5 Natural Insecticides from Actinomycetes and Other Microbes for Vector Mosquito Control

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Abstract

Mosquitoes are the most dreadful bloodsucking insects in the world, and though tiny in size, they inflict most human deaths worldwide. They transmit deadly pathogens like *Plasmodium*, chikungunya virus, yellow fever virus, dengue virus, Japanese encephalitis virus and West Nile virus. Worldwide, there are 3500 species of mosquitoes grouped into 41 genera, but only 100 species are reported as vectors of human and other vertebrate diseases. India contributes nearly 34 % of global dengue and 11 % of global malaria cases. During the year 2012, nearly 1.13 million people were infected with dengue, malaria and chikungunya in India, and 766 succumbed to these diseases. In India, three genera, namely, *Aedes*, *Anopheles* and *Culex*, are the most common groups of mosquitoes found almost in all regions. *Aedes* spp. transmit dengue, chikungunya and yellow fever, *Anopheles* spp. transmit malaria, and *Culex* spp. transmit filariasis and Japanese encephalitis. In recent years, a decrease in the malaria and filariasis cases has been reported, but the number of infected cases and mortality due to dengue is steadily increasing. The failure in mosquito control is mainly due to the inefficiency of synthetic pesticides and repellents. Mosquitoes have developed resistance to almost all types of chemical insecticides. The increasing number of mosquito breeding sites and the destruction of mosquitoes' natural enemies are also contributing to the sudden rise in mosquito population and mosquito-borne diseases. Application of synthetic chemicals in water bodies is unsafe to humans

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and nontarget organisms. Microbial pesticides and botanical pesticides are eco-friendly and target specific compared to synthetic pesticides. Microbial pesticides obtained from actinomycetes, *Bacillus thuringiensis* (*Bt*), *B. sphaericus* (*Bs*) and many other microorganisms are reported as eco-friendly alternatives for mosquito control. A large number of *Bt* strains have been reported to possess insecticidal properties against different groups of insects. *B. thuringiensis israelensis* (*Bti*) is an important pathogenic bacterium to mosquitoes. The secondary metabolites of some microorganisms are potential toxins against mosquito larvae at very low concentrations. Spinosad, a potent insecticide, has been isolated from the actinomycete bacterium *Saccharopolyspora spinosa*. In this review, potentially effective actinomycetes and other microorganisms against mosquito larvae and their effective bioactive compounds are described. The review also presents up-to-date information on the efficacy of microbial pesticides in mosquito control, their biosafety, field efficacy and commercial applications.

5.1 Introduction

Mosquitoes, the tiny dipteran insects, are known as the deadliest insects in the world, because they transmit lethal pathogens from one human to the other and kill millions of people every year. They have killed more people than all the wars in history. Malaria is the most dreadful mosquito-borne disease in the world, and in 2012, there was an estimated 627,000 malaria deaths and about 207 million malaria cases in the world (WHO 2013). Moreover, tens of millions of people are killed and harmed by other mosquito-borne diseases, namely, dengue, encephalitis, yellow fever, filariasis and chikungunya. Mosquitoes are highly adaptable to anthropogenic impacts on their habitats. Unlike other aquatic insects, mosquitoes can utilize a variety of aquatic habitats such as freshwater pools, ponds, brackish water, overhead tanks, sewage waters, rain water in small containers and tyres and drainage water from refrigerators and air conditioners for their development.

Several vector-borne diseases are emerging in new areas in the world, mainly due to the increasing anthropogenic activities and climate change (Patz et al. 2005; Pascual et al. 2006; Nerio et al. 2010). Outbreak of many vector-borne diseases like malaria and dengue is on the rise in the developing world. Man is fighting against mosquitoes for many centuries, but the war is not winnable. To escape from mosquito-borne diseases, we are following two main measures, namely, mosquito population eradication and personal protection. In mosquito eradication programmes, they are killed at their adult stage or immature stages. Adulticiding is mainly done in malaria control programmes, and larval control is done to eradicate filariasis, dengue and encephalitis (Mulla 1991).

Mosquito eradication and personal protection are largely relying upon synthetic chemicals. Controlling mosquito larvae at their breeding site depends on the application of chemical larvicides to water. The early larvicides such as DDT, BHC and methoxychlor were found to be ineffective after some years due to the development of pesticide resistance in mosquitoes. Synthetic chemicals such as chlorpyrifos, diflubenzuron, malathion, methoprene, pyriproxyfen, permethrin, petroleum oils, temephos and resmethrin are used to eradicate mosquitoes at the larval and adult stages (Brattsten et al. 2009).

