspergillus flavus isolates

A total of 38 selected isolates of *A. flavus* previously isolated from groundnut seed samples collected from different regions of India and identified as aflatoxigenic and non-aflatoxigenic were used in the present study Navya *et al*. (2013). All strains were routinely maintained on PDA and 40% glycerol was used to store the conidial suspension for longer duration at -80 °C.

Morphological characterization

Aspergillus flavus strains were characterized morphologically by growing them on CZA (Czapek's Dox agar), PDA (Potato dextrose agar), YESA (Yeast extract sucrose agar) and AFPA (*Aspergillus flavus* and *parasiticus* agar). Each strain was inoculated at the centre of 90 mm Petri plate containing each medium and incubated for 7 days at 28 ± 2 °C. After incubation period, the growth of *A. flavus* strains was observed and recorded.

Physiological characterization

Physiological characterization of selected 38 *A. flavus* strains was done by growing them on modified PDA media. The pH of the medium was adjusted to 6, 8 and 10 using 1 M HCl and 1 M NaOH. Petri plates containing medium were inoculated at the centre and incubated for 7 days at 28 ± 2 °C under dark condition. Towards the end of the incubation period plates were observed for the growth of *A. flavus*. Further, the response of these strains to different temperatures was studied by growing them on PDA medium at different incubation temperatures (4, 15, 30 and 40 °C) over a period of 7 days at dark.

Biochemical characterization

Amylase plate assay

Screening of *A. flavus* strains for the amylase activity was done by plate assay as explained by Balkan and Ertan (2005). Initially, the pH was adjusted to 6.0 using 1 M HCl and the medium was autoclaved at 121 °C for 15 min. *Aspergillus flavus* strains were inoculated on the medium and incubated at 28 ± 2 °C for 5-7 days. Starch degrading activities of strains were estimated by measuring the clear zone formed by the degradation of starch when exposed to iodine solution.

Lipase plate assay

The ability of *A. flavus* strains to produce lipase enzyme was performed by the method explained by Sierra (1957). The pH of the medium was maintained to 6.0 using 1 M HCl. Petri plates containing sterile solidified medium were inoculated with *A. flavus* and incubated at 28 ± 2 °C for 7 days. Appearance of visible precipitate around a fungal colony due to complete degradation of salt of the fatty acid determines lipolytic activity of strains.

Pectinase plate assay

Pectinase activity of *A. flavus* strains was determined according to the method of Hankin and Anagnostakis (1975). Culture plates were inoculated with *A. flavus* strains and incubated for 3-5 days at 28 ± 2 °C. At the end of incubation period, the plates were flooded with 1% aqueous hexadecyltrimethyl ammonium bromide (HDTMA, Sigma, USA) to precipitate the non-degraded pectin. A clear zone around fungal colony is indicative of the pectinase activity.

The activity of amylase, lipase and pectinase enzyme in each isolate was calculated by,
Index of relative enzyme activity, I = Diameter of clear zone/Diameter of fungal colony *i.e*., (DCZ/DFC).

Protease plate assay

Protease activity of *A. flavus* strains was determined by inoculating the strains in CZ medium amended with 1% skimmed milk powder (Gupta *et al*., 2002). The plates were incubated at 28 ± 2 °C for 5 days under dark condition. After incubation, the plates were observed for the clear zone around the fungal colony which indicates the degradation of skimmed milk by *A. flavus* and reported as positive for protease activity.

Statistical analysis

All data were analyzed separately for each experiment and were subjected to arcsine transformation and analysis of variance (ANOVA) (SPSS, version 16). Significant effects of treatments were determined by the F values ($P \leq 0.05$). Treatment means were separated using Turkey's HSD test.

RESULTS

Morphological and physiological characterization of Aspergillus flavus *isolates*

A total of 38 *A. flavus* isolates were characterized morphologically by four different culture media *viz.*, PDA, CZ, YESA and AFPA (Figure 1). As given in Table 1, colonies on each medium were recorded for their diameter. Based on the growth performance upto 7 days of incubation, the isolates were categorized as fast (> 6 cm), medium (4-6 cm) and slow (< 4 cm). Fungal cultures found growing faster and also sporulated well on PDA. The growth and sporulation was poor on AFPA in comparison with other media.

Aspergillus flavus isolates showed a very broad range of tolerance with respect to the incubation temperature. At 15 °C, growth rate was very less (1.5-4.5 cm) with minimum sporulation and at 4 °C growth of fungus was completely ceased. But at 40 °C though there was slow growth of fungal colony but sporulation was good. However, both growth and sporulation of *A. flavus* was maximum at 30 °C (Figure 2, Table 2). Similarly, at pH 8 profused growth and sporulation was observed in most of the strains followed by pH 6, control and pH 10 (Figure 3, Table 3).

