Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(6): 2237-2244

Received: 25-09-2017
Accepted: 29-10-2017

Liz J Mampallil
College of Agriculture,
Kerala Agricultural University,
Vellayani, Thiruvananthapuram,
Kerala, India

MH Faizal
College of Agriculture,
Kerala Agricultural University,
Vellayani, Thiruvananthapuram,
Kerala, India

KN Anith
College of Agriculture, Kerala
Agricultural University,
Vellayani, Thiruvananthapuram,
Kerala, India

Correspondence
Liz J Mampallil
College of Agriculture,
Kerala Agricultural University,
Vellayani, Thiruvananthapuram,
Kerala, India

Bacterial bioagents for insect pest management

Liz J Mampallil, MH Faizal and KN Anith

Abstract
Biological control of crop pests and diseases has found to play significant role in reducing the over reliance on chemical pesticides. The control of pests by entomopathogenic and plant associated bacteria is an alternative that may contribute to reduce or eliminate the pesticide use. Entomopathogenic bacteria which cause diseases in insects kill the host by septicaemia and toxin production. Plant associated bacteria such as rhizospheric, endophytic and phylloplane bacteria are involved in biocontrol by the production of certain defensive chemicals leading to the induction of systemic resistance.

Keywords: entomopathogenic bacteria, rhizosphere, endophytes, phylloplane bacteria

Introduction
Control of insect pests in agriculture is mainly achieved using chemical insecticides. To mitigate the crop loss by pests, farmers often resort to frequent application of chemical pesticides in large quantities. Inspite of heavy insecticide application, the crop loss increases due to various reasons like development of resistance, pest resurgence and pest replacement besides negatively influencing environment and human health by way of leaving toxic residues. Hence there is a need to evolve eco-friendly management strategies. Biological control of pests and diseases affecting cultivated plants has gained much attention in the past two decades as a way of reducing the use of chemical pesticides in agriculture. Macrobial control of pests is mediated by natural enemies like predators and parasitoids and microbial control is achieved utilizing beneficial microbes such as insect pathogenic bacteria, fungi, viruses, protozoa and nematodes.
Bacteria are one of the most exploited microbial agents of insect pest management programmes. The biological control paradigm changed, when the potential of entomopathogenic bacteria was discovered, especially species belonging to the genus *Bacillus* (Glare and O'Callagan, 2000) [1]. Initially, the species *Bacillus popilllae* Dutky was introduced for the management of the Japanese beetle *Popillia japonica* Newman, but more concrete results were achieved with the discovery of new *Bacillus thuringiensis* (*Bt*) strains showing high toxicity against specific insects at a competitive level compared to conventional insecticides in terms of efficacy and costs of production. Besides the entomopathogenic bacteria, there exist certain bacteria that can effectively colonise plants by occupying different sites such as roots, leaves or as inter or intracellular colonisers. The control of pests by entomopathogenic and plant associated bacteria is an alternative that may contribute to reduce or eliminate the use of pesticides.

Entomopathogenic bacteria
Bacteria cause diseases in insects upon oral ingestion. They have evolved a multiplicity of strategies to invade the host, to overcome its immune responses, to infect and to kill it. They infect through the midgut epithelial cells resulting in death of the host by septicaemia and production of toxins. Entomopathogenic bacteria include spore formers as well as non spore formers. All spore forming bacteria produce endospores which allow them to persist in a dormant stage outside the host. Upon ingestion, spores germinate in the gut. In case of crystalliferous spore formers, in addition to endospore, they produce parasporal crystal in the sporangium. Non spore formers exhibit insecticidal activity by production of insecticidal toxins.

Major groups of entomopathogenic bacteria
Majority of the entomopathogenic bacteria belong to the following families Bacillaceae, Pseudomonadaceae and Enterobacteriaceae. Members of Streptococcaceae and micrococcaceae

families also exhibit entomopathogenic activity, but only to a lesser extent.

