



ORIGINAL ARTICLE

Lipase Activity of some Fungi Isolated from Otitis Media

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ABSTRACT

Lipase activity of four fungi (*Alternaria humicola*, *Aspergillus niveus*, *Fusarium moniliforme*, and *Bipolaris spicifera*), isolated from Otitis Media (OM) of agricultural workers and reported as new causal agents of otomycoses were explored. *Alternaria humicola* and *Fusarium moniliforme* showed highest lipase activity i.e. 30 and 44 unit mg⁻¹ protein respectively in 10 days at 30°C. However, *Bipolaris spicifera* showed highest lipase activity i.e. is 68 unit mg⁻¹ protein in 30 days at 37°C. On the other hand, lowest lipase activity (6 unit mg⁻¹ protein) was recorded in *Alternaria humicola* at 30°C. Fungal virulence in relation to lipase activity was discussed in the pathogenicity of otomycoses.

KEY WORDS: Otitis Media, Lipase activity, *Bipolaris spicifera*.

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INTRODUCTION

Otomycosis (fungal otitis externa) has been described as fungal infection of the external auditory canal with infrequent complications involving the middle ear [1]. Many species of fungi have been identified to cause of otomycosis. *Aspergillus niger* and *Candida albicans* are the most common causative agents of otomycosis [2]. *Aspergillus* is considered the predominant causal organism in tropical and subtropical regions. Wadhvani and Srivastava [3] reported twenty four fungi isolated from ear wax of otitis media (OM) of agricultural field workers, of which 18 are reported for the first time from India. Germination of some fungal spores in OM under varied relative humidity at 37°C proved themselves as most potential agent for causing otomycosis, otherwise reported fungistatic effects of OM is confirmed, as spores of most of the isolated fungi could not germinated [4]. *Alternaria humicola* and *Aspergillus niveus* are added to the literature as new etiologic agents causing otomycosis [5]. Lipases (EC 3.1.1.3) catalyze the hydrolysis of triacylglycerides to diacylglycerides, monoacylglycerides, free fatty acids, and glycerol at the oil-water interface reactions [6, 7].

The labourers working in the agricultural field, is exposed to air and soil, which harbor innumerable of many saprophytic and pathogenic fungi. They also suffer from maladies of ear, ranging from minor pains to exudation along with inflammation, especially in rainy season. *Aspergillus terreus*, *Scolopuriopsis polyacillium*, *Candida albicans* and species of *Mucor* and *Rizopus* were the main etiological agents and their origin was either from air or from soil [3, 8]. Lipase activity of *Candida albicans* and some species of *Aspergillus* were reported.

Lipase activity of four fungi viz. *Alternaria humicola*, *Aspergillus niveus*, *Fusarium moniliforme* and *Bipolaris spicifera* are assayed in the present paper.

MATERIALS AND METHODS

Isolation of fungi from otitis media (OM): OM of fifty agricultural field workers was taken out with the help of sterile Johnson's buds and were immediately placed in plugged sterilized culture tubes, separately. A suspension of OM was made in 4 ml of sterile distilled water. 1 ml of suspension was plated in each petridish containing sabouraud's glucose agar medium. For each subject four replicates were used. The Petri-dishes were incubated at 30 and 37°C. Those incubated at 30°C served as controls. After four days, fungi appearing were isolated and for slow growing forms, these were kept under observations for six weeks. Dominating species were maintained in sabourauds agar media for lipase activity.

Essay of extracellular lipase activity: The extracellular lipase activity in culture filtrates of four fungi were measured following the method of Somkyti and Babel [9] by growing them on medium consisting of

20% (w/v) suspension of dried oatmeal chaffs supplemented with 0.05% yeast extract. The dried oatmeal chaffs were obtained by flushing out the starch contents of Champions oats with distilled water and drying the remaining chaffs at 80°C for 48 hrs. Thirty ml portions of the medium were dispensed into each 250 ml. Erlenmeyer flasks and autoclaved at 15 lbs for 20 min. These flasks were inoculated with 2.0 mm discs of mycelium of each fungus of pure culture on agar medium separately for thirty days. Five replicates were used. The reaction mixture contained olive oil 1 ml, Sodium Taurochloride 0.4 g, Calcium Chloride 0.1 ml 0.1 M (pH 5.8) in 250 ml Erlenmeyer flasks. Three ml of culture filtrates was added in the reaction mixture incubated at 30 and 37°C for 2 hrs. At the end of incubation period 40 ml of absolute alcohol was added and the liberated fatty acid titrated with 0.02 N NaOH using 2 drops of Phenolphthalin as indicator (12/500 ml of 50% Ethanol). The reaction mixture with 3 ml of boiled extract served as control. The difference between the volume of alkali used for titrating an assay mixture and control was taken as amount of free fatty acids liberated during incubation.

