J.Mycopathol.Res. 62(3) : 489-493, 2024, ISSN : 0971-3719 (Print), 2583-6315 (Online) 10.57023/JMycR.62.3.2024.489

Review

Filamentous Fungi : Pathobiology to Biotechnology

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Received : 23.07.2024 Accepted : 14.08.2024 Published : 30.09.2024

Filamentous fungi are a diverse group of economically important fungi mostly belonging to Ascomycota and Basidiomycota. These have both positive as well as detrimental economic impact. Majority of common crop diseases are caused by filamentous fungi. The outcome of disease progression is dependent on the early events of infection. Therefore studying the behaviour of the fungus and its interaction with the host during the initial phases of infection is important for studying plant diseases and means to control the disease progression. Similarly the host defense response to the filamentous fungus, specially comparative studies done in tolerant versus susceptible host background, give us an insight into disease tolerance. On the counter side, these filamentous fungi can be utilized for economic gains including green synthesis of noble metal nanoparticles. These noble metal nanoparticles can be used as antimicrobial agents along with other therapeutic applications. Filamentous fungi have the advantage of being used in fermenters for production of nanoparticles. With standardized methods of synthesis, protein-capped nanoparticles can be produced which have diverse fields of application. These nanoparticles not only have antimicrobial properties but also can be used for gene/drug delivery into cancer cells.

Keywords : Antimicrobial, cancer cell, crop protection, Filamentous Fungi, fungal diseases, gene delivery, nanobiotechnology, nanoparticles, transgenics

INTRODUCTION

Filamentous fungi include a diverse group of fungi having great economic importance, having both beneficial and detrimental facets. The Filamentous fungi include harmful candidates like phytopathogens to useful ones like edible mushrooms. Therefore this heterogeneous group of fungi attracts research interest from diverse fields of science. This review will discuss the different aspects of filamentous fungi from pathobiology to biotechnology.

Filamentous fungi and crop diseases

Fungi are responsible for about 42 % of plant diseases, compared to 37% bacterial, 18% viral diseases and 13% diseases caused by nematodes (Arun and Sreena, 2022). Out of these fungal diseases a great majority are filamentous fungi, specially those belonging to the two major divisions, Ascomycota and Basidiomycota.

The phytopathogenic filamentous fungi can be of three types based on their mode of nutrition; the biotrophic, hemi-biotrophic and necrotrophic (Chowdhury *et al*. 2017a). This nature of the fungi influence the host pathogen interaction during the infection and establishment phases. During the early period after initial infection, the pathogen adopts different means to evade the host defense system and gain access to the host tissue (Chowdhury *et al.* 2009). On the counter side plants adopt timely activation of suitable phytohormone signaling depending on the pathogen's lifestyle so as to restrict the infection process (Chowdhury *et al*. 2017a). The early host pathogen interaction dictates these R- genes, the phytohormones pathways and their cross-talk; therefore study of early time points is critical (Chowdhury *et al*. 2009, Mondal *et al*. 2017).

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25th Prof.S.N.Banerjee Memorial Award Lecture of IMS, Kolkata, delivered on 23rd February, 2024, at Department of Botany, University of Calcutta

Phytohormone-mediated defense signaling involving Jasmonic acid (JA), Ethylene (ET) and Salicylic acid (SA) is an integral component of multilayered host-defense system against these fungal pathogens (Chowdhury *et al*. 2017a).

The charcoal root rot disease of sesame caused by *Macrophomina phaseolina* causes great economic losses for the oil seed crop. Study of early infection stages of sesame with *Macrophomina phaseolina* showed a biotrophic phase followed by a necrotrophic phase (Chowdhury *et al*. 2017a). The tolerant variety of sesame (Nirmala) showed higher defense parameters compared to the susceptible variety (VRI1). Under scanning electron microscopy the hypahe were found to inter-cellular growth along the borders of the host cells as well as showing intra-cellular growth. Studies of sesame roots infected with *M. phaseolina* showed significantly higher mycelial load even at early a time points in the susceptible host compared to the resistant one (Chowdhury *et al*. 2014a).

