Review Article

J Vect Borne Dis 42, September 2005, pp 81–86

Occurrence and diversity of mosquitocidal strains of *Bacillus thuringiensis*

K. Balaraman

Division of Product Development, Vector Control Research Centre (ICMR), Indira Nagar, Pondicherry, India

Ever since the discovery of the first *Bacillus thuringiensis* strain capable of killing mosquito larvae, namely, *B. thuringiensis* var *israelensis*, there are several reports from different parts of the world about the occurrence of mosquitocidal strains belonging to different subspecies/serotypes numbering thirty-six. The main sources of these wild type strains are soils/sediments, plants, animal feces, sick/moribund insects and waters. The toxicity of the strains within a subspecies/serotype varied widely. Some of the strains exhibited toxicity to mosquitoes as well as lepidopterans and dipterans (including mosquitoes) as well as plant parasitic nematodes.

Key words *Bacillus thuringiensis* – diversity – mosquitoes

Bacillus thuringiensis Berliner 1915, a Gram positive, rod-shaped, spore-forming bacterium produces parasporal bodies (inclusions) during the process of sporulation. These inclusions are pro-toxins which when ingested by a susceptible insect (larva) are activated into toxin(s) (δ-endotoxin) in the midgut. This is followed by binding of the δ-endotoxin to the receptors in the midgut brush border membrane, leading to paralysis of mouth parts and gut, and eventually death of the larva. Most strains of *B. thuringiensis* are specific largely to lepidopteran insects. However, several isolates of *B. thuringiensis* were found to have some activity on mosquitoes, but at concentrations which are too high for offering any operational promise[1]. The first strain of *B. thuringiensis*, ONR-60A, demonstrating a high level of activity against mosquitoes (larvae) was isolated from soil samples in Israel[2]. This strain belonged to a new flagellar (H) antigenic type (H14) and assigned the name *Bt* var *israelensis*[3]. Its inclusions have been found to contain four protein fractions, cyt 1A (27.3 kDa), cry 4A (128 kDa), cry 4B (134 kDa) and cry 11A (72 kDa)[4]. Subsequently, there has been several reports of isolation of mosquito-active strains of *B. thuringiensis* and the present review gives a brief account of these observations.

Soils/Sediments

A new subspecies of *B. thuringiensis* (serovar *kyushuensis*) possessing H11a:11c flagellar antigen and toxic to *Culex tritaeniorhynchus* was isolated from silkworm litter of a sericulture farm[5]. Three isolates of *Bt* var *darmstadiensis* (H10) were obtained in Japan[6]. Two of them (producing spherical crystals) were toxic to *Cx. tritaeniorhynchus* and *Cx. molestus* and *Aedes aegypti* but non-toxic to lepidopterans, whereas the reference strain of *Bt* var *darmstadiensis* is toxic to lepidopterans and non-toxic to mosquitoes, and the third isolate (producing irregular shaped inclusions) was non-toxic to both the groups. A potent strain of *Bt* var *israelensis* (H14) toxic to *Culex* and *Anopheles* mosquitoes was isolated from India[7]. *Bt* var *morrisoni* (H8a:8b) was isolated from Philippines and found to be toxic only to mosquitoes (*Culex* and *Aedes*)[8] whereas the reference strain was toxic only to silkworm. Out of 85 isolates reported from Nigeria, five strains belonging to the serotype, *israelensis* (serotype H14), *canadensis* (H5a:5c) and *morrisoni* (H8a:8b) were found active against *Cx. p. autogenicus*[9]. One isolate of *Bt* var *israelensis* was obtained from silkworm rearings in Vietnam[10]. In India, 44 *B. thuringiensis* isolates toxic to *Cx. quinquefasciatus* were obtained out of 710 samples screened [11]. Among these, 40 were *Bt* var *israelensis.* Of the remaining four strains, one was a new record, and assigned the name *Bt* var *pondicheriensis* (H20a:20c) which falls within the subspecies *yunnanensis* (H20a:20b). Three out of four isolates of *B. thuringiensis* toxic to *Ae. aegypti* were isolated in Israel[12] three of these reacted with the flagellar antigens of serotype H14 as well as serotype H17 and were assigned the name *Bt* var *israelensis* ± *Bt* var *aizawai* and one isolate was serotyped as *Bt* var *entomocidus* (H6) and was found to be highly toxic to *Ae. aegypti*. Thirteen isolates of *Bt* var *fukuokaensis* (H3a:3d:3e) toxic to *Ae. aegypti*, *Ae. albopictus* and *Cx. tritaeniorhynchus* and non-toxic to lepidopterans were reported from Japan whereas the type strain of *Bt* var *fukuokaensis* is toxic to lepidopterans only[13]. Two strains of *Bt* var *israelensis,* active against *Ae. aegypti*, *Cx. pipiens*, *Cx. quinquefasciatus*, *An. gambiae* and *An. stephensi* were isolated in Egypt[14]. A novel isolate highly toxic to mosquitoes was isolated in Malaysia which displayed a new subfraction (H28a:28c) of the H28 flagellar antigen and designated as *Bt* var *jegathesan*[15].

