Interaction of Bacillus thuringiensis with Pythium ultimum and Fusarium oxysporum f. sp. lycopersici: Possible role in biological control

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Bacillus thuringiensis (Bt) suppressed the growth of Pythium ultimum and Fusarium oxysporum f. sp. lycopersici and lysed the mycelium. Scanning electron micrographs showed the attachment of Bt cells to the hyphae of host pathogens. Bt cells attached to hyphae of P. ultimum polarly and externally, causing its deformation and lysis. In case of F. oxysporum f. sp. lycopersici, the attachment was random and at a few places Bt cells entered the hyphae and caused lysis.

In the search for new eco-friendly approaches to pest and pathogen management, use of biological control agents has been much studied. Researches on biological control of soil-borne plant pathogens have been making great strides since the first symposium 'Ecology of soil-borne plant pathogens – Prelude to biological control'.

Bacillus thuringiensis, which is unique in the bacterial world because of production of many chemical compounds designed for use in controlling economically and biomedically important insects, is being tested against fungal pathogens. A nematode fungal disease complex of tomato was reported to be controlled by dipping seedlings in the suspension of B. thuringiensis. Some bacteria like Enterobacter cloacae protect the plants against Pythium pre-emergence damping off caused by P. ultimum by binding to fungal hyphae. However, the mechanism by which B. thuringiensis protects the plants/seedlings from soil-borne fungal pathogens is unknown. Therefore, a study was undertaken to investigate interaction between B. thuringiensis and P. ultimum and Fusarium oxysporum f. sp. lycopersici, causing wilting of seedlings of various crops, which could be utilized as a potential biocontrol agent.

A strain of B. thuringiensis obtained from Division of Entomology, Indian Agricultural Research Institute, New Delhi, was maintained on nutrient agar medium for the present studies.

Cultures of P. ultimum and F. oxysporum f. sp. lycopersici were isolated from soil by the dilution plate method and maintained on potato dextrose agar (PDA) slants. The cultures were identified by Indian Type Culture Collection (ITCC), New Delhi.

Potato dextrose agar plated petri plates were seeded at four corners with actively growing mycelial disc of P. ultimum and F. oxysporum f. sp. lycopersici. A loopful of 24 h growth of B. thuringiensis was seeded in the centre of each petri plate. The inoculated plates were incubated at 25°C for 4 days and mycelium from the interaction zone was processed for scanning electron microscopy.

The procedure given by Weidenborner et al. was followed. Agar discs of 1 mm thickness were cut off from interaction zone and placed on cover glasses which were exposed to 2% osmium tetroxide for 24 h at 20°C. The samples were transferred to copper stubs over double adhesive tape, coated with gold in JEOL, JFC-1100E sputter coater and scanned in a SEM (JEOL-JSM 5200) at 25 kV and micrographs were taken.

In dual culture, the growth of P. ultimum and F. oxysporum f. sp. lycopersici was suppressed and mycelium was seen lysed. In case of P. ultimum, a narrow inhibition zone was also observed (Figure 1 a), while no clear inhibition zone was seen in F. oxysporum f. sp. lycopersici (Figure 1 b).

Scanning electron micrographs of the paired culture of F. oxysporum f. sp. lycopersici and B. thuringiensis showed attachment of around 9–10 bacterial cells to the hyphae of the fungal pathogen. The bacterial cells were attached more or less over the hyphal surface and at the site of attachment the hyphae were lysed (Figure 2 a, b). At a few places, bacterial cells seemed entering the mycelium as cracks in the wall were observed at
Figure 1. Dual culture of Bacillus thuringiensis with (a), Pythium ultimum and (b), Fusarium oxysporum f. sp. lycopersici.

Figure 2. Scanning electron micrographs of interaction between B. thuringiensis and F. oxysporum f. sp. lycopersici showing random attachment of Bt cells to host mycelium, (a, ×1000), (b, ×7500); Bt cells entering hypha (c, ×10000), (d, ×20000).

the point of attachment. It was observed that when bacterial cells came in contact with host cytoplasm, lysis of mycelium took place (Figure 2 c, d). In case of P. ultimum, the binding of B. thuringiensis cells was polar, i.e. the cells were seen attached towards the growing tip of the hypha and the attachment to the hyphae was only external. At a few places deformed mycelium was also observed (Figure 3 a, b).
riaceae to attach to surfaces, including fungal hyphae has been reported earlier.\(^7\,^8\)

The present study suggests that there is a relationship between hyphal attachment by bacteria and fungal growth inhibition. As *B. thuringiensis* cells were seen attached to the host mycelium, this appears to be a critical step in establishing an antagonistic relationship with *P. ultimum* and *F. oxysporum f. sp. lycopersici*. Bowen and Theodorou\(^9\) observed the interactions between bacteria and ectomycorrhizal fungi but the relationship between the growth inhibition and attachment was not investigated.

In conclusion, the results show that the adherence mechanism between bacteria and fungi may lead to the biological control of soil-borne fungal diseases. Moreover, other important factors in the use of *B. thuringiensis* are that it releases a large number of exo- and endotoxins\(^10\) and its relative ease of mass production in submerged fermentation culture on relatively cheap media\(^11\,^12\).


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