Biochemical characterization of Aspergillus flavus isolates

Among the 38 isolates of *A. flavus* screened for amylase production, most of the isolates showed lower amylase activity (Figure 4). The highest DCZ/DFC ratio of 1.2 recorded in the *A. flavus* isolates AFG49 and least ratio of 1.0 was recorded in isolates AFG15, AFG29, AFG37, AFG52, AFGS5 and AFGS15 (Figure 5).

Lipolytic activity corresponded to the white precipitate resulting from deposition of crystals of calcium salt formed by the fatty acid liberated by the enzyme (Figure 6). The highest

activity as a means of DCZ/DFC ratio of 1.55 was recorded in the isolate AFG8 and least activity of 1.01 was observed in the isolates AFG28, AFG64, AFG69 and AFGS17 (Figure 7). Pectinase activity in all the tested isolates was almost similar (Figure 8) which ranged from 1 to 1.1 (Figure 9). Except for two isolates, AFG15 and AFG28, all other were found positive for protease activity by degrading skimmed milk powder (Figure 10, Table 4).

DISCUSSION

Aspergilli are ubiquitous in nature, geographically distributed and have been observed in a broad range of habitats because of their ability to colonize on a wide variety of organic substrates. Present investigation was aimed to know the optimum media, temperature and pH for *A. flavus* growth and to analyze variability in extracellular enzyme activities among the isolates of *A. flavus* infecting groundnut in relation to toxigenicity.

Thirty-eight isolates of *A. flavus* including aflatoxigenic and non-aflatoxigenic strains isolated from groundnut seed samples collected from different agroclimatic regions of India were used in this study. Profuse growth of isolates was achieved on PDA with good sporulation followed by CZ, YESA and AFPA. *Aspergillus* species in general are more tolerant to alkaline pH (Wheeler *et al*., 1991). In our study also pH-8 was found suitable for the growth of *A. flavus* where it endowed maximum growth. As observed by Sanchis and Magan (2004) optimum temperature was found to be 30 °C. Similar kind of work attempted by Malama *et al*. (1987) also showed that the colonies of three *Aspergillus* spp. reached highest radial growth rate at 30-40 °C. Study carried out by Achar and Sanchez (2006) was also in support of our findings, they observed vigorous growth of *A. flavus* in PDA medium at temperature 27 °C and 30 °C. Earlier findings reports that *A. flavus* grew and sporulated best at 35 °C and the optimum pH for growth was 7.5 and for sporulation 6.5 (Olutiola, 1976).

For successful infection, establishment and seed deterioration, the pathogen has to overcome host barriers, which also act as a source for the growth of pathogen during infection process. Groundnut is very rich in components like cellulose, lipids and protein, degradation and utilization of these components by pathogen by producing respective enzymes helps in colonization, infection and deterioration of seeds (Agrios, 2005). Hydrolytic enzymes like cellulase, pectinase, amylase, protease, lipase are the virulent tools for infection and seed deterioration (Agrios, 2005). Vidhyasekaran *et al*. (1966) claimed that the production of extracellular hydrolytic enzymes by *F. moniliforme* and *A. flavus* was found to be responsible for the spoilage of paddy seeds. Fungi associated with seed in ill storage condition uses the seed content and release the extracellular hydrolytic enzymes, which cause seed deterioration.

Hydrolytic enzyme production is one of the best early indicators of fungal spoilage prior to visible signs of growth

