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Research Article

The Identification and Microbial Characterization of Aspergillus Species Isolated from Soil Samples

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Abstract

Aspergillus species are common soil fungi, important both as decomposers and as opportunistic pathogens and mycotoxin producers. In this work, soil samples from Bhopal were used to isolate filamentous fungi, focus on Aspergillus. Fungi were isolated on Potato Dextrose Agar (PDA) using serial dilutions, purified and examined by colony and microscopic morphology (Lactophenol Cotton Blue staining). Selected isolates were grown in Potato Dextrose Broth, genomic DNA was extracted by a phenol–chloroform–isoamyl alcohol (PCI) method, and the ITS region of rDNA was amplified by PCR and sequenced. The sequences showed 99–100% identity with Aspergillusnigerfumigatus in GenBank. The study shows a simple but reliable workflow for isolation and molecular identification of Aspergillus from soil, and confirms two medically and industrially important species in local soils.

Keywords:
Aspergillus Niger, Aspergillus Fumigatus, Soil Fungi, ITS, PCI Extraction, LPCB

1. Introduction

Fungi are eukaryotic microorganisms you find everywhere soil, water, plants and even in the air. In soil, filamentous fungi do most of the breaking down work and also include many plant and animal pathogens (Hibbett,2026). Here we concentrate on Aspergillusniger, a very common soil saprophyte that seems to grow just about anywhere and often shows up when you culture soil samples. A. niger can tolerate wide ranges of temperature, pH and moisture, makes huge numbers of airborne conidia, and easily colonizes plant debris, stored crops and house dust so people and animals get exposed to it all the time (Horn &Dorner, 1998; Kwon-Chung &Sugui, 2013).

Many strains of A. niger are actually useful; they're used industrially to produce enzymes (glucoamylase, pectinases, phytases) and organic acids like citric acid, which makes the species economically important (Frisvad& Larsen, 2016). Still, some isolates can cause problems ear infections, skin infections, and rarely invasive disease in people with weak immunity and they can make

secondary metabolites that might affect health. So its presence in the environment is both a boon and a worry (Kwon-Chung &Sugui, 2013; Frisvad& Larsen, 2016).

Identifying Aspergillus by how it looks on plates is handy but not always reliable, because many species look very similar (Balajee et al., 2007). A. niger typically shows rapid growth, dark black conidial heads, rough spores and biseriatephialides on globose vesicles but those traits can overlap with other "black aspergilli." That's why molecular methods are recommended: PCR and sequencing of the ITS region of rDNA give a much clearer picture and help tell A. niger apart from close relatives and cryptic species (White et al., 1990; Melchers et al., 1994). In practice, combining culture, careful morphology and ITS sequencing gives the best confidence that an isolate really is A. niger (Balajee et al., 2007; Peterson, 2008).

Soil samples (about 5–10 g) were collected from 5–10 cm depth at several sites around Bhopal using sterile spatulas, put into sterilized cotton-plugged tubes, labeled and brought to the lab. For each sample 1 g soil was mixed into 9 mL sterile distilled water and vortexed, then ten-fold serial dilutions were made up to 10^{-5} (Ben-David & Davidson, 2014). From the 10^{-5} dilution 0.1 mL was spread onto Potato Dextrose Agar (PDA) plates. PDA was prepared from potato extract, dextrose (20 g/L) and agar (15–20 g/L), adjusted to pH 5.6 ± 0.2 and autoclaved at 121 °C for 15–20 min (Sharma &Pandey, 2010). Plates were incubated at 25–30 °C for 3–5 days and colonies with Aspergillus-like appearance (rapid growth, powdery surface, pigmented spores) were picked and sub-cultured repeatedly on fresh PDA to obtain pure cultures. A little aerial mycelium from pure colonies was mounted in Lactophenol Cotton Blue (LPCB) and observed at 40× and 100× to note septate hyphae, conidiophores, vesicles, phialides and spore arrangements (Leck, 1999).