Study of early infection events of the sheath blight pathogen *Rhizoctonia solani* on a tolerant rice variety Swarnadhaan (IET 5656) and a susceptible variety Swarna (MTU 7029) showed that the pathogen preferred the susceptible variety compared to the tolerant variety (Basu *et al*. 2016). The *R. solani* hyphal growth was scanty and the hyphae avoided the leaf surfaces of tolerant variety of rice during growth where as in the susceptible variety, the hyphae grew in abundance close to the host surface. Interestingly, the pathogen hyphae could intercept stomatal openings on the rice leaves from a distance. The *R. solani* hyphae grew along the length of the leaf and was able to intercept the stomata from a distance then entered the stomatal opening directly with the help of rice leaf papillae for support. After entering the stomata the hyphae formed a plug around the stomatal opening and entered into the underlying tissues (Basu *et al*. 2016). Along with detailed study of the early phases of host- pathogen interaction in the two varieties of rice, assays using whole plants showed that disease severity was higher in Swarna (almost double) than Swarnadhaan. *R. solani* mycelia formed more inter-and intra-cellular structures, and greater sclerotial development in the susceptible host compared to the tolerant one. Another rice pathogen, the filamentous fungus *Magnaporthe oryzae,* also showed differential behaviours in tolerant versus the susceptible rice varieties (Sarkar *et al*. 2010, Mondal *et al*. 2017).

Early infections events during interaction of the necrotrophic pathogen *Alternaria solani* and tomato showed stomatal interception by the pathogen hyphae (Ray *et al*. 2015). Observations in terms of the production and localization of phenolics, hydrogen peroxide, superoxide dismutase, polyphenol oxidases, protein cross-linking, in a tolerant (Pusa Ruby) versus a susceptible (Pusa Early Dwarf) variety, showed differential regulations. Comet assays showed different rates of cell death, with the tolerant variety harbouring less damage (Ray *et al*. 2015, Ghosh *et al*. 2022). The expression of the tomato defense gene, *TPK1b*, was found to be significantly up regulated in the tolerant variety within the first day of infection where as in the susceptible variety, it remained low. The inoculum load also influenced disease establishment in tomato (Deb *et al*. 2024). In another study with different tobacco verities with different susceptibility status, *R. solani* showed differential behaviour showing preference for the susceptible host (Saha *et al*. 2022a). The behaviour of *R. solani* was also found to be influenced by different sources of nutrients *in vitro* (Saha *et al*. 2022b). In most cases, the up-regulation of host defense genes was seen at later time points after infection compare to the susceptible varieties due to delayed infection in these varieties (Basu *et al*; 2016, Chowdhury *et al*. 2017a; Koley *et al*. 2022).

Priming with phytohormones is also known to impart tolerance against filamentous fungi (Chowdhury *et al*. 2017b; Koley *et al*. 2022). In sesame plants, priming with different phytohormones resulted in increased tolerance against *M. phaseolina* (Chowdhury *et al*, 2017b). In tomato, priming with Salicylic acid and Jasmonic acid resulted in altered behaviour in the pathogen and improved tolerance against *R. solani* through altered regulation of several defense- related genes (Koley *et al*. 2022).

Biotechnological approaches for crop protection against diseases caused by filamentous fungi

Since the use of chemical fungicides is hazardous for the environment and human health, other safe options are currently the topic of intense research. This includes both transgenic approach to generate fungus-resistant crop plants and also the use of nanoparticles to reduce fungicide use. Our laboratory is working on some of these aspects as discussed below.

Cloning of a new defense-related protein gene with fungicidal properties and its use to generate disease tolerant transgenic crop lines

A new osmotin-like protein from *Solanum nigrum* (SindOLP) was found to exhibit significant antifungal properties *in vitro* (Chowdhury *et al.*2015). The *SindOLP* gene was cloned and overexpressed in *E. coli*. The full-length intron-less gene of 744 bp was cloned (Kundu S. *et al*. GenBank number: BankIt1588324 Solanum KC292261), which encoded a mature protein of 247 amino acids with a molecular mass of 26 kDa. It was demonstrated to possess antifungal activity against three filamentous fungal phytopathogen and could compromise hyphal walls and spores. SindOLP was found to be most active at pH 8, 25 °C and its antifungal activity is retained even after exposure to 75 °C for 30 min. SindOLP inhibited fungal spore germination *in vitro* (Chowdhury *et al.* 2015). This new *Sind OLP* gene was subsequently used to generate fungus tolerant transgenic sesame lines which was a pioneering work (Chowdhury *et al*. 2014b). The gene conferred tolerance against fungal pathogens along with conferring drought and salinity tolerance (Chowdhury *et al*. 2017b). The transgenesis of rice variety Ratna was also standardized with the view to generating disease resistant rice lines (Basu *et al*. 2014).