Table 1 Growth of *Aspergillus flavus* on different culture media

Sl. No.	Isolate Code	Colony diameter (cm)			
		CZA	PDA	YESA	AFPA
1	AFG1	4.5±0.11^{efgh}	5.9±0.28^{ij}	5.2±0.23^{hi}	4.7±0.28^{fgh}
2	AFG8	4.8±0.26^{ef}	5.9±0.23^{ij}	5.6±0.23^{ef}	5.8±0.34^{cd}
3	AFG11	4.7±0.11^{efg}	6.8±0.28^{ef}	5.4±0.05^{fg}	5.7±0.23^{cde}
4	AFG12	6.6±0.11^{ab}	7.0±0.11^{de}	5.6±0.11^{ef}	4.0±0.11^{jk}
5	AFG13	4.5±0.23^{efgh}	5.8±0.34^{jk}	5.8±0.05^{de}	4.6±0.11^{ghi}
6	AFG14	4.0±0.23^{fgh}	6.0±0.11^{hi}	5.5±0.11^{efg}	4.4±0.05^{ijk}
7	AFG15	4.0±0.11^{fgh}	6.7±0.23^{ef}	5.9±0.17^{cde}	5.0±0.11^{efg}
8	AFG16	4.0±0.057^{fgh}	6.6±0.05^{efg}	6.1±0.08^{cd}	5.7±0.11^{cde}
9	AFG18	4.9±0.057^{e}	7.6±0.05^{bc}	6.4±0.05^{bc}	3.9±0.05^{k}
10	AFG19	4.2±0.08^{fgh}	6.3±0.08^{gh}	5.7±0.11^{def}	4.8±0.05^{fgh}
11	AFG20	4.1±0.05^{fgh}	6.4±0.23^{fgh}	5.8±0.11^{de}	5.6±0.17^{cde}
12	AFG24	4.0±0.11^{fgh}	6.6±0.05^{efg}	6.4±0.05^{bc}	4.6±0.11^{ghi}
13	AFG25	4.8±0.11^{ef}	6.4±0.11^{fgh}	5.3±0.17^{gh}	5.0±0.11^{efg}
14	AFG26	4.0±0.11^{fgh}	6.4±0.05^{fgh}	6.0±0.11^{cde}	4.9±0.11^{efg}
15	AFG27	4.8±0.17^{ef}	5.4±0.11^{mn}	5.0±0.17^{ijk}	5.8±0.23^{cd}
16	AFG28	3.5±0.11^{i}	6.0±0.11^{hi}	5.2±0.23^{hi}	4.6±0.17^{ghi}
17	AFG29	3.9±0.05^{ghi}	6.8±0.11^{ef}	6.0±0.11^{cde}	2.8±0.11^{l}
18	AFG30	4.2±0.05^{fgh}	7.3±0.17^{cd}	6.6±0.11^{b}	5.3±0.17^{def}
19	AFG33	3.7±0.05^{hi}	6.4±0.11^{fgh}	5.4±0.17^{fg}	5.5±0.05^{def}
20	AFG35	4.4±0.05^{efgh}	6.3±0.17^{gh}	6.4±0.05^{bc}	5.0±0.11^{efg}
21	AFG37	4.1±0.11^{fgh}	5.7±0.28^{kl}	5.5±0.11^{efg}	5.4±0.23^{def}
22	AFG39	4.8±0.28^{ef}	6.8±0.11^{ef}	6.1±0.05^{cd}	5.4±0.11^{def}
23	AFG48	5.6±0.11^{d}	5.5±0.28^{lm}	4.9±0.23^{kl}	5.4±0.17^{def}
24	AFG49	4.2±0.11^{fgh}	6.5±0.23^{fg}	6.5±0.11^{bc}	5.4±0.17^{def}
25	AFG50	4.8±0.17^{ef}	6.0±0.17^{hi}	5.4±0.17^{fg}	4.8±0.17^{fgh}
26	AFG52	6.0±0.05^{bcd}	5.7±0.11^{kl}	5.0±0.11^{ijk}	6.9±0.05^{b}
27	AFG54	7.2±0.17^{a}	8.2±0.23^{ab}	7.4±0.23^{ab}	7.8±0.11^{a}
28	AFG57	6.0±0.11^{bcd}	7.4±0.17^{bcd}	6.1±0.11^{cd}	5.0±0.11^{efg}
29	AFG62	6.0±0.17^{bcd}	8.2±0.17^{ab}	8.0±0.17^{a}	4.9±0.11^{efg}
30	AFG64	4.3±0.17^{efgh}	6.0±0.23^{hi}	4.8±0.17^{l}	5.4±0.17^{def}
31	AFG65	3.5±0.23^{i}	6.0±0.17^{hi}	5.4±0.17^{fg}	5.9±0.05^{c}
32	AFG66	5.8±0.17^{cd}	8.1±0.14^{abc}	7.8±0.17^{a}	5.3±0.17^{def}
33	AFG69	3.8±0.17^{hi}	6.2±0.17^{gh}	6.1±0.11^{cd}	5.7±0.17^{cde}
34	AFG71	6.5±0.28^{bc}	5.7±0.11^{kl}	6.0±0.11^{cde}	4.9±0.11^{efg}
35	AFGS15	6.0±0.17^{bcd}	7.0±0.11^{de}	5.5±0.11^{efg}	4.5±0.05^{hij}
36	AFGS17	4.4±0.05^{efgh}	5.3±0.05^{n}	5.3±0.17^{gh}	4.9±0.11^{efg}
37	AFGS31	6.0±0.25^{bcd}	8.5±0.17^{a}	6.4±0.05^{bc}	5.5±0.11^{def}
38	AFGS33	6.0±0.11^{bcd}	7±0.11^{de}	6.2±0.05^{bcd}	4.9±0.51^{efg}

and mycotoxin production (Jain and Lacey, 1991; Magon, 1993). The activities of amylase, protease and lipase produced by *A. flavus* are responsible for the deterioration of commodities. However, no significant variations in extracellular enzyme activities among aflatoxigenic and non-aflatoxigenic isolates of *A. flavus* under plate culture assays was recorded.