Pure isolates were also grown in Potato Dextrose Broth (PDB; potato extract + 20 g/L dextrose) for 5–7 days at 25–30 °C to produce sufficient mycelial biomass (Zhao &Shamoun, 2005). Mycelia were harvested, washed, ground with sand in lysis buffer and genomic DNA extracted by a PCI method using SDS and Proteinase K, followed by phenol–chloroform–isoamyl alcohol extraction, isopropanol precipitation, 70% ethanol wash and resuspension in nuclease-free water (Rohland&Hofreiter, 2007; Sambrook& Russell, 2001). DNA quality was checked on 0.8–1% agarose gels. The ITS region was amplified using ITS1/ITS4 primers (White et al., 1990) in 20 µL reactions (2× buffer, dNTPs, 0.5 µM primers, Taq, ~1 µL template). Cycling: 95 °C 3 min; 30–35 cycles of 95 °C 30 s, 52–58 °C 30 s, 72 °C 45–60 s; final extension 72 °C 5–10 min (Lorenz, 2012). Clear ITS bands were gel-purified and Sanger sequenced; sequences trimmed and BLASTed against NCBI GenBank for species ID (Balajee et al., 2007; Peterson, 2008).

3. Results and discussion

Soil samples yielded numerous filamentous colonies on PDA; after purification several isolates displayed the classic black, powdery colonies

typical of Aspergillusniger. Colonies were rapid-growing, with dense conidial layers and dark brown to black reverse pigmentation. LPCB mounts revealed septate, hyaline hyphae and stout conidiophores terminating in large globose vesicles densely covered by biseriatephialides; conidia were rough-walled and produced radiate heads—morphology matching descriptions for A. niger (Afzal et al., 2013). Because these macroscopic and microscopic traits were consistent across isolates from different sampling sites, we focused subsequent molecular work on representative cultures.Genomic DNA extracted by a phenol–chloroform–isoamyl (PCI) protocol produced high-molecular-weight bands with minimal degradation, indicating good template integrity for PCR (Rohland&Hofreiter, 2007; Sambrook& Russell, 2001). ITS amplification with ITS1/ITS4 primers yielded single, crisp products of ~500–600 bp; Sanger sequencing and BLASTn searches returned top hits to Aspergillusniger with 99–100% identity, confirming the morphological identification and demonstrating concordance between phenotype and genotype (White et al., 1990; Melchers et al., 1994).

The frequent recovery of A. niger from the study soils is in line with numerous surveys that report this species as a common culturable component of agricultural and disturbed soils (Horn &Dorner, 1998; Hussein &Yousef, 2011). Ecologically, A. niger's success likely reflects several adaptive traits: rapid vegetative growth, prolific conidiation that aids dispersal, tolerance of wide temperature, pH and water activity ranges, and a versatile enzymatic repertoire for degrading complex plant polymers. These features enable occupation of decomposing plant material, stored produce and anthropogenic wastes, positioning A. niger as an important agent in soil nutrient cycling and organic matter turnover.The presence of A. niger has both beneficial and cautionary implications. On the positive side, many strains are industrially exploited for glucoamylases, pectinases and citric acid production, and native isolates may harbour useful enzymatic activities worthy of screening for local biotechnological applications (Jahangeer et al., 2005; Frisvad& Larsen, 2016). Several studies also indicate potential roles in biodegradation of pollutants and plastics, suggesting possibilities for bioremediation trials using indigenous strains (Yang et al., 2011).