Eight isolates forming spherical parasporal bodies and exhibiting low to moderate activities for *An. stephensi* and *Cx. p. molestus*, but not for *Telmatoscopus albipunctatus* (Diptera), or *Bombyx mori* and *Hyphantria cunea* (Lepidoptera) were obtained in Japan[16]. Their toxicity to anophelines was 10 times greater than that for culicines and these strains were assigned the name of new subsp *higo* (H44). Eight isolates, toxic to *Cx. quinquefasciatus, Ae. aegypti* and *An. hyrcanus* were obtained in China[17]. Four strains belonging to the serovars *thompsoni*, *malaysiensis*, *canadensis*, *jegathesan* and two more strains (untypable) were found to be highly toxic to the mosquitoes. *Bt* var *wratislaviensis* (H47), isolated from garden, flower-bed and park areas in Poland was reported to be poorly toxic to *Ae. aegypti*[18]. Of 1313 colonies of *B. cereus/B. thuringiensis* group obtained from mangrove sediments, 1.7% were allocated to *B. thuringiensis*[19]. Of these, 10 were assigned to eight serovars (*kurstaki, sumiyoshiensis, sotto, aizawai, darmstadiensis, thompsoni, neoleonensis* and *higo)*; and insecticidal activities (toxic to both lepidoptera and diptera) were associated with two *Bt* var *kurstaki* isolates and one *Bt* var *higo* isolate (Diptera-specific). Out of 809 samples collected from Japan, 13.2% were found to be *B. thuringiensis*[20]. Among these, the predominant H-serotype were H5a:5c/21 (serovar *canadensis/colmeri*), followed by H3ad (serovar *sumiyoshiensis*), H16 (serovar *indiana*) and H10ac (serovar *londrina*). All these isolates were mosquito-active and produced spherical parasporal inclusions. From intertidal brackish water sediment samples of mangroves, 18 *B. thuringiensis* isolates were recovered[21]. Among these, two isolates of *Bt* var *israelensis*/*tochigiensis* (H14/19) exhibited high toxicity to *Cx. p. molestus*. An isolate of *Bt* var *kurstaki* (H3a:3b:3c) toxic to both *Spodoptera exigua* and *Cx. pipiens* was reported from Korea[22]. One strain of *Bt* var *thompsoni* (H-12), highly toxic to mosquito larvae was isolated from India[23] and the protein profile of its parasporal body was comparable with that of *Bt* var *israelensis*. *B. thuringiensis* var *finitimus* toxic to larvae of *Ae. aegypti* was isolated from soils of Poland[24]. A novel isolate of the subspecies *jordanica* (H71), toxic to *melanogaster* and to the juveniles of root-knot nematodes (*Meloidogyne javanica* and *M. incognita*) was obtained in Jordan[25] it was poorly active towards *Cx. p. molestus* and *Culiseta longiareolata*. Out of 493 samples 115 isolates have obtained from Spain[26] and among them one was highly active against mosquitoes. One isolate of the *Bt* var *israelensis*/*tochigiensis* (H14/H19) showing very high toxicity to *Ae. aegypti*, *Cx. quinquefasciatus* and *An. stephensi* was isolated from mangrove forests in India[27].