Family Bacillaceae

Bacillaceae family comprises of Gram-positive, heterotrophic, rod-shaped bacteria that may produce endospores. Members of this family include *Bacillus thuringiensis*, *B. sphaericus*, *B. popillae, B. pumilus, Brevibacillus laterosporus* etc.

1. *Bacillus thuringiensis*

The most successful insect pathogen used for insect control is the bacterium *Bacillus thuringiensis* (*Bt*), which presently is ~2% of the total insecticidal market. *Bt* is a Gram positive, soil dwelling bacterium, known by its ability to produce crystalline inclusions upon sporulation (Cry toxins) which contains insecticidal proteins called δ endotoxin. These toxins are secreted as water-soluble proteins that undergo conformational changes in order to insert into the membrane of their hosts. *Bt* is almost exclusively active against larval stages of different insect orders and kills the insect by disruption of the midgut tissue followed by septicemia caused probably not only by Bt but probably also by other bacterial species (Bravo *et al*., 2011) [2].

Most commercially available formulations are based on spore-crystal mixtures with effectiveness against different pest species. Cry toxin specificity varies with respect to subspecies and strains of *B. thuringiensis. Bt* subsp *kurstaki* (*Btk*) is generally used against young lepidopteran larvae and includes different strains with significant commercial interest like HD-1, SA-11, SA-12, PB 54, ABTS-351 and EG2348. *Btk* HD-1 strain is effective against yellow stemborer in rice. Strains of *B. thuringiensis* subsp *aizawai* (*Bta*) are used against armyworms and diamondback moth larvae. Besides, strains belonging to the subsp *israelensis* (*Bti*) and *tenebrionis* (*Btt*) have been employed in the management of mosquitoes and simulids, and against coleoptera, respectively (Glare & O'Callaghan, 2000).

All Cry toxins contain three structural domains and share a high degree of tipological similarity. Domain I, which is a bundle of 7 α helices, upon contact with the cell membrane undergoes refolding to facilitate insertion of a portion of the toxin into the membrane which results in ion channel or pore formation. Doman II & Domain III are involved in receptor binding and protection of toxin from proteases respectively (Falnes & Sandwig, 2000) [3].

Application of *Bt* has been reported against more than 40 species of insects (Burgus and Daoust, 1986) [4]. Insects susceptible to *Bt* have increased in number with the isolation of the large number of sub species and strains (Hofte and Whitely, 1989) [5]. *Bt* has been reported as a microbial control agent against many chewing pests like cotton boll weevil, *Anthonomus grandis* (Monnerat *et al*., 2012) [6], gram pod borer, *Helicoverpa armigera,* leaf caterpillar, *Spodoptera litura*, red flour beetle, *Tribolium castaneum* (Kausarmalik, 2014) [7], cabbage white butterfly *Pieris brassicae* (Mohan *et al*., 2014) [8], bihar hairy caterpillar, *Spilarctia obliqua* (Khan, 2015) [9], red flour beetle, *Tribolium castaneum* (Kausarmalik, 2014) and so on.

2. *Bacillus popilllae*

The spore-former *B. popillae* (Dutky) is the causal agent of milky disease in phytophagous coleopteran larvae. The production of parasporal inclusions within the sporangial cells has been observed in *B. popillae*, even if they are not directly responsible for the insecticidal action. After the spores are ingested by the host, they germinate in the midgut. The following pathogenicity seems to be in relation to the septicemia caused by vegetative cells (Zhang, 1997) [10].

3. *Bacillus sphaericus*

Entomopathogenic strains belonging to *Bacillus sphaericus* species group are featured in the production of spherical endospores closely associated with parasporal crystals containing an equimolar ratio of binary protein toxins (BinA and BinB). The insecticidal mode of action includes damages to the microvillar epithelial cells in the midgut comparable to the ones known for *B. thuringiensis.* The main targets of commercial formulations based on *B. sphaericus* strains are mosquitoes, blackflies and non-biting midges (Baumann *et al*., 1991) [11].