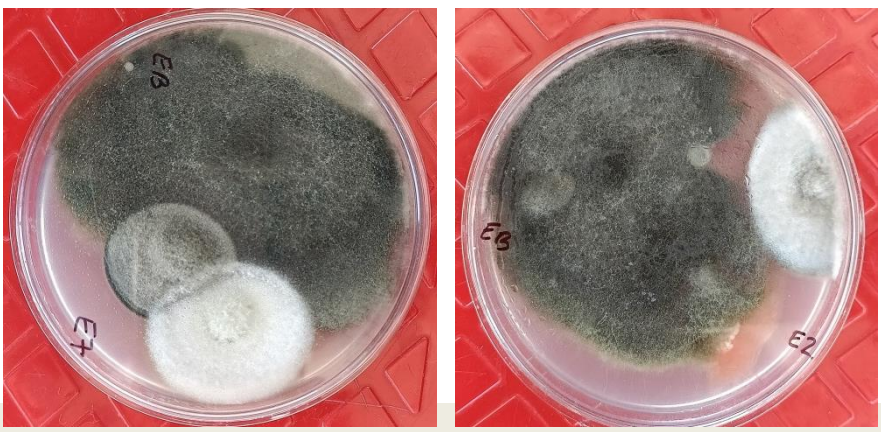

Figure 1.Mixed culture of Aspergillusniger on PDA

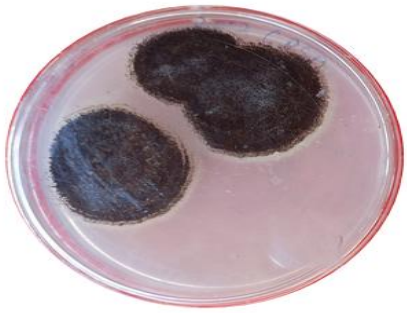

Figure 2. Pure culture of Aspergillus niger on PDA

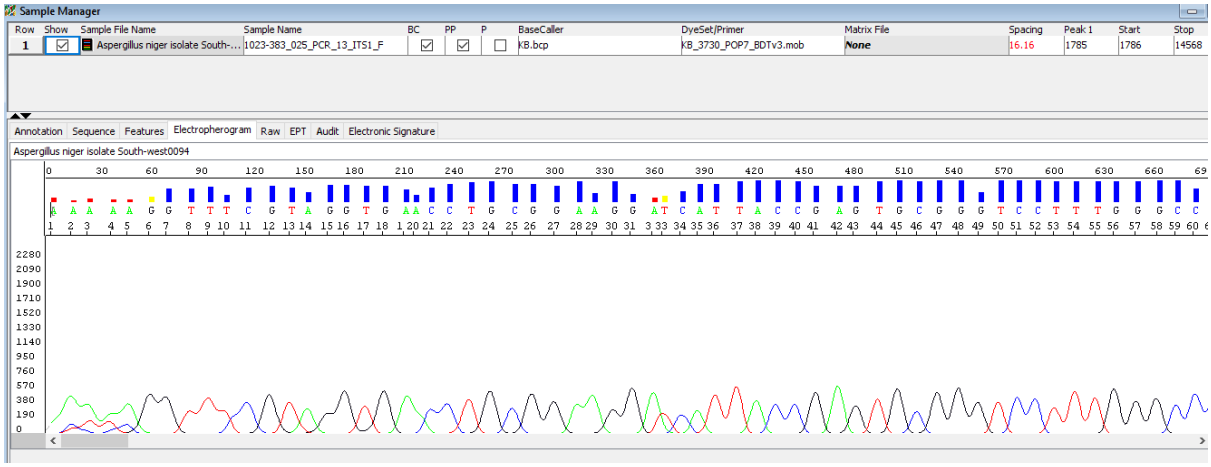

Figure 3. Figure representing Sanger sequencing chromatogram for Aspergillusniger isolate