Even though synthetic mosquitocides instantly kill mosquitoes, they leave behind many unwanted effects like environmental pollution and nontarget effects on humans and other organisms (Paulraj et al. 2011). Synthetic pesticides also cause the development of pesticide resistance in mosquitoes (Charles and LeRoux 2000). Due to these unwanted effects of synthetic chemicals,

researchers are trying to formulate eco-friendly and target-specific pesticides especially from natural resources. Plants and microbes are promising sources of natural pesticides against agricultural pests and vector insects. Mosquito control properties of plant products (Zarroug et al. 1988; Ignacimuthu 2000; de Luna et al. 2005; Maheswaran et al. 2008; Mathew et al. 2009; Patil et al. 2010; Ramar et al. 2013a, b; Rajiv Gandhi et al. 2014; Reegan et al. 2013a, 2014a, b; Sivaraman et al. 2014) and microorganisms (Des Rochers and Garcia 1983; Lee and Zairi 2006; Rydzanicz et al. 2010; Rashad et al. 2012; Poopathi et al. 2014) have been extensively studied. In this review, the toxic principles reported in mosquitocidal bacteria, their mode of action, residual toxicity, nontarget effect and their importance in eco-friendly mosquito control at present and in the future are discussed.

5.2 Biorational Mosquitocides

Nature is providing abundant sources of beneficial molecules to be utilized by man for his welfare. Plants and microorganisms are important natural sources because they possess diverse groups of molecules and are easily available. Secondary metabolites of plants and microbes show many biological properties. In plants the secondary metabolites play an important role in plant defence against pathogens and herbivory. Researchers have found that the secondary metabolites of plants and microbes can be used as potential pesticides against vector mosquitoes and agricultural pests. Plant secondary metabolites such as alkaloids, phenolics and terpenoids have been extensively studied for their mosquito control properties (Lee 2000; Bilal et al. 2012; Liu et al. 2012; Gautam et al. 2013). Larvicidal effect of plant extracts against vector mosquitoes has been reported by many investigators (de Luna et al. 2005; Maheswaran et al. 2008; Mathew et al. 2009; Patil et al. 2010; Ramar et al. 2013a, b; Rajiv Gandhi et al. 2014; Reegan et al. 2013a, 2014a, b; Sivaraman et al. 2014). Ramar et al. (2014) have reported that essential oils of aniseed, calamus, cinnamon, clove, lemon, orange, thyme, tulsi and vetiver presented larvicidal activity against *Cx. quinquefasciatus*, and the toxicity was very high in clove and tulsi oil treatments. Reegan et al. (2014a) have isolated a protolimonoid compound, niloticin from *Limonia acidissima*. This compound showed 100 % larvicidal activity against *Ae. aegypti* at 2 ppm concentration. Niloticin also showed pupicidal, ovicidal, oviposition deterrent and growth-regulating activities at 2 ppm concentration.

Biological pest control is one of the eco-friendly methods, which involves the mass culture of biocontrol agents and release in infested areas. After the work of Bassi (1836), who identified *Beauveria bassiana* as a pathogen of silkworm, and the investigations of Louis Pasteur on different diseases of the silkworm, scientists concluded that microorganisms could be used to control insect pests (Johnson 1998). Biocontrol agents of mosquitoes include viruses, bacteria, fungal pathogens, nematodes, predatory invertebrates and vertebrates like fish. Some of the biocontrol agents like nematodes, predatory invertebrates and mosquito fish are less utilizable considering the difficulties in mass multiplication (Usta 2013). But bacteria and their toxins can be produced in large quantities at laboratories and industries. Innumerable bacterial species are present on earth, which provide chances of discovering new mosquitocidal agents.

Besides plants and microbes, some more natural sources also possess mosquito larvicidal and repellent principles. Reegan et al. (2013b, 2015) have studied the larvicidal effect of marine sponge *Cliona celata* against *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes. Some synthetic derivatives of plant and microbial compounds are also reported as potential mosquito larvicides and adulticides. Paulraj et al. (2011) screened benzaldehyde and propionic acid for larvicidal, pupicidal and adult knock-down effects against *Ae. aegypti* and *Cx. quinquefasciatus*. Benzaldehyde killed 50 % populations of *Ae. aegypti* and *Cx. quinquefasciatus* at concentrations of 30.39 and 40.48 ppm, respectively. Benzaldehyde is a major compound in almond oil, and propionic acid is produced by *Propionibacterium* found in the sweat glands of humans.

5.3 Microbial Pesticides

Many bacterial strains with larvicidal activities have been identified, and some biopesticides have been formulated using their toxic principles for the eradication of mosquito larvae in their breeding places such as flood water, ponds, irrigation ditches, woodland pools, tidal water and fresh- or saltwater marshes. The most common bacterial strains that are reported as lethal to mosquitoes are *Bacillus thuringiensis (Bt)*, *B. thuringiensis* var. *israelensis* (*Bti*), *Lysinibacillus sphaericus* or *B. sphaericus*, some other strains in *B. thuringiensis* serotypes and *Clostridium bifermentans* serovar *malaysia* (Porter et al. 1993; WHO 1999; Foda et al. 2010). Among them, *B. thuringiensis israelensis* (*Bti*) and *B. sphaericus* (*Bs*) are widely exploited against different mosquito species. *Bt* was first isolated by Ishiwata from the mulberry silkworm *Bombyx mori* in 1901 (Ishiwata 1901). *Bt* was first scientifically described by Berliner in Germany in 1911. *Bti* was first discovered in 1976 in the Negev Desert of north-central Israel and was found to be useful to control mosquito and black fly (Margalit and Dean 1985). *Bti* is a spore-forming bacterium naturally found in soil and aquatic environments. *Bti* shows different levels of larval toxicity against different mosquito genera. *Culex* and *Aedes* were found to be highly susceptible to *Bti*, whereas *Anopheles* was less susceptible (WHO 1985; Charles et al. 1996). Furthermore, it shows species-specific activity within one genus of mosquito (Chui et al. 1995). *Bti* was found to be specifically toxic to larvae of 109 mosquito species Glare and O'Callaghan (1998)