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Fungal growth: fast (> 6 cm), medium (4-6 cm) and slow (< 4 cm). Values are the means ± SE from three separate experiments. Mean followed by the same letters in the column are not significantly different according to Tukey's HSD at $P \leq 0.05$.

Table 2 Growth of *Aspergillus flavus* on PDA medium at different temperature

Sl. No.	Isolate Code	Colony diameter (cm)			
		Temperature (°C)			
		4	15	30	40
1	AFG1	NG	3.8±0.17^{bc}	8.2±0.11^{abc}	6.7±0.11^{ab}
2	AFG8	NG	2.0±0.11^{klm}	4.6±0.17^{j}	4.7±0.11^{ef}
3	AFG11	NG	2.2±0.05^{jk}	4.8±0.17^{j}	4.9±0.11^{de}
4	AFG12	NG	1.9±0.11^{lm}	4.5±0.11^{j}	5.0±0.11^{de}
5	AFG13	NG	2.1±0.05^{kl}	5.0±0.17^{j}	4.8±0.17^{ef}
6	AFG14	NG	3.0±0.11^{fg}	8.6±0.17^{a}	2.6±0.05^{lm}
7	AFG15	NG	2.1±0.17^{kl}	4.7±0.17^{j}	4.6±0.23^{ef}
8	AFG16	NG	2.7±0.11^{hi}	7.3±0.17^{de}	6.2±0.11^{b}
9	AFG18	NG	2.1±0.17^{kl}	4.9±0.11^{j}	4.6±0.05^{ef}
10	AFG19	NG	2.3±0.11^{jk}	4.6±0.05^{j}	4.3±0.17^{gh}
11	AFG20	NG	2.0±0.03^{klm}	4.9±0.05^{j}	4.4±0.11^{fg}
12	AFG24	NG	1.7±0.11^{lm}	8.3±0.17^{abc}	3.0±0.11^{kl}
13	AFG25	NG	2.2±0.11^{jk}	4.8±0.23^{j}	2.7±0.17^{lm}
14	AFG26	NG	2.3±0.17^{jk}	4.6±0.23^{j}	4.5±0.11^{fg}
15	AFG27	NG	3.7±0.11^{cd}	8.2±0.11^{abc}	4.2±0.11^{ghi}
16	AFG28	NG	4.3±0.17^{ab}	8.0±0.23^{abc}	5.2±0.11^{cd}
17	AFG29	NG	1.5±0.11^{m}	6.5±0.14^{gh}	3.5±0.05^{jk}
18	AFG30	NG	2.3±0.11^{jk}	4.8±0.17^{j}	4.8±0.05^{ef}
19	AFG33	NG	3.6±0.17^{cd}	7.5±0.05^{cd}	5.0±0.17^{de}
20	AFG35	NG	3.6±0.17^{cd}	7.5±0.17^{cd}	4.5±0.23^{fg}
21	AFG37	NG	3.3±0.05^{de}	7.2±0.05^{ef}	2.3±0.11^{m}
22	AFG39	NG	2.2±0.11^{jk}	4.8±0.17^{j}	4.6±0.11^{ef}
23	AFG48	NG	2.1±0.11^{kl}	4.9±0.11^{j}	4.4±0.17^{fg}
24	AFG49	NG	4.5±0.11^{a}	6.0±0.11^{hi}	5.0±0.17^{de}
25	AFG50	NG	2.7±0.11^{hi}	6.8±0.23^{fg}	3.2±0.11^{kl}
26	AFG52	NG	3.2±0.11^{def}	7.6±0.23^{bc}	6.1±0.11^{b}
27	AFG54	NG	3.0±0.03^{fg}	8.2±0.08^{abc}	7.2±0.11^{a}
28	AFG57	NG	3.1±0.17^{efg}	7.2±0.05^{ef}	4.1±0.11^{hi}
29	AFG62	NG	2.6±0.11^{ij}	8.3±0.17^{abc}	7.1±0.05^{a}
30	AFG64	NG	2.6±0.11^{ij}	8.7±0.11^{a}	6.4±0.05^{b}
31	AFG65	NG	3.2±0.11^{def}	8.4±0.23^{ab}	5.1±0.11^{cd}
32	AFG66	NG	3.4±0.11^{de}	8.4±0.17^{ab}	5.5±0.17^{c}
33	AFG69	NG	2.3±0.11^{jk}	5.3±0.17^{ij}	4.8±0.11^{ef}
34	AFG71	NG	2.1±0.11^{kl}	5.0±0.11^{j}	4.6±0.05^{ef}
35	AFGS15	NG	2.8±0.05^{gh}	6.6±0.17^{gh}	4.8±0.11^{ef}
36	AFGS17	NG	2.0±0.11^{klm}	4.6±0.23^{j}	4.1±0.11^{hi}
37	AFGS31	NG	2.8±0.17^{gh}	6.6±0.11^{gh}	4.0±0.05^{ij}
38	AFGS33	NG	2.0±0.05^{klm}	7.3±0.11^{de}	4.5±0.11^{fg}