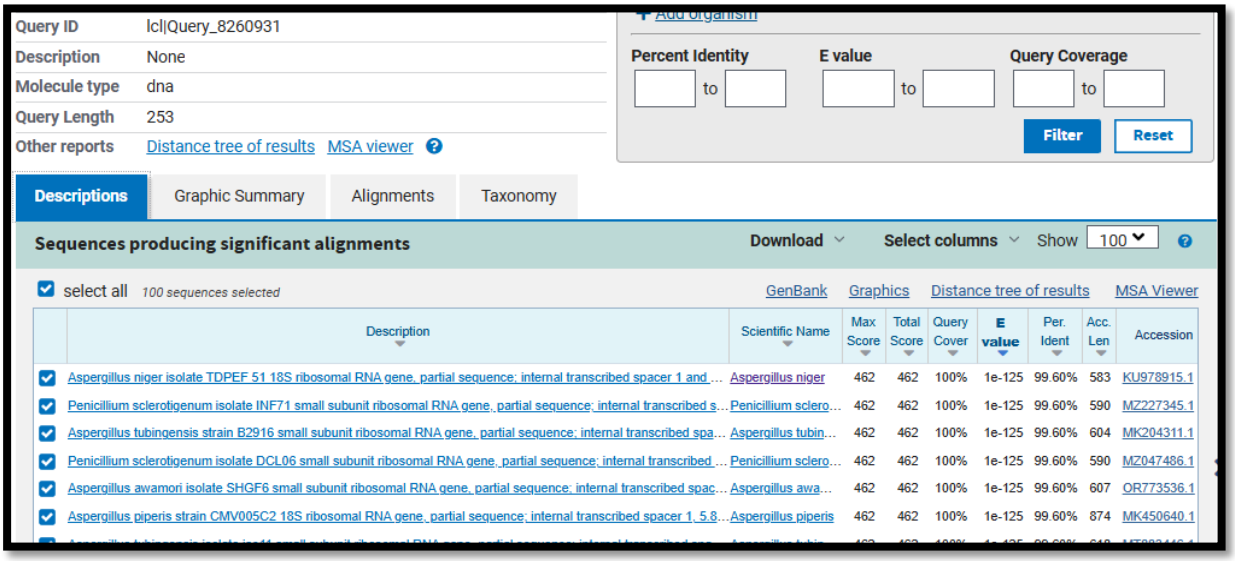

Figure 4: NCBI GenBank blast results confirmed A. niger species.

Conversely, some A. niger strains can produce secondary metabolites with health relevance and act as opportunistic pathogens causing otomycosis or invasive infections in immunocompromised persons (Kwon Chung &Sugui, 2013). Environmental abundance therefore represents an exposure route via dust and aerosols, underscoring the need for monitoring in settings with vulnerable populations.Methodologically, combining classical morphology with ITS sequencing proved reliable for routine species identification; however, morphology alone can be misleading in closely related black aspergilli, so molecular confirmation remains important (Balajee et al., 2007; Peterson, 2008). Study limitations include culture bias (many soil fungi are unculturable on PDA), modest sample size and lack of functional assays (mycotoxin profiling, enzyme activity or virulence markers). Future work should expand sampling, apply culture-independent community profiling and assess functional traits of isolates to fully evaluate ecological roles and applied potential of local A. niger strains.

Conclusion

Soil samples from Bhopal yielded filamentous fungi, including colonies with clear Aspergillusniger was consistently isolated and confirmed from local soils by morphology and ITS sequencing, showing it is a common culturable fungus in the study area. The isolates displayed typical A. niger features and yielded high quality DNA suitable for molecular work. While this species holds industrial and bioremediation potential, its environmental abundance also raises minor health concerns for vulnerable individuals. Further studies should expand sampling, include culture independent surveys and assess toxin/enzyme profiles of the isolates.

