Bacillus thuringiensis: distribution and habitat

LITERATURE REVIEW

For several decades since its discovery, formulations of Bacillus thuringiensis (B. t.) have been seen as the ideal means of controlling Lepidoteran pests in agriculture because of the many attributes that differentiate this microbial insecticide from the synthetic chemical formulations. No toxicity to mammals, environmental friendliness, apparent immunity to the pesticide resistance phenomenon (no longer true), good integration with other pest control methods and the possibility of being mass produced at farm level at low cost, all made B. thuringiensis the much-needed tool for IPM programmes in developing countries. Research of almost 85 years reveals that Bacillus spp., especially B. thuringiensis and Bacillus sphaericus are the most potent biopesticides (Boucias & Pendland, 1998). B. thuringiensis is a species of bacteria that has insecticidal properties that affects a specific range of insect orders. There are at least 34 subspecies of B. thuringiensis (also called serotypes or varieties) and possibly over 800 strain isolates (Swadener, 1994). B. thuringiensis accounts for about 5-8% of Bacillus spp. population in the environment (Hastowo et al., 1992). Till date more than 130 species of lepidopteran, dipteran and coleopteran insects are found to be controlled by B. thuringiensis (Dean, 1984).

Historical Background of B. thuringiensis

B. thuringiensis are interesting and important bacteria used in the biological control of insect pest which form toxic crystal proteins at the time of sporulation. Perhaps the most well known and widely used biopesticide comes from B. thuringiensis, a bacterium that produces insecticidal proteins during its sporulation. This common soil bacterium, most abundantly found in

grain dust from soil and other grain storage facilities, was discovered first in Japan in 1901 by Ishawata and then in 1911 in Germany by Berliner (Baum et al., 1999). It was subsequently found that thousands of strains of B. thuringiensis exist (Lereclus, 1993). The bacterium was isolated from diseased larvae of Anagasta kuehniella, and this finding led to the establishment of B. thuringiensis as microbial insecticide.

The first record of its application to control insects was in Hungary at the end of 1920, and in Yugoslavia at the beginning of 1930s, it was applied to control the European corn borer (Lords, 2005). Sporine which was the first commercial product of B. thuringiensis was available in 1938 in France (Waiser, 1986) for the control of flour moth (Jacobs, 1951). Unfortunately, the product was used only for a very short time, due to World War II (Nester et al., 2002). Formation of transgenic plant was also observed. The first reports of insertion of genes encoding for B. thuringiensis delta-endotoxins into plants came in 1987 and the first transgenic plants to express B. thuringiensis toxins were tobacco and tomato plants (van Frankenhuyzen, 1993). In 1957 pacific yeast products commercialized the first strain on B. thuringiensis, named as “ Thuricide” due to the increasing concern of biopesticide over the use of chemical insecticides.

B. thuringiensis is a gram-positive spore-forming bacterium that produces crystalline proteins called deltaendotoxins during its stationary phase of growth (Schnepf et al., 1998). The crystal is released to the environment after analysis of the cell wall at the end of sporulation, and it can account for 20 to 30% of the dry weight of the sporulated cells (Schnepf et al., 1998)

Distribution & Habitat of B. thuringiensis

This bacterium is distributed worldwide (Martin & Travers, 1989). The soil has been described as its main habitat; however it has also been isolated from foliage, water, storage grains, and dead insects, etc (Iriarte & Caballero, 2001). Isolation of strains from dead insects has been the main source for commercially used varieties, which include kurstaki, isolated from A. kuehniella; israelensis, isolated from mosquitoes, and tenebrionis, isolated from Tenebrio monitor larvae (Ninfa & Rosas, 2009; Iriarte & Caballero, 2001).. The spores of B. thuringiensis persist in soil, and vegetative growth occurs when nutrients are available (DeLucca et al., 1981; Akiba, 1986; Ohba & Aizawa, 1986; Travers et al., 1987; Martin & Travers, 1989).