B. sphaericus produces binary toxin during sporulation (Broadwell and Baumann 1986; Charles et al. 1988), and this binary toxin is composed of two polypeptides, namely, BinA (molecular weight, 41.9 kDa) and BinB (51.4 kDa) (Smith et al. 2004). The amino acid sequences of these two polypeptides are not similar to the amino acid sequence of crystal proteins of *B. thuringiensis*. The binary toxin, BinA and BinB, forms microcrystalline inclusions inside the mother cell and will be solubilized in the alkaline pH of the mosquito larval gut, if ingested (Smith et al. 2004). Rungrod et al. (2009) have stated that the mosquitocidal toxins, namely, Mtx1 and Mtx2, were species specific and very toxic against *Cx. quinquefasciatus* and *Ae. aegypti*, respectively. They cloned *mtx*1 and *mtx*2 genes into a single plasmid and expressed in *Escherichia coli*. The cells produced both Mtx1 and Mtx2 toxins and recorded high synergistic activity against *Ae. aegypti* larvae nearly 10 times more compared to the activity of a single toxin.

The toxic properties of these bacteria against mosquito larvae are due to the production of protein inclusion bodies during sporulation. These toxins are highly lethal to the larvae of mosquitoes, black flies some closely related dipteran flies when ingested (Gibbs et al. 1986). *Bt* produces 'Cry' (crystal) and 'Cyt' (cytolytic) toxins, and *Bs* produces 'Bin' (binary) and 'Mtx' (mosquitocidal) toxins (Charles et al. 1996; Charles and LeRoux 2000; Federici et al. 2003) (Table 5.1). It has been reported that *Bti* is producing different groups of toxic proteins, namely, Cry4Aa, Cry4Ba, Cry10Aa, Cry11Aa, Cyt1Aa and Cyt2Ba (Berry et al. 2002). The larvicidal effect of these bacterial strains depends mainly upon the mosquito species and the environmental conditions. One important advantage of microbial larvicides is that they can be used along with other mosquito control measures in integrated pest management (IPM) programmes.

Several *Bt* strains with mosquito larvicidal activity have been isolated after the discovery of *Bti*. The strains differ from each other by their mosquito larvicidal activity, serotype and polypeptide composition. Plenty of work has been done on isolation, larvicidal screening and residual efficacy of *Bti* and *Bs* against vector mosquitoes. Many reviews and research articles have been published on these two bacterial pesticides. The species-specific activities, nontarget effects and efficacy in integrated control strategies of these two microbes have been well documented.

In a review, Mulla (1991) has documented the larvicidal effects of *B. thuringiensis* and *B. sphaericus* against different mosquito species in laboratory and open field conditions. He also discussed the factors influencing the efficacy and

Table 5.1 Larvicidal activity of Bin, Cry and Cyt proteins against different mosquito species