Fungal growth: fast (> 6 cm), medium (4-6 cm) and slow (< 4 cm); NG-No growth. Values are the means ± SE from three separate experiments. Mean followed by the same letters in the column are not significantly different according to Tukey's HSD at $P \leq 0.05$.

Fungal growth: fast (> 6 cm), medium (4-6 cm) and slow (< 4 cm); NG-No growth. Values are the means ± SE from three

Table 3 Physiological characteristics of *Aspergillus flavus* isolates on PDA medium at different pH

Sl. No.	Isolate Code	Colony diameter (cm)			
		pH			
		Control	6	8	10
1	AFG1	7.0±0.11^{cd}	7.2±0.06^{bc}	7.7±0.11^{abc}	7.6±0.17^{abc}
2	AFG8	7.8±0.11^{a}	7.5±0.11^{abc}	7.7±0.08^{abc}	7.6±0.05^{abc}
3	AFG11	7.2±0.11^{bc}	7.1±0.11^{cd}	7.4±0.11^{bcd}	7.1±0.17^{cd}
4	AFG12	6.4±0.4^{fg}	7.4±0.17^{bc}	7.7±0.17^{abc}	7.4±0.23^{bc}
5	AFG13	6.0±0.11^{hi}	6.1±0.08^{ef}	7.1±0.05^{cde}	6.3±0.17^{fgh}
6	AFG14	7.8±0.17^{a}	7.5±0.11^{abc}	7.9±0.11^{ab}	7.6±0.11^{bc}
7	AFG15	6.2±0.05^{gh}	6.2±0.11^{ef}	6.2±0.11^{ghi}	6.0±0.11^{ghi}
8	AFG16	7.6±0.28^{ab}	7.0±0.11^{cd}	7.6±0.11^{abc}	7.2±0.05^{cd}
9	AFG18	6.6±0.17^{ef}	8.1±0.05^{a}	8.2±0.11^{a}	7.0±0.11^{cde}
10	AFG19	6.6±0.05^{ef}	7.3±0.11^{bc}	7.5±0.23^{bcd}	7.3±0.17^{bc}
11	AFG20	7.4±0.17^{ab}	7.2±0.17^{bc}	7.6±0.11^{abc}	7.2±0.05^{cd}
12	AFG24	6.6±0.15^{ef}	6.0±0.11^{ef}	6.6±0.11^{efg}	6.1±0.05^{gh}
13	AFG25	6.2±0.05^{gh}	6.2±0.11^{ef}	6.4±0.05^{fgh}	6.2±0.05^{fg}
14	AFG26	6.6±0.11^{ef}	6.5±0.05^{de}	6.8±0.23^{def}	6.1±0.05^{gh}
15	AFG27	6.6±0.17^{ef}	5.0±0.11^{i}	6.8±0.28^{def}	6.0±0.17^{ghi}
16	AFG28	6.4±0.17^{fg}	5.5±0.11^{gh}	7.2±0.11^{cde}	6.1±0.11^{gh}
17	AFG29	7.5±0.05^{ab}	7.5±0.17^{abc}	7.6±0.17^{abc}	7.5±0.23^{bc}
18	AFG30	5.7±0.11^{ij}	7.1±0.05^{cd}	7.46±0.20^{bcd}	6.0±0.11^{ghi}
19	AFG33	7.0±0.11^{cd}	7.1±0.05^{cd}	7.2±0.11^{cde}	7.1±0.11^{cd}
20	AFG35	7.4±0.23^{ab}	7.2±0.05^{bc}	7.4±0.05^{bcd}	6.6±0.11^{ef}
21	AFG37	7.2±0.17^{bc}	7.5±0.28^{abc}	7.6±0.17^{abc}	8.3±0.1^{a}
22	AFG39	7.3±0.17^{bc}	7.0±0.05^{cd}	8.0±0.11^{ab}	7.2±0.05^{cd}
23	AFG48	6.8±0.11^{de}	7.6±0.17^{abc}	7.8±0.05^{abc}	7.3±0.17^{bc}
24	AFG49	4.2±0.05^{k}	5.7±0.05^{fg}	5.76±0.18^{i}	5.6±0.05^{hi}
25	AFG50	6.4±0.05^{fg}	6.0±0.11^{ef}	6.5±0.11^{efg}	6.2±0.11^{fg}
26	AFG52	6.8±0.17^{de}	7.5±0.05^{abc}	7.7±0.11^{abc}	7.4±0.05^{bc}
27	AFG54	5.8±0.17^{hi}	6.4±0.17^{fgh}	7.4±0.17^{bcd}	7.0±0.11^{cd}
28	AFG57	7.4±0.23^{ab}	5.4±0.05^{hi}	6.0±0.17^{hi}	6.3±0.17^{fg}
29	AFG62	7.4±0.20^{ab}	7.4±0.17^{bcd}	7.8±0.11^{abc}	7.3±0.17^{bc}
30	AFG64	6.8±0.17^{de}	7.4±0.23^{bc}	7.8±0.11^{abc}	7.4±0.17^{bc}
31	AFG65	6.0±0.11^{hi}	6.0±0.17^{ef}	6.9±0.1^{ef}	5.6±0.11^{hi}
32	AFG66	7.2±0.11^{bc}	7.2±0.11^{bc}	6.2±0.05^{ghi}	6.8±0.11^{de}
33	AFG69	7.0±0.11^{cd}	7.0±0.057^{cd}	7.5±0.23^{bcd}	7.0±0.05^{cd}
34	AFG71	7.7±0.11^{ab}	7.5±0.11^{abc}	7.7±0.08^{abc}	7.4±0.17^{bc}
35	AFG15	5.5±0.11^{j}	5.9±0.11^{ef}	6.4±0.05^{fgh}	5.5±0.05^{ij}
36	AFGS17	6.0±0.11^{hi}	5.8±0.11^{fg}	6.0±0.17^{hi}	6.0±0.05^{ghi}
37	AFGS31	5.9±0.2^{8hi}	6.1±0.057^{ef}	5.8±0.11^{i}	5.0±0.11^{j}
38	AFGS33	7.3±0.17^{bc}	7.3±0.11^{bc}	7.6±0.23^{abc}	6.6±0.17^{ef}

