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Diversity, Pathogenicity and Toxicology of A. niger: An Important Spoilage Fungi

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ABSTRACT

Aspergillus niger, a worldwide distributed member of ascomycotina, has been isolated from numerous habitats. A. niger is one of the fungi that has been labelled with the GRAS (generally recognized as safe) status from the US Food and Drug Administration. This dull or dark black looking fungus has several important products in fermentation industry. But due to cosmopolitan nature, human beings gets frequently exposed to spores and vegetative forms of A. niger present in air, on foodstuffs and others stored consumables products and suffers with allergic problems. A. niger may also produce certain mycotoxins which are heptocarcinogenic, nephrogenic immunological in nature. In addition, this fungus is also causative agent for many rot diseases in plants. So, the present review article is an important step to understand the diversity, pathogenicity and toxicology of this important spoilage A. niger.

Key words: Aspergillus niger, distribution, industrial applications, pathogenicity and toxicology

INTRODUCTION

Aspergillus niger (black mold), a filamentous ascomycete having ability of fast growth and pH tolerance is most important cosmopolitan fungi associated with postharvest decay of different substrates (Pitt and Hocking, 1997; Perfect et al., 2009; Perrone et al., 2007). This organism is a soil saprobe with a wide array of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocelluloses. Because of their ability to produce extracellular organic acids some of them are commonly used in food industry. These features of A. niger enable them to cause decay of various organic substances including fruits, vegetables, nuts, beans, cereals, herbs, wood and herbal drugs. A. niger also plays a significant role in the global carbon cycle (Baker, 2006). Moreover, A. niger is one of the fungi that has been labelled with the GRAS (generally recognized as safe) status from the US Food and Drug Administration (Powell et al., 1994). But instead of the safe categorization, A. niger has been found to be a opportunistic reason for infections of humans. If inhaled, in sufficient quantity it can cause severe lung problems i.e., aspergillosis in humans. It is also associated with various plant diseases resulting in huge economic loss. Beside animal and plant pathogen, A. niger is also reported to produce ochratoxin A and fumonisin B2 and aflatoxins (Abraca et al., 1994; Schuster et al., 2002; Noonimsbe et al., 2009; Al-Abdalall, 2009) in stored commodities, which seems to be very inevitable. Mycotoxins produced by A. niger are not only linked to discoloration, quality deterioration, reduction in commercial values but can also cause

270
several ailments of liver, kidney, nervous system, muscles, skin, respiratory organs, digestive tract, genital organs, etc. (Muntanola, 1987; Purchase, 1974; Durakovic et al., 1989; Rai and Mehrotra, 2005; Truckeses and Scott, 2008). Therefore, the purpose of this review is to summarize the current knowledge like diversity, pathogenicity and toxicology about this important spoilage fungus.

**General characteristics:** *Aspergillus niger* is a versatile filamentous fungus found in soil, water, air, decaying plant material and large number of food and feeds all over the world (Pitt and Hocking, 1997). Raper and Fennell (1965) designated 15 species as comprising the *Aspergillus niger* group, which includes all of the aspergilli with black conidia, but now the concept of retention of the *A. niger* group based on black conidia seems dominant (Someren et al., 1990).

*Aspergillus niger* is both a species and a group within the genus *Aspergillus*. The taxonomic description is as follows:

**A. niger** (Tiegh.) Speg.:

- **Domain**: Eukaryota
- **Kingdom**: Fungi
- **Phylum**: Ascomycota
- **Subphylum**: Pezizomycotina
- **Class**: Eurotiomycetes
- **Order**: Eurotiales
- **Family**: Trichocomaceae
- **Genus**: *Aspergillus*
- **Species**: *niger*

The major difference between *A. niger* and other species of *Aspergillus* is the production of carbon black or very dark brown spores from biseriate phialides (Raper and Fennell, 1965). Vegetative growth is very rapid on culture media with submerged mycelium. The hyphae are septate and hyaline more or less yellow in color. The colonies are black coloured and reverse usually colourless (Fig. 2a, b). Conidiophores mostly arise directly from substratum and are smooth, septate or nonseptate, varying greatly in length and diameter, i.e., 200-400×7-10 and 20 μ, respectively. Conidial heads are fuscous, blackish-brown to purple-brown or in every shade to carbonous black, varying from small, almost columnar masses of a few conidial chains to the common globes or radiate heads, up to 300, 500 μ, or 1000 μ long. Vesicle globose, commonly 20-50 μ up to 100 μ in diameter. Phialides typically in two series, (biseriate), thickly covering the vesicle, primary greatly varies in length, secondary 6-10×2-3 μ (Fig. 1). Conidia are globose, at first smooth, but later spinose with coloring substance, mostly 2.5-4 μ (Gilman, 2001).