4. *Bacillus pumilus*

Bacillus pumilus strain 15.1 has been recently described to be toxic against larvae of the Mediterranean fruit fly, *Ceratitis capitata*, one of the most damaging pests for fruits and vegetables worldwide. During sporulation this strain forms parasporal crystals that morphologically resemble those produced by *Bacillus thuringiensis* cry proteins. *B. pumilus* 15.1 genome harbors other genes encoding well-known entomopathogenic factors, such as chitinases, metalloproteases, and cytolysins (Molina *et al.*, 2010) [12].

5. *Brevibacillus laterosporus*

The insecticidal action of different *B. laterosporus* strains have been reported against insects in different orders, including coleoptera, lepidoptera and diptera. It has the potential to produce different toxins. Certain strains showing toxicity against the corn rootworms (*Diabrotrica* spp.) and other coleopteran larvae, produce insecticidal secreted proteins (ISPs) that act as binary toxins in the insect midgut and have high homology with *B. thuringiensis* vegetative insecticidal proteins (VIPs) (Warren, 1997) [13].

Specific strains toxic to mosquitoes produce parasporal inclusion bodies similar of those produced by *B. thuringiensis*. These bodies contain proteins and their implication in the mosquitocidal action has been reported (Zubasheva *et al*., 2010) [14]. The mode of action implies histopathological changes in the midgut with disruption of the microvillar epithelium.

Family Pseudomonadaceae

Members of the family Pseudomonadaceae are strictly aerobic, gram negative, straight or curved rods with polar flagella. A number of species are pathogens and others are found commonly in the digestive tracts of insects as commensals. A number of species of *Pseudomonas* are found associated with insects either as pathogens or as commensals in the digestive tract. Though *Pseudomonas aeruginosa* is one of the commonly isolated bacteria from insects, they seldom cause epizootics in field populations (Buchner, 1963) [15]. The pathogenicity of the bacterium is correlated with the production of proteolytic enzymes and to the toxicity and clotting of the insect's haemolymph by proteases. The proteases cause degenerative changes in haemocytes and digest certain specific insect haemolymph proteins (Lysenko and Kucera, 1971) [16].

***Pseudomonas* spp.**

Pseudomonas fluorescens (Flugge) strains were found effective in killing or causing morphological defects in widely used laboratory insect (Pimenta *et al*., 2003) [17]. *P. fluorescens* was reported to cause a mortality of 70% and 56% in the larvae and adults of alder leaf beetle, *Agelastica ani* L. respectively within 7 days after treatment whereas, *Pseudomonas chlororaphis* brought about 37 and 30% mortality of larvae and adults respectively (Sezen *et al*., 2004) [18].

Sezen *et al*. (2007) [19] determined the insecticidal effect of two species of *Pseudomonas* on *Melolontha melolontha* larvae, a serious pest of hastle nuts in Turkey. The insecticidal activity of isolates at 1.8×10^9 bacteria ml^{-1} dose, within 10 days on the larvae were 40% and 50% for the two species of *Pseudomonas*. According to Meca *et al*. (2009) [20], *Pseudomonas* sp caused 70 per cent mortality of citrus leaf miner, *Phyllocnitis citrella* larvae at 72 h.

Entomopathogenic activity of different strains of *Pseudomonas* sp were tested against the 5th instar larvae of migratory locust, *Locusta migratoria* (L.) (Acrididae; Orthoptera). The results obtained 1 week after treatment showed that, treated nymphs were sensitive to the bacteria with a mortality rate of 100% and 98% for *Pseudomonas* sp strain B3 (HF911369) and strain B4 (HF911366) respectively (Mohandkaci *et al*., 2015) [21].