RESULTS

Fungi, isolated from Otitis Media (OM) of agricultural field workers, along with their percent occurrence in air and soil have been reported by the author in his previous paper. Out of 24 fungi belonging to 15 genera, maximum number of species belonged to the genus *Aspergillus* (1/3 of total fungi, ascoporic species included). Four fungi, *Aspergillus ustus*, *A. niger* and *Trichoderma glaucum* recorded high percent frequency in decreasing order. Similar trend of their occurrence was observed in air and soil also. A perusal of available literature revealed that 18 fungi (marked with single asterisk) are being reported for the first time from OM of human ear. Two osmophilic fungi *Aspergillus amsteoldami* and *A. chevalieri* belonging to *A. glaucus* group were also isolated, but no correlation could be established with OM as their spore failed to germinate in water. Sabouraud's medium and OM of two workers, who were suffering from otomycosis, *Aspergillus niveus* and *Alternaria humicola* were obtained exclusively in pure cultures.

The lipase activity on the basis of fatty acids upto 30 days at 30 and 37°C for *Alternaria humicola*, *Aspergillus niveus*, *Bipolaris spicifera* and *Fusarium moniliforme* has been expressed in fig. 1a-d. Each fungus exhibited an entirely different pattern at 30°C and 37°C. *Alternaria humicola* showed an increase in lipase production upto 30 days at 37°C but it was entirely reverse at 30°C and maximum at 10th day and gradually decreased upto 30 day (fig. 1a). *Aspergillus niveus* exhibited a downward trend at 30°C which suddenly increased at 20th day at 30°C while there was a constant decrease in enzyme production at 37°C (fig. 1b). *Fusarium moniliforme* showed a slight increase upto 20th day at 30°C which declined after, but at 37°C it showed maximum lipase production initially with constant decrease upto 30th day (fig. 1c). *Bipolaris spicifera* showed gradually increasing trend at both the temperatures. The highest activity was noted at 37°C on 30 days incubation (fig. 1d).

DISCUSSION

A random sampling and direct observation of Otitis Media (OM) indicated that there is a definite mycoflora in the external auditory meatus of agriculture field workers. Source of these fungi were air, soil or organic matter in the fields. Twenty four fungal species are being reported for the first time from OM of human ear from India [3]. The fungal flora in OM is dominated with *Aspergillus* (33.3%). These results are in accordance with the findings of others [10, 11]. So far 19 species of *Aspergillus* belonging to 9 groups have been reported. Present results elevate it to 22 species belonging to 11 groups. The new additions are *Aspergillus japonicus*, *A. niveus* and *A. ustus* of *A. niger*, *A. flavipes* and *A. ustus* groups, respectively. Grouping of *Aspergillus* species is based on Raper and Fannell's classification [12]. Ninety percent cases of fungal infection of human ear due to *Aspergilli* are commonly reported from countries with humid and warm climate but Canant *et al.* [13] recognized only 15-20% infection as true cases of otomycosis. Initially, *A. fumigatus* was most frequently isolated, because of its ability to grow under environmental conditions provided by the human body. Later reports recorded the high percent of otomycosis due to *A. niger* and *A. flavus* also.

During present investigations *Aspergillus niveus* and *Alternaria humicola* were isolated from the diseased ears of two subjects separately. Presence of conidial head of *Aspergillus* and germinating spores of *Alternaria* and the capability of their spores to germinate in OM at body temperature at elevated RH are direct evidence of their active phase in OM. Pathogenicity experiments, with two fungi were not conducted on rabbits, because, of the reported failure with other fungi [14]. The fungal infections were not secondary, as no foul smell was present in the affluent of OM. The asexual spores of hyphae of these fungi occur in dormant condition in air or soil, got lodged in the external otitis meatus of the workers [15, 16]. As their dormancy was of exogenous type, germination commenced as soon they got adequate relative humidity in the external ear (during rainy season RH of atmosphere usually ranges from 85-100%) [17], this caused itching and scratching of ear canal with match stick traumatized the skin, which

resulted in inflammation and pain [18]. Plugging of ear canal with cotton after the administration of oil or greasy paint suspended the skin causing shedding of the epithelial cells, which provided pabulum for further growth of the fungus [19]. Ismail [20] was of the view that the factors that interfere with the protective mechanism of the ear are elevated temperature and humidity. Frequent washing of ears with cotton wood applicators traumatizes the skin of external canal also. Reported 17 fungistatic effect of OM has been confirmed as spores of most of the isolated fungi could not germinate in it. At 37°C (body temperature) spore of 12 fungi *Aspergillus niger*, *A. fumigates*, *A. japonicas*, *A. terreus*, *A. niveus*, *Alternaria humicola*, *Absidia corymbifera*, *Bipolaris spicifera*, *Fusarium moniliforme*, *Mucor fragilis*, *Rhizopus arrhizus* and *Trichoderma glaucum* could germinate on Sabouraud's culture medium. Though, spores of four fungi *Alternaria humicola*, *Aspergillus niveus*, *Bipolaris spicifera* and *Fusarium moniliforme* germinated on OM also, but those of *Aspergillus niveus* failed to germinate in distilled water (control). Therefore, the effect of Relative Humidity (RH) on spore germination on OM at 37°C was studied for these four fungi only. An increase in percent spore germination was observed for *Alternaria humicola*, *Bipolaris spicifera* and *Fusarium moniliforme* with increase in relative humidity but spores of *Aspergillus niveus* required 100% RH for their germination. Thus, these genera are selected for their lipase activity in relation to their virulence potential to cause otomycoses high lipase activity at 37°C means high potential to cause otomycoses.