Plants

An isolate of subspecies *kenyae* (H4a:4c) with spherical parasporal bodies was recovered from sorghum dust in Mexico[28] and the parasporal bodies were composed of proteins of mw, 27, 65, 128 and 134 kDa which cross-reacted with the polyclonal antisera raised against the parasporal body of subspecies *israelensis*. Twenty-three isolates of *B. thuringiensis* were obtained from the rhizoplanes of aquatic plants belonging to 23 genera[29]. Among these, 20 were *Bt* var *israelensis* (H14) and one was *Bt* var *tohokuensis* (H17), and others were untypable. *B. thuringiensis* isolates of the subspecies, *yosoo, jinghongiensis* and *tochigiensis* have been reported to exhibit moderate activity towards larvae of *Ae. aegypti* were obtained from the phylloplanes of *Ouercus robur* and *Sorbus aucuparia* in Poland[24]. In Colombia, 256 *B. thuringiensis* isolates were obtained from the phylloplanes of the plant genus *Piper* (74% of 35 samples)[30]. Among these, ca. 55% presented bi-pyramidal-crystal morphology; and ca. 42% round-crystal morphology. And ca. 60% of the isolates were toxic to *S. frugiperda* and ca. 40% to *Cx. quinquefasciatus*.

Animal feces

Out of 34 samples collected from 14 species of wild mammals, 43 *B. thuringiensis* isolates were obtained in Korea[31]. Of these, 13 were assigned to nine subspecies: *sumiyoshiensis* (H3a:3d), *dakota* (H15), *tohokuensis/mexicanensis* (H17/27), *tochigiensis* (H19), *colmeri* (H21), *amagiensis* (H29), *toguchini/ muju* (H31/49), *jinghongiensis* (H42) and *higo* (H44) and the other isolates were untestable. Out of 287 *B. thuringiensis* isolates recovered from the feces of zoo-maintained animals, 188 were found to be toxic to both *B. mori* and *Ae. aegypti*[32], of these two were specific to *B. mori* and three were specific to *Ae. aegypti* and assigned to eight sero-groups including H3a:3b:3c (subsp *kurstaki*), H6 (subsp *entomocidus*). And the isolates with dual toxicity belonged to the subspecies *kurstaki* and subspecies *aizawai*. Seven out of 10 samples from deer yielded 33 *B. thuringiensis* isolates in Japan and among these only one, assigned to the serotype H3a:3b:3c (subsp. *kurstaki*), exhibited dual toxicity against *B. mori* and *Ae. aegypti*[33].

Insects

A total of 454 mosquito larval samples were screened and 11 strains of *Bt* var *israelensis,* toxic to *Cx.quinquefasciatus* were isolated[11]. Another strain of the same subspecies was isolated from the stem borer of casuarinas (*Stromatium fulvum* Vill: Coleoptera) and this was more toxic to *Ae. aegypti* than to *Cx. pipiens*[34]. From *Simulium* larvae and adults, 18 *B. thuringiensis* isolates, toxic to *Ae. aegypti* were obtained in Brazil[35].

Water

Out of 728 samples screened fifty-seven *B. thuringiensis* isolates were obtained [11]. Among these, 50 belonged to var *israelensis* (H14) and one to var *indiana* (H16) and six were untypable. In Japan, out of 107 samples 49.5% were positive for *B. thuringiensis*[36]. These isolates were assigned to 26 H-serotypes and of these, H14/36 (serovar *israelensis/malaysiensis*) was the predominant one, followed by H3abc (*kurstaki*), H27 (*mexicanensis*), H3a:3d (*sumiyoshiensis*), and H35 (*seoulensis*).

Conclusion

Mosquito-toxic strains of *B. thuringiensis* have been reported from different continents except, the Americas and Australia and the sources include soils/sediments, plants (rhizoplane of aquatic plants, phylloplanes, etc.), insects (mosquito larvae, stem borer, etc.), animal feces (wild mammals, zoo-animals and deer) and water (Table 1). These *B. thuringiensis* strains belonged to different subspecies/serotypes and the highly toxic strains are restricted not to the first recognised subspecies, *Bt* var *israelensis* (H14) alone. And they belonged to other subspecies/sero-

Table 1. Mosquitocidal *B. thuringiensis* serotypes and sources of their isolation