Gopal *et al*. (2002) [22] reported *Pseudomonas alcaligens* can cause septicaemia in rhinoceros beetle grubs under stress conditions. Of the 6627 grubs and 307 adults collected from various breeding sites of the pest, 5% of the grubs and 22% of the adults had natural viral infection caused by *Oryctes* virus, 3% larvae died of *Metarhizium anisopliae* mycosis and 20% larvae died from bacterial septicaemia. *Pseudomonas aeruginosa* isolated from dead grubs of epilachna beetle, *Henosepilachna vigintioctopunctata* was reported to cause a mortality of 73.01 per cent on these grubs (Aswathy, 2015) [23].

Family Enterobacteriaceae

The Enterobacteriaceae is a large family of Gram-negative bacteria that includes, along with many harmless symbionts, many of the more familiar pathogens, such as *Salmonella*, *Escherichia coli*, *Yersinia pestis, Klebsiella* and *Shigella*. Most widely reported entomopathogenic bacteria in enterobacteriaceae are *Serratia* and *Enterobacter.*

Serratia marcescens is a facultative anaerobe that multiplies quickly in the gut of many insect species, causing septicaemia and death. It is often isolated from dead and diseased insects. Other species such as *S. entomophila*, induces diseases in pest insects when the bacterium is ingested (Johnson *et al*., 2001) [24].

***Serratia* spp.**

Serratia entomophila and *Serratia proteamacula* are used as effective biological pesticides against Newzeland grass grubs, *Costelytra zealandica* (Hurst *et al*., 2000) [25]. The soil dwelling bacterium, *Serratia entomophila* cause natural epizootics of amber disease in grass grub larvae by means of production of toxins Sep A, Sep B and Sep C and has been developed as a commercial microbial control agent against *C. zealandica* (Johnson *et al.*, 2001) [24].

Lauzon *et al.* (2003) [26] stated that non-pigmenting strains of *S. marcescens* Bizio were pathogenic to apple maggot flies, *Rhagoletis pomonella* rendering rapid mortality within 24 hours after treatment. Reports prove that *S. marcescens* NMCC46 had potent mosquito larvicidal activity resulting from the red colour pigment prodigiosin causing 50 per cent mortality within the first 24 h of treatment (Patel *et al*., 2011) [27]. *S. marcescens* isolated from dead grub of epilachna beetle, *H. vigintioctopunctata* (Fab.) from brinjal was found to cause 93.28% mortality of the grub (Ashwathy, 2015). Pu and Hou (2016) [28] observed, *Serratia marcescens* caused a mortality of 56.75% on the fourth instar larvae of red palm weevil, *Rhynchophorus ferrugineus* Oliver.

Serratia sp and *Pseudomonas* sp have been reported as pathogens of *Anastrepha frateraculus*, *Ceratitis capitata* and *Rhynchophorus palmarum* (Briceno, 2004) [29]. Meca *et al*. (2009) [30] reported that *Serratia* sp had entomopathogenic effect on *Phyllocnistis citrella*, where it caused a per cent mortality of 80.4 per cent on the larvae between 48 and 72 h. Reports reveal significant reduction in feed consumption by lepidopteran insects (*Helicoverpa armigera*, Hubner and *Spodoptera litura*, Fab.) fed on diet supplemented with *Serratia* sp. A maximum mortality of 94.3 per cent and 92.7 per cent was noted in *H. armigera* and *S. litura* respectively at 72 h after treatment (Chattopathayay *et al*., 2012) [31].

***Enterobacter* spp.**

Enterobacter sp at high concentrations can cause mortality in fruit flies, *Ceratitis capitata* and *Anastrepha fraterculus.* The principal symptoms of infection are septcaemia, inhibition of feeding, lack of motility and death at 24 to 72 hr (Briceno, 2004). *Enterobacter aerogenes* was reported to cause mortality of 73 per cent in the larvae of *Phyllocnitis citrella* (Meca *et al*., 2009).

Other bacteria

Clostridium bifermentus (Weinburg and Seguin) was found pathogenic to mosquitoes and blackflies (Nicolas *et al*., 1990) [32]. *Burkholderia* sp has recently been asserted to affect ovipositon and fecundity of the bean bug *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae) (Kil *et al.*, 2014) [33]. Whole cell broth cultures of *B. rinojensis* (A396), rendered oral toxicity and contact effects against the beet armyworm *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae) (Kreylos *et al.*, 2013) [34].