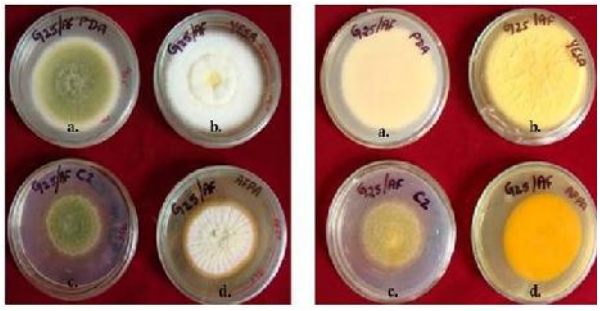


Figure 1 Morphological characteristics of *Aspergillus flavus* isolate AFG25 on a. PDA-Potato dextrose agar; b. YESA-Yeast extract sucrose agar; c. CZA-Czapek's Dox agar and d. AFPA-*Aspergillus flavus* and *parasiticus* agar.

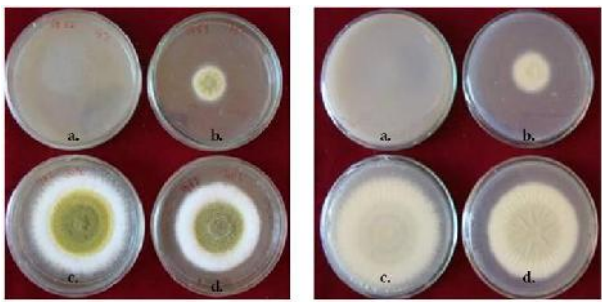
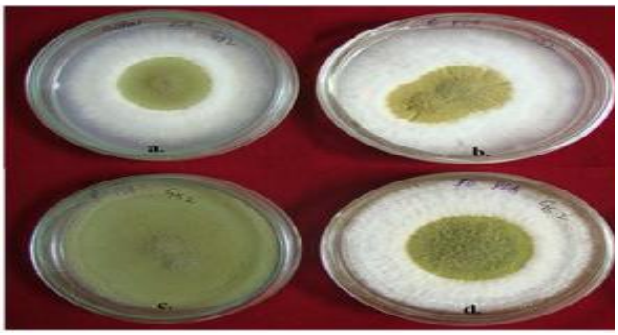