The genome size of *A. niger* is about 35.5 to 38.5 Mb composed of about 13,000 genes. Of these genes, about 8000 to 8500 genes have functional assignments. In addition, about 14,000 Open Reading Frames (ORF) were identified in the genome which could potentially encode a protein. The DNA sequence of *A. niger* consists of approximately 33.9 million base pairs. The possible function of 6500 genes could be established which is only about 45% of its total gene count. Electrophoretic karyotyping of *A. niger* allows the visualization of chromosomes separated into four separate bands. The chromosomal bands range from 3.5 to 6.6 Mb. The karyotype sequence that was obtained could be arranged into 19 separate supercontigs that correspond to eight linear chromosomes (Debets et al., 1990).
Diversity and geographical distribution: *Aspergillus niger* have the ability to grow in wide temperature (6-47°C) and pH range (1.4-9.8). The water activity limit for growth is 0.88, which is relatively high compared with other *Aspergillus* species. These abilities make ubiquitous occurrence of the species, with a higher frequency in warm and humid places (Palacios-Cabrera *et al*., 2005). It can be found anywhere in and around of us (Kozakiewicz, 1989; Abarca *et al*., 2004; Samson *et al*., 2004). Although the main source of black aspergilli is soil (Khan *et al*., 2007), it has also been isolated from various other sources like, air (Versar, 1991), food and food products (Agrawal *et al*., 1980; Bennett and Klei, 1992; Mandeel, 2005; Essono *et al*., 2007; Perrone *et al*., 2007; Reddy *et al*., 2009), herbs and herbal products (Gautam and Bhadauria, 2008; Gautam *et al*., 2009; Sareen *et al*., 2010; Gautam *et al*., 2010, Avesthi *et al*., 2010), fruits and fruits products (Magnoli *et al*., 2003), etc. Not even as saprophytic fungi, *A. niger* is also isolated as parasitic/pathogenic fungi from onion (Narayana *et al*., 2007), *Catharanthus rosea* as endophytic fungi (Kharwar *et al*., 2008) and from various other medicinally/commercially important plants (Table 1).

Ecology: *A. niger* is commonly isolated from soil, plant debris, air and indoor environments. In addition to producing extracellular enzymes and citric acid, *A. niger* is used for organic waste
Table 1: Diversity of *Aspergillus niger* on different substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>Versar (1991)</td>
</tr>
<tr>
<td>Water</td>
<td>Versar (1991)</td>
</tr>
</tbody>
</table>

management and biotransformation. The fungi is most commonly found in mesophilic environments such as decaying vegetation or soil and plants (Schuster *et al.*, 2002). *A. niger* is one of the fungi that have been labeled with the GRAS (generally recognized as safe) status according to the US Food and Drug Administration (Schuster *et al.*, 2002). The safe use of *A. niger* comes into existence from its use in the food industry for the production of many enzymes and acid proteases (Bennett, 1985; Ward, 1989). The annual production of citric acid by fermentation is now approximately 350,000 tons, using either *A. niger* or *Candida* yeast as the producing organisms. Citric acid fermentation using *A. niger* is carried out commercially in both surface culture and in submerged processes (Berry *et al.*, 1977; Ward, 1989).

By making use of industrial fermentation, *A. niger* produces many useful enzymes like amylase, amylglucosidase, cellulases, glucoamylase, lactase, invertase, pectinases, etc. Glucoamylase is a useful enzyme used in the production of high fructose corn syrup and pectinases are used in cider and wine clarification. α-galactosidase, an enzyme that breaks down certain complex sugars, is a component of Beano and other medications which the manufacturers claim can decrease flatulence. Another use for *A. niger* within the biotechnology industry is in the production of magnetic isotope-containing variants of biological macromolecules for NMR analysis (Staiano *et al.*, 2005).