Name of bacterial toxin	Bacterial strain	Target mosquito species	Reference
Bin	*Bacillus sphaericus* WBM 1-1-13	*Ochlerotatus taeniorhynchus*, *Culex quinquefasciatus*	Park et al. (2007)
BinAB (recombinant)	*B. sphaericus* 2362 SPH-28 (expressed in *Escherichia coli)*	*Culex quinquefasciatus*	Pinto da Silva et al. (2011)
Cry2Aa1	*Bacillus thuringiensis kurstaki* HD-1, HD-263	*Aedes aegypti*	Zeigler (1999)
Cry4Aa1	*Bt israelensis* 4Q2-72	*Anopheles stephensi*, *Aedes aegypti*, *Culex pipiens*	Zeigler (1999)
Cry4Ba1	*Bt israelensis* 4Q2-72	*Aedes aegypti* (Diptera: Culicidae)	Zeigler (1999)
Cry10Aa1	*Bt israelensis* ONR60A	*Aedes aegypti* (Diptera: Culicidae)	Zeigler (1999)
Cry11Aa1	*Bt israelensis* HD-567	*Anopheles stephensi*, *Aedes aegypti*, *Culex pipiens*	Zeigler (1999)
Cry11Ba1	*Bt jegathesan* 367	*Anopheles stephensi*, *Aedes aegypti*, *Culex pipiens* (Diptera: Culicidae)	Zeigler (1999)
Cry11Bb1	*Bt medellin*	*Anopheles albimanus*, *Aedes aegypti*, *Culex quinquefasciatus* (Diptera: Culicidae)	Zeigler (1999)
Cry16Aa1	*Clostridium bifermentans malaysia* CH18	*Anopheles stephensi*, *Aedes aegypti*, *Culex pipiens* (Diptera: Culicidae)	Zeigler (1999)
Cry19Aa1	*Bt jegathesan*	*Anopheles stephensi*, *Culex pipiens* (Diptera: Culicidae)	Zeigler (1999)
Cry20Aa1	*Bt fukuokaensis*	*Aedes aegypti* (Diptera: Culicidae)	Zeigler (1999)
Cry21Aa1	*Bt higo*	*Culex pipiens molestus* (Diptera: Culicidae)	Zeigler (1999)
Cry11, C*ry30*	*Bt* 147-8906	*Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles albimanus*	Ibarra et al. (2003)
Cyt1Aa1	*Bt israelensis* IPS82	*Anopheles stephensi*, *Aedes aegypti*, *Culex pipiens* (Diptera: Culicidae)	Zeigler (1999)
Cyt1Ab1	*Bt medellin* 163-131	*Anopheles stephensi*, *Aedes aegypti*, *Culex pipiens* (Diptera: Culicidae)	Zeigler (1999)
Cyt2Aa1	*Bt kyushuensis*	*Anopheles stephensi*, *Aedes aegypti*, *Culex pipiens* (Diptera: Culicidae)	Zeigler (1999)
Cyt1 and C*yt2*	*Bt* 147-8906	*Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles albimanus*	Ibarra et al. (2003)

nontarget effects of these two microorganisms. In Africa, *Bs* and *Bti* are reported as promising biocontrol agents of major vectors of malaria (Fillinger and Lindsay 2006; Fillinger et al. 2003; Majambere et al. 2007).

Studies on mosquito larvicidal potential of actinomycetes are scanty. A few studies indicate that secondary metabolites of actinomycetes are potent mosquito larvicides. Kumar et al. (2011) have isolated a compound, namely, 5-(2,4-dimethylbenzyl) pyrrolidin-2-one, from a marine *Streptomyces* sp. This compound recorded 100 % larvicidal activity against *An. stephensi* and *Cx. tritaeniorhynchus* in 24 h.

5.4 Commercial Larvicidal Products from Microbes

During the last three decades, different types of formulations were developed using different subspecies of *B. thuringiensis* against vector mos-

quitoes. Some of these biopesticides showed very high efficiency against target mosquitoes. The two species of *Bacillus*, namely, *B. thuringiensis israelensis* and *B. sphaericus*, are the main ingredients in the biolarvicides, which are commercially available to control mosquitoes. Table 5.2 shows some of the commercially available biolarvicides and their target mosquito species.

Bti was first registered in 1983 as an insecticide by the United States Environmental Protection Agency (US EPA). Nearly 25 *Bti* products have been registered in the USA. AquaBac, Teknar, VectoBac and LarvX are common trade names of some of the mosquito control products (US EPA 2000). *Bs* was first registered by US EPA in 1991 for eradicating different species of mosquitoes. VectoLex CG and WDG are registered *Bs* products, which are effective for nearly 1–4 weeks after application in the larval habitats (US EPA 2000).

Djènontin et al. (2014) have evaluated the larvicidal activity of VectoBac GR (potency 200 ITU/mg) prepared from *Bti* strain AM65-52 against *An. gambiae* and *Cx. quinquefasciatus* in simulated field and natural habitats in Benin. They found that VectoBac GR caused emergence inhibition of ≥80 % until 21 days for *An. gambiae* at 1.2 g/m^2 dose and 28 days for *Cx. quinquefasciatus* at 2 g/m^2 in simulated field habitats. They also reported that the efficacy of VectoBac GR in natural habitat was for 2–3 days against larvae and up to 10 days against pupae. Fillinger et al. (2003) have studied the larvicidal potential of VectoBac and VectoLex against *Anopheles gambiae*. They found that *An. gambiae* was more susceptible to VectoLex (*B. sphaericus* as ingredient) than VectoBac. Majambere et al. (2007) have reported that both VectoBac and VectoLex were effective in controlling *An. gambiae*.

Mousticide is a combination of TMOF (trypsin-modulating oostatic factor) and *B. thuringiensis israelensis* (Bti) serotype H-14. TMOF is a natural decapeptide hormone synthesized by the ovaries and the neuroendocrine system of mosquitoes. TMOF stops protein digestion in mosquito larvae and causes larval death. When TMOF is combined with Bti, it yields a potential product with synergistic effect of more than 200×.

Since our country has a rich source of plants and microbes, there is a scope for finding numerous active principles from plants and microbes for the purpose of mosquito eradication/management (Ignacimuthu and Paulraj 2009).