Ingestion of the pigment violecein (tryptophan derivative), synthesized by *Chromobacterium substugae* was found to cause toxic effects in different insects *viz*, Colorado potato beetle (*Lepinotarsa decemlineata* Say), western corn root worm (*Diabrotica virgifera* Le conte), southern corn root worm (*Diabrotica undecimpunctata* Mannerheim), diamond back moth (*Plutella xylostella* L.), sweet potato white fly (*Bemisia tabaci* Gennadium) and southern green stink bug (*Nazara viridula* L.) (Martin *et al*., 2007) [35].

Klebsiella pneumoniae was isolated from dead grubs of red palm weevil, *Rhynchophorus ferrugineus* Oliver (Pu and Hou, 2016). *Klebsiella pneumoniae* along with *Serratia marcescens* were isolated from the gut region of red palm weevil in the study carried out at CPCRI, Kayamkulam by Josephrajkumar *et al*. (2017) [36].

Nematode associated bacteria

***Photorhabdus* and *Xenorhabdus* species**

There is great interest in the discovery of additional bacterial toxins with insecticidal activity. *Photorhabdus* and *Xenorhabdus* spp. that live as mutualists in the intestines of entomophagous nematodes have been recognized as a source of insecticidal proteins for crop protection (Bowen *et al*., 1998) [37]. Nematodes with these bacterial mutualists actively seek out susceptible insect larvae, penetrate the cuticle, invade the blood system and regurgitate the insecticidal bacteria. The bacteria release a range of toxins that kill the insect host and convert the cadaver into a bacterial and nematodal food source and breeding ground.

Different *Photorhabdus* and *Xenorhabdus* species producing an insecticidal toxin complex (Tc) have high potential for pest management. Generally, the TCs are high-molecular weight and multi-subunit proteins that include three components, A, B and C orally active against different insects. While the mode of action is not completely understood, all these components are normally needed to achieve full toxicity. A second class comprises the 'Makes caterpillars floppy' (Mcf toxins) that are active upon injection. Another example of insecticidal proteins produced by these bacterial species is represented by the *Photorhabdus* insect related (Pir) proteins, produced by *P. luminescens* (Thomas and Poinar), that show similarity to *B. thuringiensis* delta-endotoxins and have been proposed to be mimics of the juvenile hormone esterases (JHEs) interfering with insect development regulation (Waterfield & Dowling, 2007) [38]. *Photorhabdus luminescens* and *Xenorhabdus nematophila* are toxic against tobacco hornworm *Manduca sexta* larva by means of Mcf toxin and toxin complex (TC) proteins respectively (Bowen *et al*., 1998).

Plant associated bacteria

Biological control within the broad ecological context includes plant microbe interactions also (Andrews, 1992) [39]. Many bacteria associated with plants are known to exert beneficial effects on the plants such as growth promotion (Glick, 1995) [40], induced resistance to pathogens and pest control capability against insect herbivores (Bostock *et al*., 2001) [41]. Bacteria that associate with plants in their natural environment are called by different names such as rhizosphere bacteria, endophytic bacteria and phylloplane bacteria depending on the sites they inhabit.

Mode of action by which beneficial bacteria bring about insect control

The association of beneficial bacteria with plant triggers induced resistance in the latter. This form of induced systemic resistance via various signal transduction pathway leads to the production of various defense enzymes & pathogenesis related proteins (PR proteins) in the plant like chitinases, peroxidases (PO), polyphenol oxidases (PPO), lipoxygenase, 1,3-glucanases etc. The defense enzymes and PR proteins thus formed will defend the plant from harmful pathogens and herbivory.