DeLucca et al., (1981) found that B. thuringiensis represented between 0. 5% and 0. 005% of all Bacillus species isolated from soil samples in the USA. Martin & Travers (1989) recovered B. thuringiensis from soils globally. Meadows (1993) isolated B. thuringiensis from 785 of 1115 soil samples, and the percentage of samples that contained B. thuringiensis ranged from 56% in New Zealand to 94% in samples from Asia and central and southern Africa. Ohba & Aizawa (1986) isolated B. thuringiensis from 136 out of 189 soil samples in Japan.

There are several theories on the ecological niche filled by B. thuringiensis. Unlike most insect pathogenic microbes, B. thuringiensis generally recycle poorly and rarely cause natural epizootics in insects, leading to speculation that B. thuringiensis is essentially a soil micro-organism that possesses incidental insecticidal activity (Martin & Travers 1989). Evidence to support this view is that B. thuringiensis are commonly reported in the environment

independent of insects and there is a lack of association between occurrence and insect activity (van Frankenhuyzen 1993). Meadows (1993) suggested four possible explanations for the presence of B. thuringiensis in soil: 1) rarely grows in soil but is deposited there by insects; 2) may be infective to soil-dwelling insects (as yet undiscovered); 3) may grow in soil when nutrients are available; and 4) an affinity with B. cereus.

B. thuringiensis has been found extensively in the phylloplane. Numerous B. thuringiensis subspecies have been recovered from coniferous trees, deciduous trees and vegetables, as well as from other herbs (Smith & Couche, 1991; Damgaard et al., 1997). B. thuringiensis deposited on the upper side of leaves (exposed to the sun) may remain effective for only 1-2 days, but B. thuringiensis on the underside of leaves (i. e. protected from the sun) may remain active for 7-10 days (Swadner, 1994).

B. thuringiensis kurstaki has been recovered from rivers and public water distribution systems after an aerial application of Thuricide 16B (Ohana, 1987).

Crystal Composition and Morphology

The existence of parasporal inclusions in B. thuringiensis was first noted in 1915 (Berliner, 1915), but their protein composition was not delineated until the 1950s (Angus, 1954). Hannay (1953) detected the crystalline fine structure that is a property of most of the parasporal inclusions. B. thuringiensis subspecies can synthesize more than one inclusion, which may contain different ICPs (Hannay, 1953).

Depending on their ICP composition, the crystals have various forms (bipyramidal, cuboidal, flat rhomboid, or a composite with two or more crystal types) (Bulla et al., 1977; Höfte & Whiteley, 1989). A partial correlation between crystal morphology, ICP composition, and bioactivity against target insects has been established (Bulla et al., 1977; Höfte & Whiteley, 1989; Lynch & Baumann, 1985).

Classification of B. thuringiensis subspecies

The classification of B. thuringiensis subspecies based on the serological analysis of the flagella (H) antigens was introduced in the early 1960s (de Barjac & Bonnefoi, 1962). This classification by serotype has been supplemented by morphological and biochemical criteria (de Barjac, 1981). Until 1977, only 13 B. thuringiensis subspecies had been described, and at that time all subspecies were toxic to Lepidopteran larvae only. The discovery of other subspecies toxic to Diptera (Goldberg & Margalit, 1977) and Coleoptera (Krieg et al., 1983) enlarged the host range and markedly increased the number of subspecies. Up to the end of 1998, over 67 subspecies based on flagellar H-serovars had been identified.

Genetics of ICP

In the early 1980s, it was established that most genes coding for the ICPs reside on large transmissible plasmids, of which most are readily exchanged between strains by conjugation (González & Carlton, 1980; González et al., 1981). Since these initial studies, numerous ICP genes have been cloned, sequenced and used to construct B. thuringiensis strains with novel insecticidal spectra (Höfte & Whiteley, 1989).