5.5 Actinomycetes: Promising Sources of Active Compounds for Mosquito Control

Actinomycetes are gram-positive soil bacteria. They contain high GC content in their DNA. Actinomycetes, particularly the genus *Streptomyces*, produce many economically important secondary metabolites (Subramani and Aalbersberg 2012). Very few actinomycetes like *Mycobacterium tuberculosis* are pathogenic to humans. But a large number are very useful to humans, because they produce useful compounds with antibiotic, antifungal, antitumor, immunosuppressive and pesticidal properties. The active compounds of actinomycetes are present in the extracellular metabolites secreted by them in the culture media (Bode et al. 2002). Actinomycetes synthesize the secondary metabolites when their growth is slowing or stopped (Doull and Vining 1990; Sanchez and Demain 2002).

The antimicrobial properties of secondary metabolites of actinomycetes are well studied (Chaudhary et al. 2013; Rana and Salam 2014; Phongsopitanun et al. 2014). In recent years, researchers are interested to examine the acute and chronic toxicities of actinomycetes on different vector mosquitoes. Many studies have proven that actinomycetes were toxic to different mosquito spp. Vijayakumar et al. (2010) screened 30 actinomycetes isolated from soil samples from Muthupet mangrove forest, Tiruvarur District, against *Anopheles* mosquito larvae. They used the culture filtrate for larvicidal screening and found that 23 isolates presented larvicidal activity, among which 2 isolates were significantly effective.

Table 5.2 Some of the *Bt*-based commercial microbial larvicides used against vector mosquitoes

Sl. no.	Trade name of microbial pesticide	Formulation type	Active ingredient	Name of the manufacturer	Target mosquito species
1	AquaBac® XT	AS	*Bacillus thuringiensis* subsp. *israelensis*, serotype H-14, strain BMP-144	Becker Microbial Products, Inc., USA	*Aedes* spp., *Culex* spp., *Psorophora columbiae*
2	AquaBac® (200G)	G	*Bacillus thuringiensis* subsp. *israelensis*, serotype H-14, strain BMP-144	Becker Microbial Products, Inc., USA	*Aedes* spp., *Culex* spp., *Psorophora columbiae*
3	Bacticide®	WP	*Bacillus thuringiensis* var. *israelensis*, serotype H-14, strain 164	Biotech International Ltd., India	*Aedes* spp., *Anopheles* spp., *Culex* spp., *Culiseta* spp., *Psorophora* spp., *Uranotaenia* spp., *Mansonia* spp.
4	Bactimos®	Pellets, tablets	*Bacillus thuringiensis* var. *israelensis*	Valent BioSciences Corporation	Mosquitoes and black flies
5	Bti (AS, WP)[a]	AS, WP	Bti	Kilpest India Ltd.	*Aedes*, *Culex*, *Anopheles*
6	Introban®	AS	Bti	Valent BioSciences Corporation, USA	Mosquitoes
7	Mousticide™	WP	*Bacillus thuringiensis israelensis* (Bti) serotype H-14	EntoGeneX, Malaysia	Mosquitoes
8	Skeetal®	FC	*Bacillus thuringiensis* var. *israelensis*	Renovita	Mosquitoes
9	Sphericide®	WP	*Bacillus sphaericus*; serotype H-5a, 5b; strain B-101	Biotech International Ltd., India	*Aedes* spp., *Anopheles* spp., *Culex* spp., *Culiseta* spp., *Psorophora* spp., *Uranotaenia* spp., *Mansonia* spp.
10	Teknar®	SC	*Bacillus thuringiensis* var. *israelensis*	Valent BioSciences Corporation	Mosquitoes
11	VectoBac®	WDG	*Bacillus thuringiensis* subsp. *israelensis*, serotype H-14, strain HD-14	Valent BioSciences Corporation	Mosquitoes and black flies
12	VectoBac®	AS	*Bacillus thuringiensis* subsp. *israelensis* strain AM65-52	Valent BioSciences Corporation	Mosquitoes and black flies
13	VectoLex®	WDG	*Bacillus sphaericus*	Valent BioSciences Corporation	*Culex* spp., *Psorophora* spp., *Culiseta* spp.
14	VectoMax®	WSP	*Bacillus thuringiensis* var. *israelensis*, *Bacillus sphaericus*	Valent BioSciences Corporation	Mosquitoes
15	VectoPrime®	FG	*Bti* strain AM65-52 with (S)-methoprene	Valent BioSciences Corporation	Mosquitoes

AS aqueous suspension, *FG* fine granules, *G* granules, *SC* suspension concentrate, *WDG* water-dispersible granules, *WP* wettable powder, *WSP* water-soluble pouch

[a]No trade name

In India, some investigators have explored the anti-insect properties of actinomycete metabolites against insects including mosquitoes. Mishra et al. (1987) have reported that metabolites of actinomycetes are potential alternatives to synthetic insecticides. Rao et al. (1990) have reported the isolation of active compounds from actinomycetes against mosquitoes. Vijayan and Balaraman (1991) have studied the ovicidal, larvicidal and adulticidal activities of the secondary metabolites of fungi and actinomycetes against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*. They reported that the metabolites of 34 fungi and 3 actinomycetes, 133 fungi and 35 actinomycetes and 17 fungi were found to kill the eggs, larvae and adults, respectively. Dhanasekaran et al. (2010) have isolated 30 actinobacteria from Muthupet mangrove environment. Four isolates belonging to the genera *Streptomyces*, *Streptosporangium* and *Micropolyspora* showed strong larvicidal activity against *Anopheles* larvae.