Besides the production of useful enzymes, various strains of *A. niger* used in the industrial preparation of citric acid and gluconic acid, which have been assessed as acceptable for daily intake by the World Health Organisation (WHO) and Food and Drug Administration (FDA) (Schuster *et al.*, 2002). It is primarily used for the production of many enzymes such as amylase, amylglucosidase, cellulases, lactase, invertase, pectinases and acid proteases (Bennett, 1985; Ward, 1989). In addition to production of enzymes and acids through fermentation, *A. niger* has some uses as the organism itself. Due to its ease of visualization and resistance to several antifungal agents, is used to test the efficacy of preservative treatments (Jong and Ganitt, 1987). Due to exquisitely sensitiveness to micronutrient deficiencies, *A. niger* can be utilized for soil testing (Raper and Fennell, 1965). Besides, RNase produced by *A. niger* called actinase has antiangiogenic and anticarcinogenic characteristics (Schwartz *et al.*, 2007).

Other properties of this species include spoilage and production of secondary metabolites, such as aflatoxins, fumonisins and ochratoxins (Abraca *et al.*, 1994; Noonimabc *et al.*, 2009; Edwin *et al.*, 2010) that are toxic. The mycotoxin fumonisins B2 was recently found to be produced by *A. niger* (Noonimabc *et al.*, 2009). Metabolite production, involvement in spoilage of food and other commodities, simply being a pathogen makes the genome sequencing of this important fungus essential to biological applications (Takahashi *et al.*, 1991; May and Adams, 1997).
Pathogenicity and toxicology: *A. niger* is relatively harmless as compared to other filamentous fungi. Despite this fact, there have been some medical cases that have been accounted for, such as lung infections or ear infections in patients that have weakened immune system or an immune system that has been impaired by a disease or medical treatment (Schuster et al., 2002; May and Adams, 1997). Besides human pathogenicity, *A. niger* can cause various plant diseases also.

*Aspergillus niger* as plant pathogenic fungi: *A. niger* has been isolated from a variety of substrates but, these reports involve co-isolation with other perhaps more destructive microorganism or isolation from a stored product. The organism is considered as a strict saprophyte (Farr et al., 1989). There are reports of *A. niger* being as plant pathogen (Fig. 3a, b, Table 2). This fungus can cause rotting of numerous fruits, vegetables and other food products, thus causing substantial economic loss. There are many examples of plant diseases caused by *A. niger*. Black rot of onions associated with *A. niger* is responsible for serious losses of onion bulbs in the field and storage (Narayana et al., 2007). Other plant pathogenic reports of *A. niger* are, spoilage of mangos (Prakash and Raoof, 1989), grapes (Sharma and Vir, 1986), Tomatos (Sinha and Saxena, 1987), Shallot; stem rot of *Dracaena* (Abbasi and Aliabadi, 2008); root stalk rot of Sansevieria; and boll rot of Cotton; spoilage of cashew kernels, dates, figs, vanilla pods and dried prune (Bobberala et al., 2000). *A. niger* can induce a crown rot of peanuts due to *A. niger*-infected seed under specific hot, humid growth conditions (Anderegg et al., 1976). Kharwar et al. (2008) isolated *A. niger* from *Catharanthes rosea* as an endophytic fungi which can alter its metabolite production.

<table>
<thead>
<tr>
<th>Name of disease</th>
<th>Host</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black rot of onions</td>
<td><em>Allium cepa</em> L. (Onion)</td>
<td>Narayana et al. (2007)</td>
</tr>
<tr>
<td>Crown rot of peanuts</td>
<td><em>Pisum Sativum</em> L. (Peanut)</td>
<td>Anderegg et al. (1976)</td>
</tr>
<tr>
<td>Tuber rot of yam</td>
<td><em>Dioscorea</em> sp. (Yam)</td>
<td>Awuah and Akrasi (2007)</td>
</tr>
<tr>
<td>Stem rot of <em>Dracaena</em></td>
<td><em>Dracaena sanderiana</em> Mast.</td>
<td>Abbasi and Aliabadi (2008)</td>
</tr>
<tr>
<td>Black mold rot of cherry</td>
<td><em>Prunus avium</em> L. (Cherry)</td>
<td>Lewis et al. (1953)</td>
</tr>
<tr>
<td>Kernel rot of maize</td>
<td><em>Zea mays</em> L. (Corn)</td>
<td>Faleza et al. (2010)</td>
</tr>
<tr>
<td>Fruit rot of grapes</td>
<td><em>Vitis</em> sp. (Grape)</td>
<td>Sharma and Vir (1986)</td>
</tr>
<tr>
<td>Fruit rot of banana</td>
<td><em>Musa</em> sp. (Banana)</td>
<td>Adesosin et al. (2006)</td>
</tr>
<tr>
<td>Rot of Tomatoes</td>
<td><em>Solanum lycopersicum</em> L. (Tomato)</td>
<td>Sinha and Saxena (1987)</td>
</tr>
</tbody>
</table>