H serotype	Subspecies	Source(s) of isolation	H serotype	Subspecies	Source(s) of isolation
H2	*finitimus*	Soils	H19	*tochigiensis*	Soils, plant, animal feces
H3a:3b:3c	*kurstaki*	Soils, water, animal feces	H20a: 20c	*pondicheriensis*	Soils
H3a:3d:3e	*fukuokaensis*	Soils	H21	*colmeri*	Animal feces
H3a:3d	*sumiyoshiensis*	Soils, water, animal feces	H24a:24b	*neoleonensis*	Soils
H4a:4c	*kenyae*	Plants	H27	*mexicanensis*	Water, animal feces
H4a:4b	*sotto*	Soils	H28a:28c	*jegathesan*	Soils
H5a:5c	*canadensis*	Soils	H29	*amagiensis*	Animal feces
H6	*entomocidus*	Soils, animal feces	H35	*seoulensis*	Water, animal feces
H7	*aizawai*	Soils, animal feces	H42	*jinghongiensis*	Plant, animal feces
H8a:8b	*morrisoni*	Soils	H44	*higo*	Soil, animal feces
H10	*darmstadiensis*	Soils	H47	*wratislaviensis*	Soils
H10a:10c	*londrina*	Soils	H71	*jordanica*	Soils
H11a:11c	*kyushuensis*	Soils	H5a:5c/H21	*canadensis/colmeri*	Soils
H12	*thompsoni*	Soils	H14/36	*israelensis/ malaysiensis*	Water, animal feces
H14	*israelensis*	Soils, plants, water, insects	H14/19	*israelensis/ tochigiensis*	Soils (from mangrove)
H15	*dakota*	Animal feces	H17/H19	*tohokuensis/ tochigiensis*	Plants
H16	*indiana*	Soils, water, animal feces	H17/27	*tohokuensis/ mexicanensis*	Animal feces
H17	*tohokuensis*	Plant	H31/49	*toguchini/muju*	Animal feces
H18a:18c	*yosoo*	Plant			

types also, namely, *Bt* var *canadensis* (H5a:5c), *Bt* var *morrisoni* (H8a:8b), *Bt* var *darmstadiensis* (H10), *Bt* var *thompsoni* (H12) and *Bt* var *jegathesan* (H28a:28c). Within each subspecies/serotype there are strains which exhibited different levels of toxicity against the same target species (in terms of LC_{50} values)—very high → high → moderate →poor → non-toxic and certain subspecies showed dual toxicity— *kurstaki* (H3a:3b:3c), *israelensis*/*tochigiensis* (H14/H19) to mosquitoes (Diptera) and *Spodoptera* species (Lepidoptera) and *Bt* var *jordanica* (H71) to mosquitoes and *D. melanogaster* (Diptera) and to root-knot nematode parasites of plants (*Meloidogyne* spp.). Although the reference strains of the subspecies/ serotypes, such as *Bt* var *fukuokaensis* (H3a:3d:3e), *Bt* var *morrisoni* (H8a:8b) and *Bt* var *darmstadien-*

sis (H10) are known to be non-toxic to mosquitoes, few other strains were found to be toxic.

Thus, so far around 36 subspecies/serotypes of *B. thuringiensis* toxic to different species of mosquitoes were reported from different parts of the world. A classical feature of all the mosquitocidal *B. thuringiensis* strains is that they all possess a large transferable plasmid which is responsible for the toxicity and it carries *cry* and *cyt* genes that code for the cry and cyt toxins[37,38]. The vast variation observed in the toxicity of different strains may likely be due to the presence or partial presence or absence of the *cry* and/or *cyt* gene(s). Loss of plasmid from *B. thuringiensis* strains is likely to make them avirulent/non-pathogen/non-toxic.