Figure 2 Growth characteristics of *Aspergillus flavus* isolate AFG62 on PDA medium at different temperature. a. 4 °C; b. 15 °C; c. 30 °C; d. 40 °C.

separate experiments. Mean followed by the same letters in the column are not significantly different according to Tukey's HSD at $P \leq 0.05$.

Table 4 Protease activity of *Aspergillus flavus* isolates

Sl. No.	Isolate code	Protease
1	AFG1	+ve
2	AFG8	+ve
3	AFG11	+ve
4	AFG12	+ve
5	AFG13	+ve
6	AFG14	+ve
7	AFG15	-ve
8	AFG16	+ve
9	AFG18	+ve
10	AFG19	+ve
11	AFG20	+ve
12	AFG24	+ve
13	AFG25	+ve
14	AFG26	+ve
15	AFG27	+ve
16	AFG28	-ve
17	AFG29	+ve
18	AFG30	+ve
19	AFG33	+ve
20	AFG35	+ve
21	AFG37	+ve
22	AFG39	+ve
23	AFG48	+ve
24	AFG49	+ve
25	AFG50	+ve
26	AFG52	+ve
27	AFG54	+ve
28	AFG57	+ve
29	AFG62	+ve
30	AFG64	+ve
31	AFG65	+ve
32	AFG66	+ve
33	AFG69	+ve
34	AFG71	+ve
35	AFGS5	+ve
36	AFG S17	+ve
37	AFG S31	+ve
38	AFGS33	+ve



Obverse view

Reverse view

Figure 3 Growth characteristics of Aspergillus flavus isolate AFG52 on PDA medium at different pH. a. control; b. pH 6; c. pH 8; d. pH 10

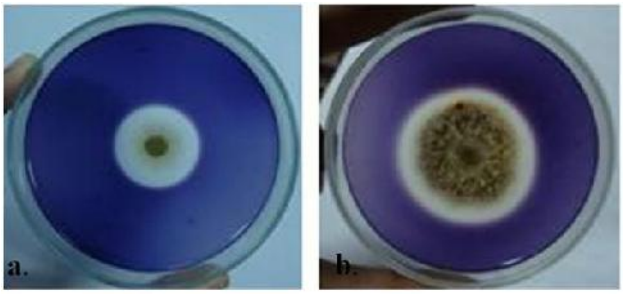


Figure 4 Screening for amylase activity of *Aspergillus flavus* isolates a. AFG16 and b. AFG49 in culture plate on starch containing medium.

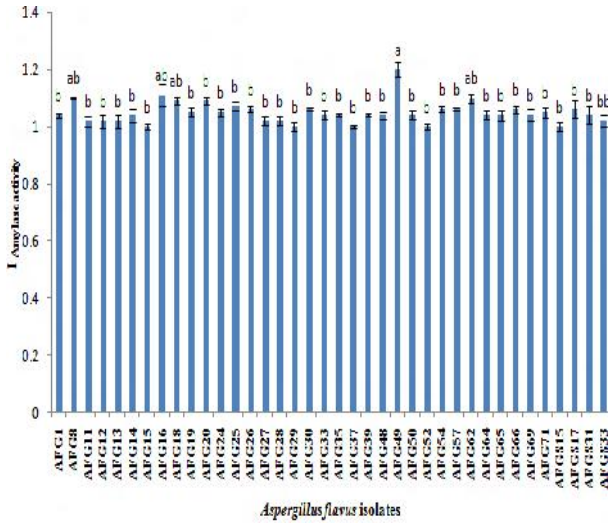


Figure 5 Index of relative amylase activity in different *Aspergillus flavus* isolates. Values represent the average of three replicates. Column followed by the same letters are not significantly different according to Tukey's HSD at $P \leq 0.05$. Vertical bars indicates standard errors of three replications