The currently known crystal (cry) gene types encode ICPs that are specific to either Lepidoptera (cryI), Diptera and Lepidoptera (cryII), Coleoptera (cryIII), Diptera (cryIV), or Coleoptera and Lepidoptera (cryV) (Höfte & Whiteley, 1989). All ICPs described to date attack the insect gut upon ingestion. To date, each of the proteolytically activated ICP molecules with insecticidal activity has a variable C-terminal domain, which is responsible for receptrecognition (host susceptibility), and a conserved N-terminal domain, which induces pore formation (toxicity) (Li et al., 1991).

Most naturally occurring B. thuringiensis strains contain ICPs active against a single order of insects. However, conjugative transfer between B. thuringiensis strains or related species can occur, resulting in new strains with various plasmid contents (González & Carlton, 1980). Thus the mobility of the cry genes and the exchange of plasmids may explain the diverse and complex activity spectra observed in B. thuringiensis (González & Carlton, 1980; González et al., 1981; González et al., 1982; Reddy et al., 1987; Jarrett & Stephenson, 1990). New B. thuringiensis strains have been developed by conjugation that is toxic to two insect orders.

Nutritional status of B. thuringiensis

Since sporulation and germination in bacilli are dependent on the nutritional status of the organism (Hardwick & Foster, 1952), a study of the nutritional requirement of B. thuringiensis var. thuringiensis is important for delineating the control mechanisms which regulate spore and parasporal crystal formation. Certain amino acids support growth, sporulation and crystal formation of B. thuringiensis var. thuringiensis, while others inhibit the growth (Singer et al., 1966; Singer & Rogoff, 1968; Bulla et al., 1975;

Nickerson & Bulla, 1975; Rajalakshmi & Shethna, 1977). A lower concentration of cystine (Nickerson & Bulla, 1975) or cysteine (Rajalakshmi & Shethna, 1977) promotes growth, sporulation and crystal formation in Î’. thuringiensis, while at a higher concentration of cys/cysSH, only the vegetative growth was observed, (Rajalakshmi & Shethna, 1977).

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The classification of B. thuringiensis subspecies based on the serological analysis of the flagella (H) antigens was introduced in the early 1960s (de Barjac & Bonnefoi, 1962). This classification by serotype has been supplemented by morphological and biochemical criteria (de Barjac, 1981). Many strains of B. thuringiensis have been isolated and classified within more than 20 different varieties by serological techniques. On the basis of their potency for insect these varieties have been grouped into five pathotypes:

- Lepidopteran-Specific (e. g. B. thuringiensis . var Kurstaki)
- Dipteran-Specific (e. g. B. thuringiensis . var israelensis)
- Coleopteran-Specific (e. g. B. thuringiensis . var. tenebrionis)
- Those active against Lepidoptera and Dipter(e. g. B. thuringiensis . var. aizawai)
- Those with no toxicity recorded in insects (e. g. B. thuringiensis . var. Dakota)

Mode of Action

The ICP structure and function have been reviewed in detail by Schnepf et al., (1998). Binding of the ICP to putative receptors is a major determinant of

ICP specificity and the formation of pores in the midgut epithelial cells is a major mechanism of toxicity (Van Frankenhuyzen, 1993). After ingestion of B. thuringiensis by insect the crystal is dissolved in the insect's alkaline gut. Then the digestive enzymes that are present in insect's body break down the crystal structure and activate B. thuringiensis's insecticidal component, called the delta-endotoxin (Swadner, 1994). The delta-endotoxin binds to the cells lining the midgut membrane and creates pores in the membrane, upsetting the gut's ion balance. The insect soon stops feeding and starves to death (Gill et al., 1992).

Target Organisms

In the past decades, B. thuringiensis Cry toxins were classified according to the target pest they attacked (Hofte & Whiteley, 1998); however, due to the dual toxic activity exhibited by some cry genes and the inconsistencies in the original classification proposed by Höfte and Whiteley(1998), Crickmore et al., (1998) proposed a revision of the nomenclature for insecticidal crystal proteins, based on the ability of a crystal protein to exhibit some experimentally verifiable toxic effect in a target organism (Crickmore et al., 1998; Höfte & Whiteley, 1998). The diversity of B. thuringiensis is demonstrated in the almost 70 serotypes and the 92 subspecies described to date (Galan-Wong et al., 2006).