Induced resistance, defined as an enhancement of the plant's defensive capacity is of two types via pathogen induced Systemic acquired resistance (SAR) and beneficial bacteria mediated Induced systemic resistance (ISR) (Van Loon *et al*., 1998) [42]. JA and related members of jasmonate family plays an important role in defense related responses to insect attack. JA formed from systemin (membrane lipids) induces defense proteins like lipoxygenase, arginases, ascorbate oxidases and induce proteinase inhibitors to control insects. It also induce the production of chitinases which act on the exoskeleton of insects.

Role in pest control

Rhizosphere bacteria

Bacteria that survive in association with the rhizosphere are known as rhizosphere bacteria (Schroth and Hancock, 1982) [43]. About 2 - 5 per cent of rhizobacteria show a beneficial effect on plant growth and hence these bacteria are termed as plant growth promoting rhizobacteria (PGPR) (Kloepper *et al*., 1995) [44]. PGPR enhance plant growth directly by facilitating nutrient uptake and indirectly by protecting the plant from pathogens and insect pests. Systemic resistance induced by these microbes enhance plant defense against pests and pathogens thereby plays significant role as efficient biocontrol agents (Van Loon *et al*., 1998) [42].

Reports show PGPRs like *Pseudomonas, Bacillus* and *Serratia,* are efficient root colonisers and protect plants from different crop pests (Tomczyk, 2006 [45]; Hanafi *et al.*, 2007 [46]; Siddiqui *et al.*, 2007) [47].

Pseudomonas

Rhizospheric *Pseudomonas maltophila* was found to affect the growth of corn ear worm, *Helicoverpa zea,* a major polyphagous pest of many agricultural crops contributing more than 60% reduction in adult emergence of the pest (Bong and Sikorowski, 1991) [48]. *Pseudomonas gladioli* was found to affect the relative growth rate, consumption rate and digestibility of feed by *Helicoverpa armigera* in cotton (Qingwen *et al*., 1998) [49].

Plant growth promoting *Pseudomonas fluorescens* strains Pf1, TDK 1 and PY 15 rendered notable reduction in the leaf folder damage in rice plants which is attributed to the enhanced activity of polyphenol oxidase, lipoxygenase, chitinase and proteinase inhibitors in PGPR treated plants. Besides this, increased natural enemy population in the PGPR treated plots was also noticed (Saravankumar *et al.*, 2007) [50].

Melvin and Muthukumaran (2008) [51] observed that tomato leaves treated with combined foliar application of defense inducer, jasmonic acid (JA) and *Pseudomonas aeruginosa* recorded maximum larval mortality of *Spodoptera litura* under the pot culture condition. Combined treatment resulted significant reduction in pupation rate, adult emergence and adult longevity of the pest. The activity of proteinase inhibitor, polyphenol oxidase (PPO) and lipoxygenase molecules was promoted by JA treatment.

Maria *et al*. (2008) [52] attributed the insecticidal property of two strains CHAO and pf-5 of rhizospheric *Pseudomonas fluorescens* to a large protein toxin termed Fit (*P. fluorescens* insecticidal toxin). Haemocoelic injection of even low doses of *P. fluorescens* CHA0 or Pf-5 was observed efficient in inducing mortal effects on larvae of the tobacco hornworm *Manduca sexta* and the greater wax moth *Galleria mellononella*.

Bacillus

Zehnder *et al.* (1996) [53] proved experimentally that PGPR treated cucumber plants caused significant reduction in feeding by dibroticine cucumber beetles due to reduction in cucurbitacin, which act as a phagostimulant for the betetle. *Bacillus pumilus* strain INR – 7 was effective against striped cucumber beetle, *Acalyma vittatum* and the spotted beetle, *Diabrotica undecimpunctata howardi* as per their study.

A delay in population growth and population size of cotton aphids *Aphis gossypii,* on cucumber plants treated with PGPR (*Bacillus* spp.) was reported by Stout *et al*., (2002) [54]. Hanafi *et al.* (2007) observed significant reduction in proliferation of *Bamisia tabaci* on *Bacillus subtilis* treated tomato plants. A significant increase in the mortality rate of cow pea aphid was reported in PGPR treated cowpea plants than the control where, *Bacillus subtilis* was found the most efficient among the different PGPRs used (Kavitha, 2010) [55].