Filamentous Fungi and Biotechnology: Use of filamentous fungi for synthesis of noble metal nanoparticles

The use of filamentous fungi for production of nanoparticles has several advantages since fungi are excellent sources of extracellular enzymes that are required for biosynthesis of nanoparticles (Kundu, 2017) . The use of filamentous fungi is a popular means of green synthesis of nanoparticles which results in less toxic by products. These have the advantage of being inexpensive and the synthesis process can be done in a larger scale in fermenters which allows for easy replenishment of raw materials (Kundu, 2017). In our laboratory, several phytopathogenic filamentous fungi as well as filamentous edible mushroom species have been used for synthesis of noble metal nanoparticles (Das *et al*. 2023). These provide eco-friendly and low-cost method of biosynthesis of nanoparticles which has the potential to be produced on a larger scale.

The cell-free filtrate of *M. phaseolina* has been used to produce silver nanoparticles with desirable properties (Chowdhury *et al*. 2014c). UV-visible spectrum showed a peak at 450 nm which corresponds to the plasmon absorbance of silver nanoparticles. Scanning electron microscopy, atomic force microscopy and transmission electron microscopy showed that the nanoparticles were spherical and of the size range 5 to 40 nm. Most importantly these nanoparticles were protein capped resulting in stabilization of the silver nanoparticles. These silver nanoparticles had potent antimicrobial activities. Silver nanoparticles have been also produced with the help of the rice pathogen *Curvularia affinis* (Halder *et al*. 2018). Scanning Electron Microscopy showed that the silver nanoparticles were mostly 10-15nm and spherical in shape. These nanoparticles had inhibitory and delaying effect on growth of first, second and third order branching of *A. solani* (Halder *et al*. 2018). This inhibitory effect was directly proportional to nanoparticle concentration. Thus these nanoparticles made with filamentous fungi have the potential to be used as nano-fungicide.

Protein-coated silver and gold nanoparticles have been also been produced for the first time from the edible, mycorrhizal fungus *Tricholoma crassum* (Berk.) Sacc (Ray *et al*., 2011, Basu *et al*. 2018).The silver nanoparticles were spherical to hexagonal in shape and had potent antimicrobial effect (Ray *et al*. 2011). Whereas, characterization of the gold nanoparticles showed that these were of different geometric shapes and of size range 5–25 nm (Basu *et al*. 2018). These gold nanoparticles had potent antimicrobial activity against bacteria and fungi. Comet assays showed that these had apoptotic properties on eukaryotic cells. Moreover, the particles were capped with a 40 kDa protein which was utilized as attachment sites for delivery of reporter gene constructs into sarcoma cancer cells (Basu *et al*. 2018).

Platinum nanoparticles were also successfully made with cell-free filtrate of the filamentous fungus, *C. affinis*. These nanoparticles were subsequently used for *in vitro* experiments with sarcoma cells line and in *in vivo* experiments with mouse models for control of cancerous tumors (Bhattacharya *et al*. 2022a, b).

CONCLUSION

On the whole it can be said that filamentous fungi are a diverse group of economically important fungi mostly belonging to Ascomycota and Basicipmycota. These have both positive and negative economic impact. Majority of common crop diseases are caused by filamentous fungi. The outcome of disease progression is dependent on the early events of infection. Therefore studying the behaviour of fungi during initial phases of infection is important for finding means to control plant diseases. On the counter side these filamentous fungi can be utilized for economic gains including green synthesis of noble metal nanoparticles. Filamentous fungi have the advantage of being used in fermenters for production of nanoparticles. With standardized methods of synthesis, protein-capped nanoparticles can be produced which have diverse fields of application. These nanoparticles have antimicrobial properties and can be used for gene/drug delivery into cancer cell lines.

DECLARATION

Conflict of Interest. The author declares no conflict of interest

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