References

1. WHO data sheet on the biological control agent *Bacillus thuringiensis* 1982. *WHO/VBC/79.75.Rev.1.NBC/BCDS79.01*
2. Goldberg L, Edward M, Margalit J. Bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii*, *Uranotaenia unguiculata*, *Culex univittatus*, *Aedes aegypti* and *Culex pipiens*. *Mosq News* 1977; *37:* 355–8.
3. deBarjac, H. Une nouvelle variete de *Bacillus thuringiensis* tres toxique pour les moustiques: *Bacillus thuringiensis* var *israelensis* serotype 14. *CR Acad Sci (Paris)* 1978; *286D:* 797–800.
4. Brown KL, Whiteley HR. Isolation of a *Bacillus thuringiensis* RNA polymerase capable of transcribing crystal protein genes. *Proc Natl Acad Sci USA* 1988; *85:* 4166–70.
5. Ohba M, Aizawa K. A new subspecies of *Bacillus thuringiensis* possessing 11a:11c flagellar antigenic structure: *Bacillus thuringiensis kyushuensis*. *J Invert Pathol* 1979; *33:* 387–8.
6. Padua LE, Ohba M, Aizawa K. The isolates of *Bacillus thuringiensis* serotype 10 with a highly preferential toxicity to mosquito larvae. *J Invert Pathol* 1980; *36:* 180–6.
7. Balaraman K, Hoti SL, Manonmani LM. An indigenous virulent strain of *Bacillus thuringiensis,* highly pathogenic and specific to mosquitoes. *Curr Sci* 1981; *50:* 199–200.
8. Padua LE, Gabriel BP, Aizawa K, Ohba M. *Bacillus thuringiensis* isolated in the Philippines. *Philipp Entomol* 1982; *5:* 199–208.
9. Weiser J, Prasertphon S. Entomopathogenic sporeformers from soil samples of mosquito breeding habitats in northern Nigeria. *Zbl Mikrobiol* 1984; *139* : 49-55.
10. Weiser J, Mehtha V, Gelbic J, Miu UT. A mosquito pathogenic strain of *Bacillus thuringiensis* isolated from a silkworm rearing in Vietnam. *Folia Parasitologica* 1985, *32:* 284.
11. Manonmani LM, Hoti SL, Balaraman K. Isolation of mosquito pathogenic *Bacillus thuringiensis* strains from mosquito breeding habitats in Tamil Nadu. *Indian J Med Res* 1987; *86*: 462–8.
12. Brownbridge M, Margalit J. Identification of *Bacillus thuringiensis* strains toxic to mosquitoes recently isolated in Israel. *J Invert Pathol* 1987; *50*: 322–3.
13. Ohba M Aizawa K. Occurrence of two pathotypes in *Bacillus thuringiensis* subsp. *fukuokaensis* (flagellar serotype 3a:3d:3e). *J Invert Pathol* 1990; *55:* 293–4.
14. Abdel Hameed A, Leake C, Carlberg G, Niemela SL, El-Tayer OM. Studies on *Bacillus thuringiensis* H14 strains isolated in Egypt,VII. Toxicity to insect resistant and susceptible strains of mosquitoes. *Environ Toxicol Water Quality* 1994; *9:* 109–13.
15. Seleena P, Lee HL, Lecadet MM. A new serovar of *Bacillus thuringiensis* possessing 28a28c flagellar antigenic structure: *Bacillus thuringiensis* serovar *jegathesan*, selectively toxic against mosquito larvae. *Mosq News* 1995; *11*: 471–3.
16. Ohba M, Saitoh H, Miyamoto K, Higuchi K, Mizuki E. *Bacillus thuringiensis* serovar *higo* (flagellar serotype 44), a new serogroup with a larvicidal activity preferential for the anopheline mosquito. *Lett Appl Microbiol* 1995; *21:* 316–8.
17. Sun Ming, Luo Xixia, Daj JingYuan, Qu KeHul, Liu Zido, Yu Ling, Chen Yahua, Yu ZiNiu. Evaluation of *Bacillus thuringiensis* and *Bacillus sphaericus* strains from Chinese soils toxic to mosquito larvae. *J Invert Pathol* 1996; *68*: 74–7.
18. Lonc E, Lecadet MM, Lachowicz TM, Panek E. Description of *Bacillus thuringiensis wratislaviensis* (H47), a new serotype originating from Wroclaw (Poland) and other *Bt* soil isolates from the same area. *Lett Appl Microbiol* 1997; *24:* 467–73.
19. Maeda M, Mizuki E, Nakamura Y, Hatano T, Ohba M. Recovery of *Bacillus thuringiensis* from marine sediments of Japan. *Curr Microbiol* 2000; *40*: 418–22.

20. Ohba M, Wasano N, Mizuki E. *Bacillus thuringiensis* soil populations naturally occurring in the Ryukyus, a subtropic region of Japan. *Microbiol Res* 2000; *155:* 17–22.

21. Maeda M, Mizuki E, Hara M, Tanaka R, Akao T, Yamashita S, Ohba M. Isolation of *Bacillus thuringiensis* from intertidal brackish sediments in mangroves. *Microbiol Res* 2001; *156:* 195–8.

22. Lee IH, Je YH, Chang JH, Roh JY, Oh HW, Lee SG, Shin SC, Boo KS. Isolation and characterization of a *Bacillus thuringiensis* sp. *kurstaki* strain toxic to *Spodoptera exigua* and *Culex pipiens*. *Curr Microbiol* 2001; *43:* 284–7.