Serratia

Serratia sp. isolated from maize rhizosphere was found entomopathogenic on maize rootworms *Diabrotica virgifera virgifera*, and the rootworm infestation subsequently increased the bacterial population (Prischmann *et al*., 2008) [56]. Rhizosperic *Serratia marcescens* was found effective against pod bug, *Riptortus pedestris* in cow pea (Kavitha, 2010).

Endophytic bacteria

Endophytic bacteria are those that inhabit the interior of plants, especially leaves, branches and stems, showing no apparent harm to the host they colonize (Sturz *et al*., 2000) [57]. The presence of endophytic micro organisms in host plant can enhance plant vigour by way of rendering protection against insect pests and diseases, promoting growth and enhancing resistance in stress conditions (Azevedo *et al*., 2000) [58].

Thuler *et al*. (2006) [59] reported that strain EN5 of endophytic *Alcaligenes piechaudii* reduced the incidence of *Plutella xylostella* by about 50 to 80%. *Bacillus subtilis* strains EPCO 102, EPCO 16 and *Pseudomonas fluorescens* Pf1 reduced the aphid infestation in cotton (Rajendran *et al*., 2011) [60]. Endophytic strains of *Bacillus thuringiensis*, S1905 and S2122 caused 100 per cent and strain S2124 caused 58.33 per cent mortality in third instar caterpillars of diamond back moth, *Plutella xylostella* (Praca, 2012) [61].

Fahey (1991) [62] genetically modified xylem-inhabiting endophytic bacteria *Clavibacter xyli* subsp *cynodontis*, by the introduction of delta endotoxin gene of *Bacillus thuringiensis*, and the recombinant provided resistance against lepidopteran and coleopteran pests. Gaofu *et al.* (2012) [63], effectively modified an endophyte, *Bacillus subtilis* WH2, isolated from rice seedlings by introducing *Pinellia ternata* agglutinin (PTA) gene with insecticidal properties and found it effective in controlling white backed plant hopper (WBPH) in rice.

Phylloplane bacteria

Phylloplane is a natural habitat on leaf surface which supports growth of wide range of microorganisms. According to Beattie and Lindow (1995) [64], phylloplane bacteria survive on the leaf surfaces colonizing on sites such as trichomes, stomata and epidermal cellwall junctions.

Andrews (1992) [39] reported that microorganisms that stably colonize the surface of plant leaves act as potential biological agents to suppress foliar pathogens and insect defoliators. Mostly being commensalistic, some of the phylloplane bacteria can produce extracellular chitinase which in turn degrades the peritrophic membrane of chewing insects, thereby making them good biocontrol agents (Aggarwal *et al*., 2015) [65].

Bacillus thuringiensis is indigenous to diverse environments such as soil, insect cadavers and leaves of plants (Smith and Couch, 1991) [66]. *Bt* has been reported as a phylloplane inhabitant in addition to entomopathogen and soil inhabitant (Meadows, 1993) [67].

Otsu *et al*. (2003) [68] proved scientifically that the phytophagous epilachna beetles can be biologically controlled by chitinase secreting strain KPM-012A of *Alcaligens paradoxus* isolated from tomato phylloplane, which caused the degradation of peritrophic membrane in *Henosepilachna vigintioctopunctata*. Otsu *et al*. (2004) [69] provided an experimental basis for the biological control of herbivorous insect pests using leaf inhabiting, entomopathogenic strain of *Pseudomonas fluorescens*. The strain KPM-018P isolated from tomato leaves caused 70.5±21.5% mortality in larvae of *H. vigintioctopunctata*. This method was thus proved effective for decreasing the population of larvae and adults of the pest in the subsequent generation.