Gadelhak et al. (2005) have isolated three efficient chitinase enzyme producing actinomycetes from 38 different strains collected from the United Arab Emirates soil. They found that the application of two isolates *Streptomyces clavuligerus* and *Actinoplanes philippinensis* in combination gave higher effects as this treatment reduced the pupation of *Drosophila melanogaster*. The compounds, namely, tetranectin (Ando 1983), avermectins (Pampiglione et al. 1985), faeriefungin (Anonymous 1990) and macrotetrolides (Zizka et al. 1989), are produced by *Streptomyces aureus*, *Streptomyces avermitilis*, *Streptosporangium albidum* and *Streptomyces griseus*, respectively. These compounds were reported to be lethal to different mosquito species.

There is a big scope for isolating potential actinomycete strains with significant mosquito control property from forest, desert, mangrove and marine environments. Our recent studies have resulted in the identification of 8 potential actinomycete isolates from a total of 283 pure isolates obtained from soil samples collected from Nilgiris and Kalakkad Mundanthurai Tiger Reserve in Tirunelveli District. An important finding in this study was that the active isolates showed species-specific activity against different mosquito species. Among the eight active isolates, CFR-16 (collected from Coonoor forest soil, Nilgiris) was found to be the most effective isolate against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Based on 16S rRNA characterization studies, the most effective isolate (CFR-16) was identified as a *Streptomyces* sp. (Fig. 5.1) (unpublished data).

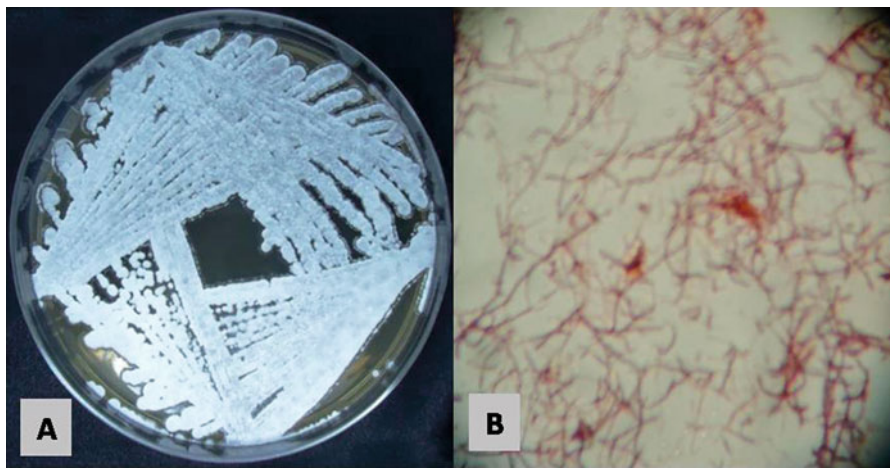

Fig. 5.1 The active isolate, *Streptomyces* sp. (CFR-16 Strain) collected from Coonoor Forest soil, Nilgiris, Tamil Nadu. (**a**) Surface colony morphology of *Streptomyces* sp. (CFR-16) AIA. (**b**) Gram stained photomicrograph of *Streptomyces* sp. (CFR-16) (100×)

5.6 Spinosad: A Promising Molecule from Actinomycetes for Mosquito Control

Spinosad is a biorational insecticide produced during the fermentation of the actinomycete *Saccharopolyspora spinosa*. Spinosad is a mixture of two tetracyclic macrolide neurotoxins, namely, spinosyns A and D. It targets the nicotinic acetylcholine and GABA receptors of the insect's nervous system, which leads to paralysis and death (Salgado 1997, 1998).

Spinosad is currently used to control coleopteran, dipteran, lepidopteran and thysanopteran pests of agricultural and forestry plants in different countries (Biondi et al. 2012). Spinosad has very little toxicity to vertebrates and has been approved for use against mosquito larvae in drinking water (WHO 2010). The United States Environmental Protection Agency (US EPA) has classified spinosad as a reduced-risk material due to its very low mammalian toxicity and favourable ecotoxicological profile (Thompson et al. 2000). Spinosad was registered in 1997 under the trade name Tracer®. It was found to be effective in reducing the development of immature stages of *Ae. aegypti*, *Ae. albopictus*, *An. gambiae*, *An. pseudopunctipennis*, *An. albimanus*, *Cx. pipiens* and *Cx. quinquefasciatus* (Hertlein et al. 2010). Spinosad is primarily a stomach poison with some contact activity and is particularly active against Lepidoptera, Diptera, some Coleoptera, termites, ants and thrips (Bret et al. 1997). Exposure resulted in cessation of feeding followed later by paralysis and death. Due to its selective toxicity and its favourable environmental profile, spinosad is considered by IPM practitioners as an important new-generation biorational pesticide (Schneider et al. 2004).