Reference

Hawksworth, D. L. (1991). The fungal dimension of biodiversity: Magnitude, significance, and conservation. Mycological Research, 95(6), 641–655. https://doi.org/10.1016/S0953-7562(09)80305-0

Horn, B. W., &Dorner, J. W. (1998). Soil populations of Aspergillus species from section Flavi along a transect through peanut-growing regions of the United States. Mycologia, 90(5), 767–776. https://doi.org/10.1080/00275514.1998.12026985

Kwon Chung, K. J., &Sugui, J. A. (2013).Aspergillusfumigatus—what makes the species a ubiquitous human fungal pathogen? PLoS Pathogens, 9(12), e1003743. https://doi.org/10.1371/journal.ppat.1003743

Frisvad, J. C., & Larsen, T. O. (2016).Extrolites of Aspergillusfumigatus and other pathogenic species in Aspergillus section Fumigati. Frontiers in Microbiology, 6, 1485. https://doi.org/10.3389/fmicb.2015.01485

Balajee, S. A., Houbraken, J., Verweij, P. E., Hong, S. B., Yaguchi, T., Varga, J., & Samson, R. A. (2007). Aspergillus species identification in the clinical setting. Studies in Mycology, 59, 39–46. https://doi.org/10.3114/sim.2007.59.06

White, T. J., Bruns, T., Lee, S., & Taylor, J. W. (1990).Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In M. A. Innis, D. H. Gelfand, J. J. Sninsky, & T. J. White (Eds.), PCR Protocols: A Guide to Methods and Applications (pp. 315–322). Academic Press.

Melchers, W. J., Verweij, P. E., Van den Hurk, P., Van Belkum, A., De Pauw, B. E., Hoogkamp-Korstanje, J. A., &Meis, J. F. (1994). General primer-mediated PCR for detection of Aspergillus species. Journal of Clinical Microbiology, 32(7), 1710–1717. https://doi.org/10.1128/JCM.32.7.1710-1717.1994

Peterson, S. W. (2008). Phylogenetic analysis of Aspergillus species using DNA sequences from four loci. Mycologia, 100(2), 205–226. https://doi.org/10.3852/07-188

Ben-David, A., & Davidson, C. E. (2014).Estimation method for serial dilution experiments. Journal of Microbiological Methods, 107, 214–221. https://doi.org/10.1016/j.mimet.2014.08.009

Sharma, G. P. R. R., &Pandey, R. R. (2010). Influence of culture media on growth, colony character and sporulation of fungi isolated from decaying vegetable wastes. Journal of Yeast and Fungal Research, 1(8), 157–164.

Leck, A. (1999). Preparation of lactophenol cotton blue slide mounts. Community Eye Health, 12(30), 24.

Zhao, S., &Shamoun, S. F. (2005).Effects of potato dextrose broth and gelatin on germination and efficacy of Phomaexigua, a potential biocontrol agent for salal (Gaultheria shallon). Canadian Journal of Plant Pathology, 27(2), 234–244. https://doi.org/10.1080/07060660509507205

Rohland, N., &Hofreiter, M. (2007).Comparison and optimization of ancient DNA extraction.BioTechniques, 42(3), 343–352. https://doi.org/10.2144/000112383

Sambrook, J., & Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor Laboratory Press.

Lorenz, T. C. (2012). Polymerase chain reaction: Basic protocol plus troubleshooting and optimization strategies. Journal of Visualized Experiments (JoVE), (63), e3998. https://doi.org/10.3791/3998

Afzal, H., Shazad, S., Qamar, S., &Nisa, U. (2013). Morphological identification of Aspergillus species from the soil of Larkana District (Sindh, Pakistan). Asian Journal of Agriculture and Biology, 1(3), 105–117.

Jahangeer, S., Khan, N., Jahangeer, S., Sohail, M., Shahzad, S., Ahmad, A., & Khan, S. A. (2005). Screening and characterization of fungal cellulases isolated from the native environmental source. Pakistan Journal of Botany, 37(3), 739–744.

Yang, Y. S., Zhou, J. T., Lu, H., Yuan, Y. L., & Zhao, L. H. (2011). Isolation and characterization of a fungus Aspergillus sp. strain F-3 capable of degrading alkali lignin. Biodegradation, 22(6), 1017–1027. https://doi.org/10.1007/s10532-011-9458-9