Lipases are produced by plants, animals, bacteria and molds. Plant enzymes are not used commercially while animal, bacterial and mold enzymes are used extensively. Fungal lipases are derived mainly from *Candida* and *Aspergillus* sp. [6, 7] and *Geotrichum* sp. [21]. Typical substrates are vegetable oil, animal fat, fish oil, olive oil, butter oil (milk fat) and synthetic TAG such as triolein. Among the desirable characteristic that commercially important lipases should exhibit are, alkali tolerance and thermostability. Most lipases reported so far are active at neutral pH.

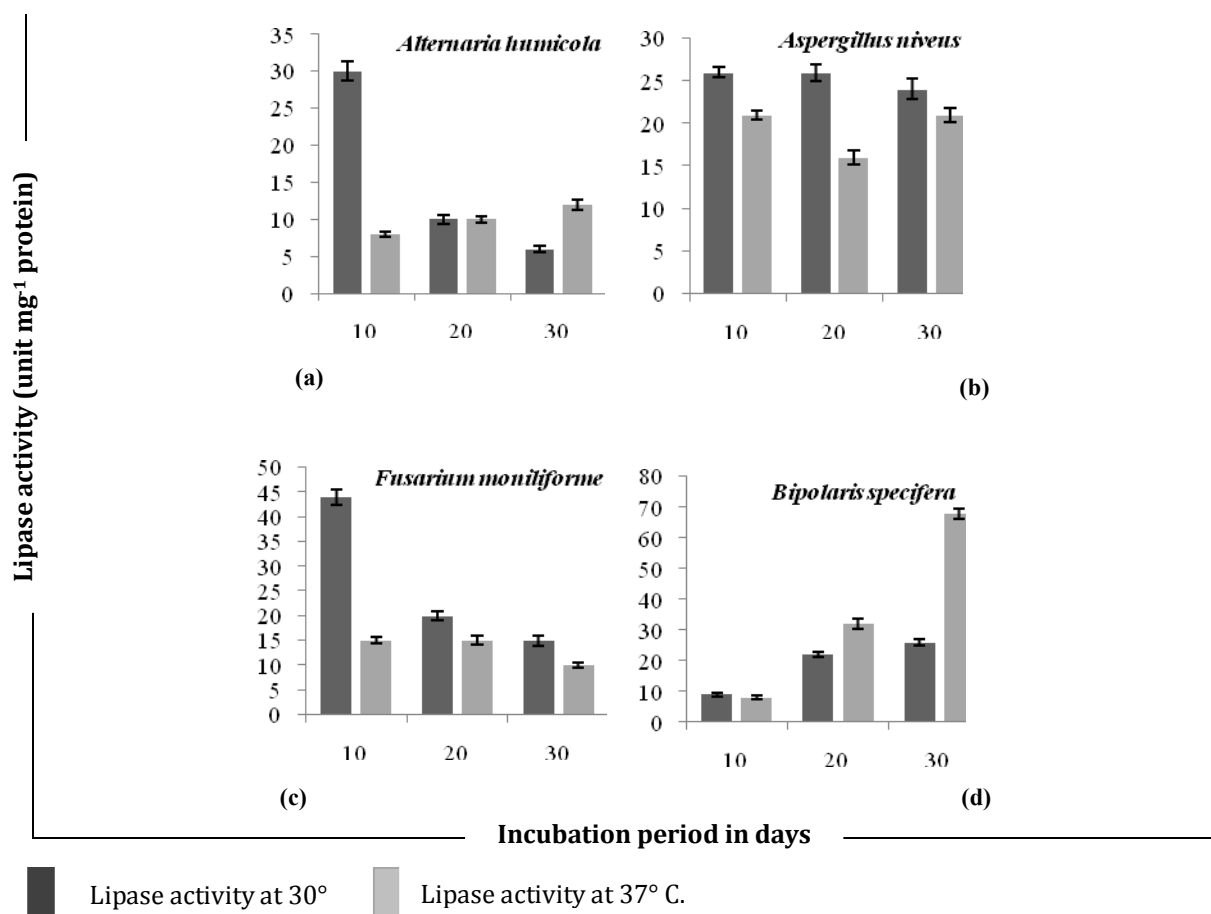


Fig. 1 Lipase activity by (a) *Alternaria humicola*, (b) *Aspergillus niveus* (c) *Fusarium moniliforme* (d) *Bipolaris specifera* at 30 and 37°C.

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