7. Findings of the Scientific Review Panel on the Report on Diesel Exhaust as adopted at the Panelis 22 April 1998 Meeting, California Air Resources Board, 1998, *mimeo*.
8. Enya, Takeji *et al.*, *Environ. Sci. Technol.*, 1997, **31**, 2772–2776.
9. Jones, R. R., *Br. Med. J.*, 1996, **312**, 1605–1606.
10. Unpublished data on PM10 levels in Delhi, Central Pollution Control Board, 1998.
11. Shah, J. and Nagpal, T. (eds), *Urban Air Quality Management Strategy in Asia (URBAIR)*, The World Bank, 1996.
12. Raju, B. S. N., *Fundamentals of Air Pollution*, Oxford and IBH, New Delhi, 1997.
13. Unpublished data on NO_2 levels in Delhi, Central Pollution Control Board, 1998.
14. Schwartz, J., *Environ. Res.*, 1989, **50**, 309–321.
15. *Air Quality Guidelines for Europe*, WHO Regional Publications, Copenhagen, Europe series, 1987, vol. 23, pp. 45–58.
16. Singh, A. *et al.*, *Atmos. Environ.*, 1997, **31**, 3421–3427.
17. Nadis, S., *Environ. Health Perspect.*, 1996, **104**, 138–139.
18. Sharma, A. and Roychowdhury, A., *Slow Murder, The Deadly Story of Vehicular Pollution in India*, Centre for Science and Environment, New Delhi, 1996.
19. Akbar, S. and Ashmore, M. R., Paper presented at the National Conference on Health and Environment organized by the Centre for Science and Environment, New Delhi, 1998, *mimeo*.
20. Reynolds, A. *et al.*, Summary of an Australian Conservation Foundation Discussion Paper, 1999, *mimeo*.

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SCIENTIFIC CORRESPONDENCE

Susceptibility of brinjal shoot and fruit borer to the δ-endotoxins of *Bacillus thuringiensis*

Brinjal (egg plant) is one of the most important vegetable crops of India. It is widely consumed by all sections of the population and is relatively inexpensive and is available throughout the year[1]. Brinjal is infested by a lepidopteran insect called brinjal shoot and fruit borer (BSFB *Lucinodes orbonalis* Gueenee) which causes extensive damage to the growing shoot tips and fruits, thereby drastically reducing the marketable fruit yield[2]. It is very difficult to control the pest because of its burrowing nature. Organic pesticides are widely used to control BSFB, which may adversely affect human health and environment[3]. Safe, effective and eco-friendly strategies to control insect pests include genetic engineering of brinjal using genes encoding insecticidal proteins[4]. *Bacillus thuringiensis* (Bt), a gram-positive soil bacterium, synthesizes insecticidal crystal proteins or δ-endotoxins during sporulation[5]. It was observed that many of the δ-endotoxins were lepidopteran-specific and were active at very low concentrations[6]. In the present study, we tested the efficacy of seven lepidopteran-specific δ-endotoxins of Bt towards the second instar larvae of BSFB. An artificial diet was formulated to rear BSFB larvae and perform insect bioassays.

Seven lepidopteran-specific Bt δ-endotoxin genes, viz. *cry1Aa, cry1Ab, cry1Ac, cry1B, cry1C, cry1E*, and *cry2Aa* cloned in *Escherichia coli* expression vectors were obtained from Donald Dean (Ohio State University, Columbus). The *E. coli* cultures were grown for 48 h at 37°C in LB medium and δ-endotoxins were purified as described by Lee *et al.*[7]. The crystal proteins were solubilized in buffer containing 50 mM sodium carbonate (pH 9.5), and 10 mM dithiothreitol, at 37°C for 3 h. The proteins were electrophoresed by SDS-PAGE and the δ-endotoxin fraction of *E. coli* protein was quantified by laser densitometery[8]. Insect bioassays were done by coating the proteins on to BSFB artificial diet. A modified semisynthetic diet was used for bioassays[9]. The diet consisted of 120 g black gram flour, 40 g wheat germ, 1.5 g ascorbic acid, 3 g sorbic acid, 3 g methyl-*p*-hydroxybenzoate, 10 g Wesson salt mixture[10], 3 g aureomycin, 10 ml vitamin mixture[11], 5 ml formaldehyde, 32 g yeast and 16 g agar in one litre of distilled water. The mixture was poured into 24 well tissue culture plates (ICN, USA). The diet was allowed to solidify and different Bt toxins were coated on the diet. Six concentrations of each protein were tested. Two second instar larvae were released into each well of the tissue culture plate. The plates were incubated at 25°C under light : dark regime (12 : 12 h). Larval mortality was recorded after every 24 h and final mortality count was taken on the fourth day. Thirty larvae were tested for each protein and the experiment was repeated three times. The results of the bioassays were evaluated by probit analysis[12].

Table 1 shows the relative efficacy of Bt toxins against second instar larvae of BSFB. The protein Cry 2 Aa, was the most potent toxin tested followed by Cry

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.

1. Bose, T. K., Som, M. G. and Kabir, J., *Vegetable Crops*, Naya Prakashan, Calcutta, 1983.
2. Atwal, A. S., *Agicultural Pests of India and South East Asia*, Kalyani Publishers, New Delhi, 1976.
3. Rataul, H. S., *Indian J. Entomol.*, 1986, **48**, 406–412.
4. Kumar, P. A., Mandoakar, A. and Sharma R. P., *Agril. Biotech News Info.*, 1998, **10**, 329–331.
5. Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D. Baum, J., Feitelson, J., Zeigler, D. R. and Dean, D. H., *Microbiol. Mol. Biol. Rev.*, 1998, **62**, 775–806.
6. Kumar, P. A., Sharma, R. P. and Malik, V. S., *Adv. Appl. Microbiol.*, 1996, **42**, 1–43.
7. Lee, M. K., Milne, R. E., Ge, A. Z. and Dean, D. H., *J. Biol. Chem.*, 1992, **167**, 3115–3121.
8. Chakrabarti, S. K., Mandaokar, A., Kumar, P. A. and Sharma, R. P., *J. Invertebr. Pathol.*, 1998, **72**, 336–337.
9. Patil, P. D., *J. Entomol. Res.*, 1990, **14**, 164–172.
10. Reinecke, J. P., *Nutrition: Artificial diets, Comprehensive Insect Physiology, Biochemistry and Pharmacology*, Pergamon Press Ltd, Oxford, 1984, vol. 4, pp. 391–419.
11. Vanderzant, E. S. *Annu. Rev. Entomol.*, 1974, **19**, 139–160.
12. Finney, D. J., *Probit Analysis*, Cambridge University Press, Cambridge, 1972.
13. Hofte, H. and Whiteley, H. R., *Microbiol. Rev.*, 1989, **53**, 242–255.
14. Kumar, P. A., Mandaokar, A., Sreenivasu, K., Chakrabarti, S. K., Sharma, S. R., Bisaria, S., Kaur, S. and Sharma, R. P., *Mol. Breed.*, 1998, **4**, 33–37.
15. McGaughey, W. H., Gould, F. and Gelernter, W., *Nature Biotech.*, 1998, **16**, 144–145.

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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).