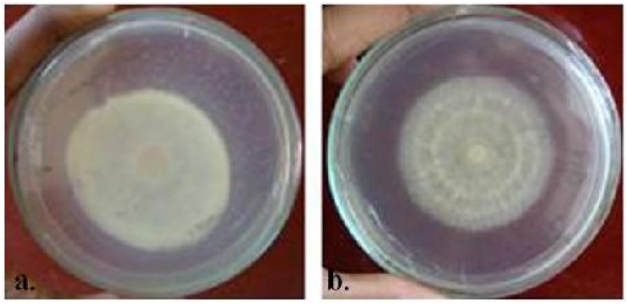


Figure 6 Screening for lipase activity of *Aspergillus flavus* isolates; a. AFG33 and b. AFG29 in Tween-20 containing medium

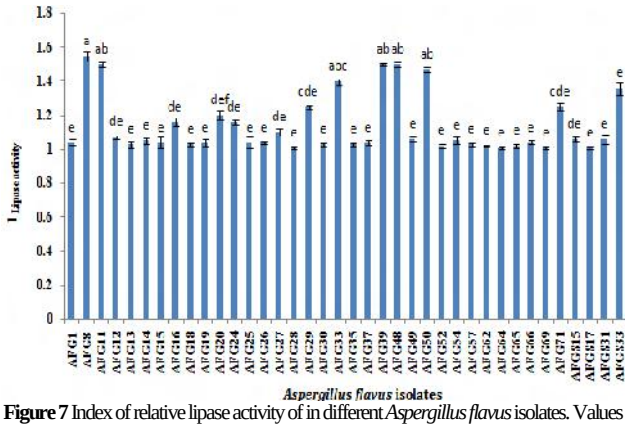


Figure 7 Index of relative lipase activity of in different *Aspergillus flavus* isolates. Values represent the average of three replicates. Column followed by the same letters are not significantly different according to Tukey's HSD at $P \leq 0.05$. Vertical bars indicates standard errors of three replications.

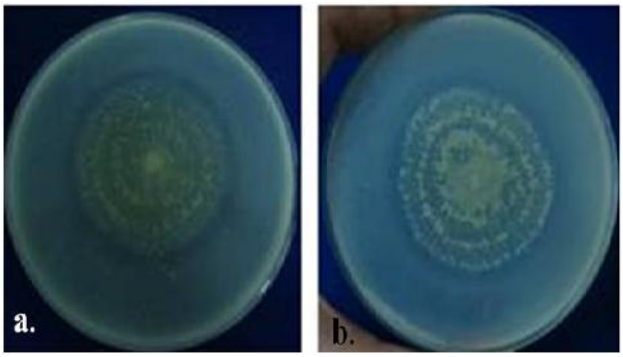


Figure 8 Screening for pectin degrading activity of *Aspergillus flavus* isolates; a. AFG28, b. AFG35 in pectin amended medium

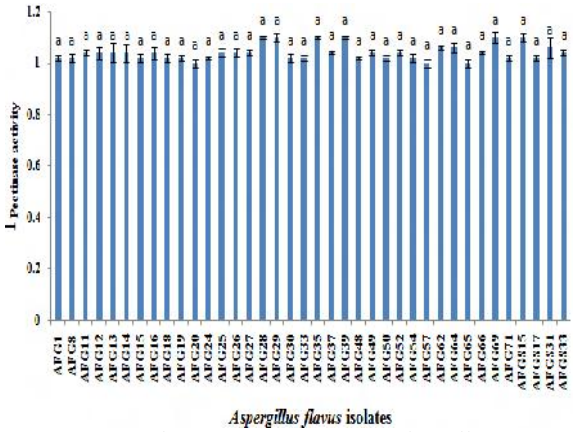


Figure 9 Index of relative pectinase activity of in different *Aspergillus flavus* isolates. Values represent the average of three replicates. Column followed by the same letters are not significantly different according to Tukey's HSD at $P \leq 0.05$. Vertical bars indicates standard errors of three replications

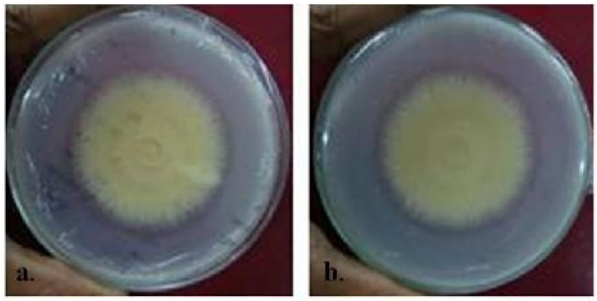


Figure 10 Screening for protease activity of *Aspergillus flavus* isolate; a. AFG11 and b. AFG27 on skimmed milk containing medium

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