It is well known that many insects are susceptible to the toxic activity of B. thuringiensis; among them, lepidopterans have been exceptionally well studied, and many toxins have shown activity against them (Jarret & Stephens., 1990; Sefinejad et al., 2008). Order Lepidoptera encompasses the majority of susceptible species belonging to agriculturally important families

such as Cossidae, Gelechiidae, Lymantriidae, Noctuidae, Pieridae, Pyralidae, Thaumetopoetidae, Tortricidae, and Yponomeutidae (Iriarte & Caballero, 2001).

General patterns of use:

Commercial applications of B. thuringiensis have been directed mainly against lepidopteran pests of agricultural and forest crops; however, in recent years strains active against coleopteran pests have also been marketed (Tomlin, 1997). Strains of B. thuringiensis kurstaki active against dipteran vectors of parasitic disease organisms have been used in public health programmes (Tomlin, 1997).

Applications in agriculture and forestry

Commercial use of B. thuringiensis on agricultural and forest crops dates back nearly 30 years, when it became available in France (Van Frankenhuyzen, 1993). Use of B. thuringiensis has increased greatly in recent years and the number of companies with a commercial interest in B. thuringiensis products has increased from four in 1980 to at least 18 (Van Frankenhuyzen, 1993). Several commercial B. thuringiensis products with

B. thuringiensis aizawai, B. thuringiensis kuehniella or B. thuringiensis tenebrionise have been applied to crops using conventional spraying technology. Various formulations have been used on major crops such as cotton, maize, soybeans, potatoes, tomatoes, various crop trees and stored grains. Formulations have ranged from ultralow-volume oil to high-volume, wettable powder and aqueous suspensions (Tomlin, 1997). In the main, naturally occurring B. thuringiensis strains have been used, but transgenic

microorganisms expressing B. thuringiensis toxins have been developed by conjugation and by genetic manipulation, and in some cases, these have reached the commercial market (Carlton et al., 1990). These modified organisms have been developed in order to increase host range, prolong field activity or improve delivery of toxins to target organisms. For example, the coleopteran-active cryIIIA gene has been transferred to a lepidopteran-active B. thuringiensis kuehniella (Carlton et al., 1990). A plasmid bearing an ICP gene has been transferred from B. thuringiensis to a non-pathogenic leaf-colonizing isolate of Pseudomonas fluorescens; fixation of the transgenic cells produces ICP contained within a membrane which prolongs persistence (Gelernter, 1990).

Applications in vector control

B. thuringiensis Kurstaki has been used to control both mosquitos and blackflies in large-scale programmes (Lacey et al., 1982; Chilcott et al., 1983; Car, 1984; Car & de Moor, 1984; Cibulsky & Fusco, 1987; Becker & Margalit, 1993; Bernhard & Utz, 1993). For example, in Germany 23 tonnes of B. thuringiensis Kurstaki wettable powder and 19 000 litres of liquid concentrate were used to control mosquitos (Anopheles and Culex species) between 1981 and 1991 in the Upper Rhine Valley (Becker & Margalit, 1993). In China, approximately 10 tonnes of B. thuringiensis Kurstaki have been used in recent years to control the malarial vector, Anopheles sinensis.

Resistance of Insect Populations

A number of insect populations of several different species with different levels of resistance to B. thuringiensis have been obtained by laboratory selection experiments during the last 15 years (Schnepf et al., 1998). The

species include Plodia interpunctella, Cadra cautella, Leptinotarsa decemlineata, Chrysomela scripta, Tricholplusia ni, Spodoptera littoralis, Spodoptera exigua, Heliothis virescens, Ostrinia nubilalis and Culex quinquefasciatus (Schnepf et al., 1998). The Indian meal moth, a pest of grain storage areas, was the first insect to develop resistance to B. thuringiensis. Kurstaki (Swadner, 1994).