23. Manonmani AM, Balaraman K. A highly mosquitocidal *Bacillus thuringiensis* var *thompsoni*. *Curr Sci* 2001; *80* : 779–81.

24. Lonc E, Doroszkiewicz W, Klowden MJ, Rydzanicz K, Galgan A. Entomopathogenic activities of environmental isolates of *Bacillus thuringiensis* against dipteran larvae. *J Vect Ecol* 2001; *26:* 15–20.

25. Khyami-Horani H, Hajaij M, Charles JF. Characterization of *Bacillus thuringiensis* ser. *jordanica* (serotype H71), a novel serovariety isolated in Jordan. *Curr Microbiol* 2003; *47:* 26–31.

26. Quesada-Moraga E, Garcia-Tovar E, Valverde-Garcia P, Santiago-Alvarez C. Isolation, geographical diversity and insecticidal activity of *Bacillus thuringiensis* from soils in Spain. *Microbiol Res* 2004; *159:* 59–71.

27. Prabakaran G, Geetha I, Padmanabhan V, Paily KP, Balaraman K. Isolation of *Bacillus thuringiensis* var *israelensis/ tochigiensis*, serotype H14/H19 from the phylloplane of mangrove forests (Unpublished).

28. Lopez-Meza J, Federici BA, Poehner WJ, Martinez-Castillo, Ibarra JE. Highly mosquitocidal isolates of *Bacillus thuringiensis* subspecies *kenyae* and *entomocidus* from Mexico. *Biochem Systemat Ecol* 1995; *23*: 461–8.

29. Manonmani LM, Rajendran G, Balaraman K. Isolation of mosquito-pathogenic *Bacillus sphaericus* and *Bacillus thuringiensis* from the root surface of hydrophytes. *Indian J Med Res* 1991; *93*:111–4.

30. Maduell P, Callejas R, Cabrera KR, Armengol G, Orduz S. Distribution and characterization of *Bacillus thuringiensis* on the phylloplane of species of piper (Piperaceae) in three altitudinal levels. *Microb Ecol* 2002; *44:* 144–53.

31. Lee DH, Cha IH, Woo DS, Ohba M. Microbial ecology of *Bacillus thuringiensis*: fecal populations recovered from wildlife in Korea. *Curr Microbiol* 2003;*49:* 465–71.

32. Lee DH, Shisa N, Wasano N, Ohgushi A, Ohba M. Characterization of flagellar antigens and insecticidal activities of *Bacillus thuringiensis* populations in animal feces. *Curr Microbiol* 2003; *46:* 287–90.

33. Ohba M, Lee DH. *Bacillus thuringiensis* associated with faeces of the Kerama-jika, Cervus Nippon keramae, a wild deer indigenous to the Ryukyus, Japan. *J Basic Microbiol* 2003; *43:* 158–62.

34. Alfazairy AA. The pathogenicity of *Bacillus thuringiensis* isolated from the casuarinas stem borer *Stromatium fulvum* Vill (Coleoptera: Cerambycidae) for larvae of two species of mosquitoes. *Insect Sci Appl* 1986; *7*: 633–6.

35. Cavados CF, Fonseca RN, Chaves JQ, Rabinovitch L, Araujo-Coutinho CJ. Identification of entomopathogenic *Bacillus* isolated from *Simulium* (Diptera, Simuliidae) larvae and adults. *Mem Inst Oswaldo Cruz* 2001; *7:* 1017–21.

36. Ishimastu T, Mizuki E, Nishimura K, Akao T, Saitoh H, Higuchi K, Ohba M. Occurrence of *Bacillus thuringiensis* in fresh waters of Japan. *Curr Microbiol* 2000; *40:* 217–20.

37. Kronstad JW, Schnepf HE, Whiteley HR. Diversity of location of *Bacillus thuringiensis* crystal protein genes. *J Bacteriol* 1983; *154*: 419–28.

38. Gonzalez JM Jr, Carlton BC. A large transmissible plasmid is required for crystal toxin production in *Bacillus thuringiensis* variety *israelensis*. *Plasmid* 1984; *11:* 28–38.

Corresponding author: Dr. K. Balaraman, Deputy Director (SG), Division of Product Development, Vector Control Research Centre (ICMR), Indira Nagar, Pondicherry–605 006, India
e-mail: vcrc@vsnl.com