Fig. 3: Infection of *A. niger* in plants: (a) Black rot of onion and (b) Stem rot of *Dracaena sanderiana* (Abbasi and Aliabadi, 2008)
Aspergillus niger as a human pathogen: Aspergillus niger is believed to be most common storage fungi posing serious threat to contamination of stored commodities in tropical warm regions of the world. Food and herbal drug industries are very much suffering from A. niger and its mycotoxin contamination. It is studied that less than 10% of the A. niger strains were tested positive for ochratoxin A and fumonisins under conditions that were favorable (Schuster et al., 2002). Livings beings including humans, when contacted with A. niger and mycotoxins (ochratoxin A and fumonisins) usually through consumption may cause many negative effects, i.e., immunotoxicity, carcinogenicity and hepatotoxicity. The effects on animals include decrease in antibody responses, size reduction in immune organs and an alteration in the production of cytokine which are proteins and peptides specifically used in signaling. Poultry feed if contaminated by A. niger has major affect on the poultry industry. Different animals, such as chicken, turkey and ducks, are very prone to ochratoxin (Schuster et al., 2002; May and Adams, 1997).

Aspergillus niger is commonly regarded as a pathogenic allergen generally associated with lung infections in individuals with weak immune system. Because the conidia and conidiophores are small, readily air borne, can easily breathed in and cause deep or systemic mycosis (Kierownik, 1990) (Table 3). Ear is the location of A. niger infection (Fig. 4). Local lesions in both external and middle ear, as well as in post operative cavities, can create favourable conditions for fungal growth and subsequent otomysis (Kaur et al., 2000; Kurnatowski and Kilipak, 2001). A. niger can produce a secondary metabolites include oxalic acids, kojic acids abundantly and cyclic pentapeptides having moderate to high acute toxicity (Ueno and Ueno, 1978). Oxalate crystals of oxalic acids produced by A. niger can cause pulmonary oxalosis (Nakagawa et al., 1999) (Fig. 5).

Other risks: Apart from the human and plant pathogenic effects, there are so many risks/ problems being associated with A. niger. One of the most important one is its ability to grow on a

<table>
<thead>
<tr>
<th>Name of disease</th>
<th>Target organ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak immune system</td>
<td>Any organ of the body</td>
<td>Louthrenoo et al. (1990)</td>
</tr>
<tr>
<td>Systemic mycosis</td>
<td>External body part</td>
<td>Louthrenoo et al. (1990)</td>
</tr>
<tr>
<td>Ear infection</td>
<td>External auditory system</td>
<td>Padhye (1982), Walsh and Pizzo (1988)</td>
</tr>
<tr>
<td>Aspergillosis</td>
<td>Lungs</td>
<td>Ueno and Ueno (1978), Bennett (1979), Richard et al. (2008)</td>
</tr>
<tr>
<td>Asthma and allergic alveolitis</td>
<td>Respiratory tract</td>
<td>Edwards and AlZubaidy (1977)</td>
</tr>
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</table>

Fig. 4: Ear infection by A. niger in external auditory
variety of substrates, causing deterioration of materials. This spoilage or deterioration not only reduce the quality of the substrate but also alter its active components and commercial value. For example, \textit{A. niger} causes economic losses due to spoilage of bakery products, fruit, herbal drugs and vegetables. \textit{A. niger} also damages surface layers of wood, raw cotton fibers and many other materials. However, because \textit{A. niger} is already ubiquitous, the increased environmental burden of \textit{A. niger} due to release from commercial facilities is probably negligible. Thus, the baseline risk of materials damage by \textit{A. niger} will not be affected by the use of \textit{A. niger} in commercial facilities.

CONCLUSION

\textit{Aspergillus niger} is found anywhere in and around of us. In industrial fermentation, \textit{A. niger} produces many useful enzymes like amylase, amylglucosidase, cellulases, glucoamylase, lactase, invertase, pectinases. This enhances the importance of fungi in food and drug industries. In addition to beneficial aspects, human beings get frequently exposed to \textit{A. niger} spores and vegetative forms present in air and on foodstuffs and suffers with allergic problems whereas, specific strains may produce mycotoxins, elicit allergy and carcinogenic responses to lungs, kidney and liver. Although, limited instances of adverse effects seems to be associated with a limited number of strains of \textit{A. niger}. With proper characterization of different strains, industrial exploration of this important fungus can be increased and potential adverse effects can be avoided.

REFERENCES