Resistance progresses more quickly in laboratory experiments than under field conditions due to higher selection pressure in the laboratory (Tabashnik, 1991). No indications of insect resistance to B . thuringiensis were observed in the field, until the development of resistance was observed in the diamondback moth in crops where B. thuringiensis had been used repeatedly. Since then, resistance has been observed in the laboratory in the tobacco budworm, the Colorado potato beetle and other insect species (McGaughey, 1992)

B. thuringiensis's Ecological Impacts

Some of the most serious concerns about widespread use of B. thuringiensis as a pest control technique come from the effects it can have on animals other than the pest targeted for control. All B. thuringiensis products can kill organisms other than their intended targets. In turn, the animals that depend on these organisms for food are also impacted (Swadner, 1994).

Effect on Beneficial insects:

Many insects are not pests, and any pest management technique needs to be especially concerned about those that are called beneficials, the insects that feed or prey on pest species (Swadner, 1994). B. thuringiensis has

impacts on a number of beneficial species. For example, studies of a wasp that is a parasite of the meal moth (Plodia interpunctella) found that treatment with B. thuringiensis reduced the number of eggs produced by the parasitic wasp, and the percentage of those eggs that hatched (Salama, 1993). Production and hatchability of eggs of a predatory bug were also decreased (Salama, 1991).

Other insects:

Many insects that do not have as directly beneficial importance to agriculture are important in the function and structure of ecosystems. A variety of studies have shown that B. thuringiensis applications can disturb insect communities (Swadner, 1994). Research following large-scale B. thuringiensis applications to kill gypsy moth larvae in Lane County, Oregon, found that the number of oak-feeding caterpillar species was reduced for three years following spraying, and the number of caterpillars was reduced for two years (Miller, 1990).

Birds:

Because many birds feed on the caterpillars and other insects affected by B. thuringiensis applications, it is not surprising that impacts of B. thuringiensis spraying on birds have been documented (Swadner, 1994). In New Hampshire, when B. thuringiensis-treatment reduced caterpillar abundance, black-throated blue warblers made fewer nesting attempts and also brought fewer caterpillars to their nestlings (Rodenhouse, 1992).

Effects on Humans

Eight human volunteers ingested 1 gram of a B. thuringiensis kuehniella formulation (3 Ã- 109 spores/g of powder) daily for 5 days. Of the eight volunteers, five also inhaled 100 mg of the B. thuringiensis kuehniella powder daily for five days. Comprehensive medical examinations immediately before, after, and 4 to 5 weeks later failed to demonstrate any adverse health effects, and all the blood chemistry and urinalysis tests were negative (Fisher & Rosner, 1959).

Pivovarov et al., (1977) reported that ingestion of foods contaminated with B. thuringiensis gastroenteitis at concentrations of 105 to 109 cells/g caused nausea, vomiting, diarrhoea and tenesmus, colic-like pains in the abdomen, and fever in three of the four volunteers studied. The toxicity of the B. thuringiensis gastroenteritis strain may have been due to beta-exotoxin (Ray, 1990).

In a purified form, some of the proteins produced by B. thuringiensis are acutely toxic to mammals. However, in their natural form, acute toxicity of commonly-used B. thuringiensis varieties is limited to caterpillars, mosquito larvae, and beetle larvae (Swadner, 1994).

Special Concerns about B. thuringiensis Toxicity

The earliest tests done regarding B. thuringiensis’s toxicity were conducted using B. thuringiensis var. thuringiensis, a B. thuringiensis strain known to contain a second toxin called beta-exotoxin (Swadner, 1994). The beta-exotoxin is toxic to vertebrates, with an LD 50 (median lethal dose; the dose that kills 50 percent of a population of test animals) of 13-18 milligrams per

kilogram of body weight (mg/kg) in mice when injected into the abdomen. An oral dose of 200 mg/kg per day killed mice after eight days (swadner, 1994) Beta-exotoxin also causes genetic damage to human blood cells (Meretoja, 1977).