Table 1. Relative toxicity of δ-endotoxins of *Bacillus thuringiensis* to the second instar larvae of *Lucinodes orbonalis*

δ-endotoxin	LC_{50} (ng/cm^2)	Slope (± SE)	95% Fiducial limits	
			Lower	Upper
Cry 1 Aa	24.62	1.99 (± 0.36)	19.01	32.67
Cry 1 Ab	14.86	1.56 (± 0.33)	12.20	18.20
Cry 1 Ac	10.99	2.23 (± 0.42)	8.08	14.94
Cry 1 B	17.32	2.05 (± 0.36)	13.57	22.80
Cry 1 C	10.86	2.01 (± 0.35)	8.06	14.62
Cry 1 E	24.92	1.88 (± 0.36)	19.01	32.67
Cry 2 Aa	3.08	1.42 (± 0.19)	2.31	4.11

LC_{50} denotes the concentration of the toxin protein causing 50% larval mortality.

1C, Cry 1Ac, Cry 1Ab and Cry 1B in the descending order. Cry 1Aa and Cry 1E were relatively less toxic. Cry 2Aa and Cry 1B proteins were also found to be active against dipteran insects[13]. It would be interesting to find out the toxicity of both these proteins to *Phytomyza horticola*, a dipteran pest on brinjal[2]. Cry 1Ab protein was constitutively expressed in transgenic brinjal by introducing a synthetic gene optimized for plant codon usage[14]. Significant protection against BSFB was achieved in the fruits of transgenic plants. Our results confirm the efficacy of Cry 1Ab and also suggest that, the expression of Cry 2 Aa and Cry 1C in transgenic brinjal would possibly provide better protection from this pest. Large-scale cultivation of transgenic crops in the coming years may possibly impose selection pressure on the insect pest and encourage the development of resistance in insects[4]. One of the strategies proposed to avoid/delay resistance development is to pyramid genes in the transgenic plants[15]. The information generated by us is useful in this direction. The genes can be so selected that, the respective proteins bind to different receptors in the midgut epithelial membranes of *L. orbonalis*. Investigations to characterize the Bt toxin receptors in *L. orbonalis* are necessary.

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Siderophore production by *Aspergillus niger* AN27, a biocontrol agent

Siderophores have received much attention in the past decade, largely because of their proposed role in biocontrol of soil-borne plant pathogens[1] and as a supplier of iron nutrition to crop plants[2]. Since plant pathogens may not have the cognate ferri–siderophore receptor for uptake of iron–siderophore complex, they are prevented from proliferating in the immediate vicinity because of lack of iron[3]. Hence, siderophore-producing beneficial microbes can confer a competitive advantage in interactions in the rhizosphere[4]. We report here the production of hydroxamate and catecholate groups of siderophore by a potential biocontrol agent, *Aspergillus niger* AN27 (ref. 5).

The methods of preparation of the chrome azural S agar (CAS-A) medium was after Schwyn and Neilands[6]. The methods of assay, viz. non-specific, hydroxamate and catecholate groups, were followed as mentioned by Haydon *et al.*[7], Arnow[8], and Holzberg and Artis[9], respectively. For these assays, Czapek-Dox (without iron) was used as the basal low iron medium (LIM). *A. niger* AN27 was inoculated in 250 ml flask containing 100 ml of LIM. The flasks were kept in a shaker at 150 rpm and incubated at 30°C for 7 days. The supernatants were taken by filtration through Whatman filter paper (no. 42).

Figure 1. Siderophore production by *Aspergillus niger* AN27 on CAS-A medium, 3 days after inoculation, indicated by orange red pigmentation.

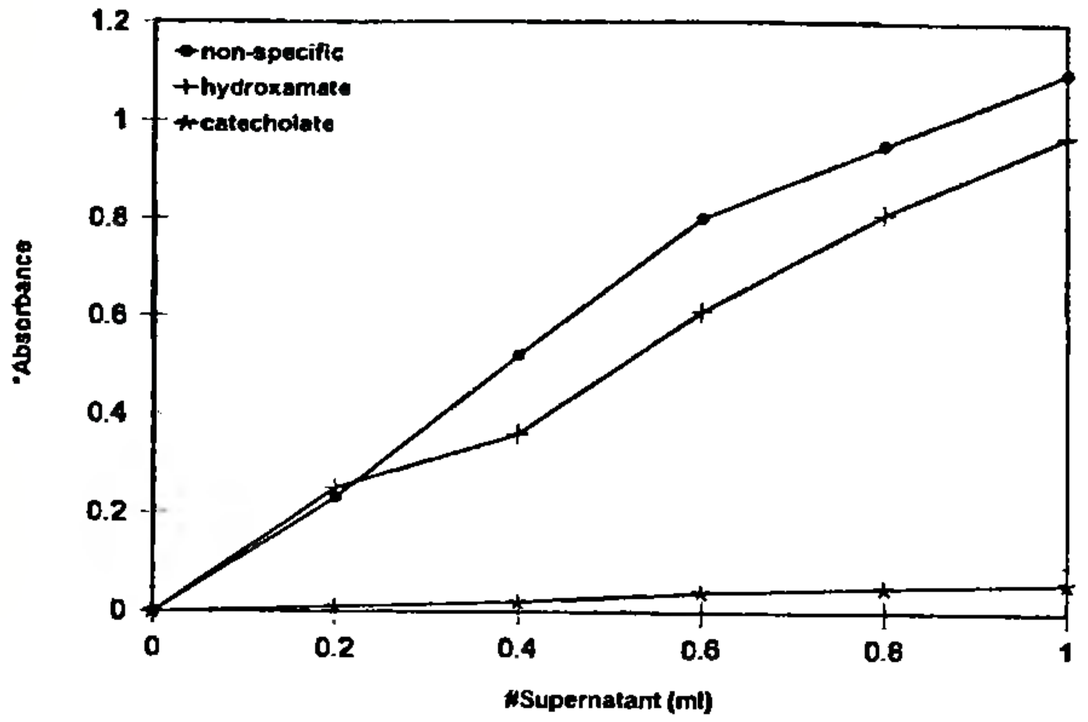

Figure 2. Iron-binding activity of supernatants, indicating the presence of secreted siderophores (#At 3 days after inoculation. *Absorbance was measured at 425, 254 and 510 nm for non-specific, hydroxamate and catecholate groups, respectively).

Production of siderophores by *A. niger* AN27 was indicated in the blue CAS-A medium by formation of orange halo around the colony (Figure 1). Non-specific tests in the LIM were also positive (Figure 2). Specific tests for hydroxamate and catecholate groups of siderophore further confirmed that *A. niger* AN27 produced both the groups of siderophores (Figure 2).

According to Srinivasan *et al.*[10], competition for iron between *Trichoderma* spp. and wood decay basidiomycetes in wood is a possible means of biocontrol of wood decay fungi. Fungistatic activity of siderophore has been reported[11]. *Rhizopus arrhizus* produces a siderophore which is related to growth promotion in tomato[12]. Fluorescent pseudomonads enhance plant growth and affect deleterious micro-organisms by producing siderophores[13]. Siderophores produced by *Pseudomonas* spp. are adequate to influence microbial interaction and hence rhizosphere competitiveness[13]. All the aforesaid attributes, viz. antagonistic activities[14], rhizosphere competence[15], and growth promotion[16] were found in *A. niger* AN27. Therefore, it can be hypothesized that siderophore production by *A. niger* AN27 might have some role in these phenomena. This forms the first report of siderophore production by a strain of *A. niger*, an effective biocontrol agent.

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