Many investigators have reported spinosad as a potentially valuable tool for the control of different vector mosquito species (Bond et al. 2004; Darriet et al. 2005; Romi et al. 2006). Marina et al. (2012) have studied the efficacy of spinosad against *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus* and *Cx. coronator* larval control in car tyres in southern Mexico. Much of the toxicity studies of spinosad on mosquitoes have been conducted under laboratory conditions; very few studies have been done in natural habitats of mosquitoes. Bond et al. (2004) have reported that spinosad at 1 ppm resulted in complete inhibition of reproduction of *Ae. aegypti* and *Culex* spp. for 8 and 15 weeks, respectively, in field trials. At 10 ppm concentration, spinosad completely eliminated reproduction of both mosquitoes during the entire period of 22 weeks.

5.7 Residual Toxicity of Bacterial Toxins on Mosquitoes

Jahan et al. (2013) have studied the residual toxicity of *B. thuringiensis* var. *israelensis* (technical powder and water-dispersible granules) and *B. sphaericus* against laboratory-reared *An. stephensi* and field-collected *Cx. quinquefasciatus* larvae. They reported that the residual toxicity decreased with decreasing concentrations. The residual activity of *B. thuringiensis israelensis* technical powder varied from 1 to 51 days against laboratory-reared *A. stephensi* larvae at 0.0001 and 100 ppm concentrations, respectively. *B. sphaericus* technical powder had a residual effect for 2–18 days at 0.0001 and 100 ppm concentrations, respectively, against the same species.

Lee and Zairi (2006) have studied the residual efficacy of *B. thuringiensis* H-14 against *Aedes* mosquitoes at field conditions with two different test designs. In one design, treated water was replenished daily with seasoned water, and in the other one, treated water was not replenished, but evaporated water was replenished. They reported that *Bt* showed a residual effect against *Aedes* mosquito larvae up to a period of 40 days with 80 % mortality, and the residual effect continued up to 60 days of study, but the larval mortality was reduced below 54 %. When the treated water was daily replenished, 100 % larval mortality was recorded for the first 3 days only. Without daily replenishment of treated water, 100 % larval mortality was recorded for the first 5 days.

Majambere et al. (2007) have tested the larval toxicity and residual effect of formulations of commercial *B. sphaericus* strain 2362 (*Bs*,

VectoLex®) and *B. thuringiensis* var. *israelensis* strain AM65-52 (*Bti*, VectoBac®) against *An. gambiae* in the Gambia. In their study, they found that *Bs* had no residual activity against anopheline larvae. But both microbes presented complete eradication of larvae when applied weekly and recorded 100 % larval mortality at 24–48 h post-application. There was 94 % reduction in pupa development at weekly retreatment intervals. Their results showed that the lethal concentration (LC) to kill 95 % of third instar larvae of *An. gambiae s.s*. after 24 h was 0.023 mg/l (14.9 BsITU/l) for *Bs* water-dispersible granules (WDG) and 0.132 mg/l (396 ITU/l) for *Bti* WDG.

5.8 Mode of Action of Microbial Toxins

The microbial toxins generally damage the gut epithelial cells of mosquito larvae. Singh and Gill (1988) and Poopathi et al. (1999a, b) have studied the cytopathological effects of microbial toxins. The Bin toxin affected the epithelial cells in the midgut of mosquito larvae by binding to Cpm1 (*Culex pipiens* maltase 1), a digestive enzyme, and causes severe intracellular damage, including a dramatic cytoplasmic vacuolation (Opota et al. 2011). Cyt toxins also affect insect midgut cells and are able to increase the insecticidal property of some Cry toxins. Moreover, the Cyt toxins are able to overcome resistance to Cry toxins in mosquitoes Soberón et al. (2013). It was found that Cyt1Aa was able to overcome the resistance to Cry4 or Cry11Aa toxins of the *Cx. quinquefasciatus* populations (Crickmore et al. 1995; Wirth et al. 1997).

5.9 Nontarget Effects of Microbial Larvicides

WHO (1999) has reported that biocontrol agents are better than chemical larvicides since they are very species specific and environmentally safe. Many studies have proven that microbial and botanical larvicides are non-toxic or less toxic to nontarget organisms like natural enemies (Theiling and Croft 1988). All microbial pesticides are thoroughly screened for their safety to nontarget organisms prior to registration. Extensive testing showed that microbial larvicides are safe to wildlife, to nontarget organisms and to the environment. The *Bti* or *B. sphaericus* products are non-toxic to humans when they are used according to the directions given in the label (Miura et al. 1980). An isolate of *B. thuringiensis* designated as PG-14 obtained from the Philippines was highly toxic to the mosquitoes *Ae. aegypti* and *Cx. molestus* but non-toxic to the silkworm, *Bombyx mori*, and adults of a daphnid. The degree of toxicity to mosquito larvae was the same as that of the reference strain of *B. thuringiensis* subsp. *israelensis* (serotype 14) (Padua et al. 1984).

5.10 Development of Actinomycete-Based Pesticides

Development of microbial pesticides, especially actinomycete-based pesticides, involves many steps. The sequence of the steps is sampling, isolation of actinomycetes, preliminary bioassay using optimized culture media, mass production of promising isolate, crude extraction, bioassay of crude extract, bioassay-guided fractionation and isolation of active compound, structural elucidation and identification of active compound, preparation of pesticidal formulation using the active compound, toxicological studies and registration.

The places of sampling of actinomycetes are generally chosen on the basis of certain evidences of the presence of beneficial microorganisms, such as dead arthropods, disease-suppressive soils or healthy plants in epidemic areas (Montesinos 2003). Extreme environments may contain useful actinomycetes. Pilot trials with

pesticide formulation under real conditions of application are very important in which biosafety and nontarget effects of the microbial pesticide should be given priority.

5.11 Registration and Commercialization of Microbial Pesticides

Application of microbial pesticides in mosquito breeding sites is an eco-friendly and efficient way of prevention of mosquito-borne diseases. In India, the manufacture, commercial use, transport, import and distribution of microbial pesticides or any biopesticide fall under the Insecticide Act (1968) under which microbial pesticides should be registered with the Central Insecticides Board (CIB) of the Ministry of Agriculture (Anonymous 2013). Registration of microbial pesticides is mandatory for commercialization in India since 2006. As of October 2009, 14 primary microbial pesticide products and their formulations were registered in India, and nearly 150 companies were involved in the production of microbial pesticides (Devi et al. 2012).

Commercial production of microbial pesticides needs large-scale production of microbes, their preservation, storage at optimum conditions and formulation (Powell and Jutsum 1993). A pesticidal formulation is the process of converting an active compound into a product that can be applied by practical methods to permit its effective, safe and economic use (Taborsky 1992). Before registration of the formulated microbial pesticide, it should be studied for nontarget effects on fishes, birds, earthworms, honeybees and silkworm and for its ecotoxicity. After the completion of required studies, the microbial pesticide formulation should be patented for legal protection. Taborsky (1992) has given a detailed account on production techniques and commercialization of microbial pesticides at small scale.

Cost effectiveness is an important criterion for any pesticide. Economic feasibility is one of the important advantages of microbial pesticides compared to chemical pesticides. Very few investigators have studied the cost effectiveness of microbial pesticides for mosquito control. Fillinger and Lindsay (2006) have reported that the cost of providing protection to human population from *Anopheles* by using *B. thuringiensis* var. *israelensis* and *B. sphaericus* was less than US$0.09/person/year.

5.12 Limitations of Microbial Pesticides and Possible Solutions to Overcome

Some investigators have proposed that environmental factors may affect the effectiveness of microbial pesticides. According to Boisvert (2005), the activity of *Bti* or *Bs* against target organisms can be influenced by environmental factors such as organic pollution, water temperature and the presence of colloidal particles. Rydzanicz et al. (2010) found that sunlight decreased the activity of *Bti* and *Bs* against *Ochlerotatus caspius* mosquitoes.

Another important concern with microbial pesticides is that mosquitoes are developing resistance to certain bacterial toxins. But a study indicated that a combination of *B. sphaericus* 2362 in a 10:1 ratio with a strain of *B. thuringiensis* subsp. *israelensis* that produces Cyt1A reduced resistance by >30,000-fold. Resistance was suppressed completely when *B. sphaericus* was combined with purified Cyt1A crystals in a 10:1 ratio (Wirth et al. 2000).

5.13 Future Prospects of Microbial Control of Mosquitoes

Mulla (1994) has stated that microbial control agents will become important components in vector mosquito control during the first quarter of the twenty-first century. Due to their target-specific activities, non-toxicity to vertebrates and human beings and economic feasibility, microbial pesticides are considered as the most reliable mosquito control agents. The limitations of these

excellent biopesticides should be succeeded in the future. The persistence of microbial pesticides in all types of aquatic habitats for longer duration and their UV stability should be improved. So research should be focused on these aspects in the future.

5.14 Conclusion

In conclusion, microbial pesticides are reliable control agents for mosquito population due to their target-specific effect. Microbial pesticides can be produced in large quantities without disturbing natural resources, and so it will ensure a continuous supply at low cost. Future research should focus on reducing the limitations of microbial pesticides particularly to avoid the pesticide resistance caused by some bacterial toxins by novel techniques. Government should give priority to such research activities to strengthen the mosquito control programme throughout the country.

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