A study conducted by Maduell *et al*. (2002) [70] reported 60 per cent of total 256 isolates of *Bacillus thuringiensis* isolated from phylloplane of *Piper* sp, toxic to *Spodoptera fugiperda* and 40 per cent were toxic to *Culex quinquefasciatus. B. thuringiensis* isolated by Gonzalez and Molla (2011) [71] from the phylloplane of tomato plants could effectively control the tomato leaf miner, *Tuta absoluta.* Aswathy (2015) reported that *P. fluorescens* isolated from the phylloplane of brinjal, caused 63.25 per cent mortality of grubs of *H. vigintioctopunctata* (Fab.).

Conclusion

Bacterial bioagents offer immense potential in pest management. Entomopathogenic as well as plant associated bacteria if utilized wisely, can become a better strategy for the control of insect pests over chemical pesticides. *Bacillus thuringiensis* covers 1 per cent of the total agrochemical market and *Bt* products on a global basis, represent more than 50 per cent of the total microbial pesticides because of its significant role in pest control. Other than *Bacillus* sp, non spore forming bacteria are also found promising in pest control. But entomopathogens like *Serratia* sp are not much recommended commercially because of their opportunistic nature. Plant associated bacteria inhabiting rhizosphere, endophytic regions and phylloplane of plants can be well exploited against a wide range of pests.

Future prospects

Gentically engineered strains of *Bacillus thuringiensis* with novel toxin combinations is an ongoing field of research to delay resistance development in insects against *Bt.* Attempts are being made to genetically engineer rhizosphere, phylloplane and endophytic bacteria by incorporating exogenous toxin genes to develop them as biocontrol agents.

References

1. Glare TR, O'Callaghan M. *Bacillus thuringiensis*: *Biology, Ecology and Safety* [book on-line]. Wiley, Chichester, New York, 2000. *Available:* https://books.google.co.in/books?isbn=1402085605. [21 July 2000].
2. Bravo A, Likitvivatanavong S, Gill SS, Soberon M. *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect Biochem. Molec. Biol. 2011; 41:423-431.
3. Falnes PO, Sandwig K. Penetration of protein toxins into cells. Curr. Opin. Cell Biol. 2000; 12:407-413.
4. Burges HD, Daoust RA. Current status of the use of bacteria as biocontrol agents. In: Fundamental and applied aspects of invertebrate pathology. Soc. Invertebr. Pathol. Wageningen. 1986; 3:514-517.
5. Hofte H, Whiteley HR. Insecticidal crystal proteins of Bacillus thuringiensis. Microbial Rev. 1989; 53:242-255.
6. Monnerat R, Martins E, Praca l, Dumas V, Berry C. Activity of a Brazilian strain of Bacillus thurigiensis israelensis against the cotton boll weevil Anthomonas grandis Boheman (Coleoptera: Tenebrionidae). Neotropical Entomol. 2012; 41(1):62-67.
7. Kausarmalik, Riasat R. Study of combined effect of locally isolated Bacillus thurigiensis and turmeric powder on red flour beetle (*Tribolium castaneum*). 2014; 3(4):760-773.
8. Mohan M, Rangeshwaran R, SivaKumar G, Verghese A.

Relative toxicity of subspecies of Bacillus thurigienis against lepidopterous insect pests of agricultural importance. J Biol. Control. 2014; 28(4):197-203.
9. Khan MA. Larval susceptibility of Spilarctia oblique against Bacillus thuringiensis isolates. Ann. Plant Prot. Sci. 2015; 23(2):273-276.
10. Zhang J, Hodgman TC, Krieger L, Schnetter W, Schairer HU. Cloning and analysis of the cry gene from Bacillus popilllae. J Bacteriol. 1997; 179:4336-4341.
11. Baumann P, Clark MA, Baumann L, Broadwell AH. *Bacillus sphaericus* as a mosquito pathogen: Properties of the organism and its toxins. Microbiol. Rev. 1991; 55:425-436.
12. Molina CA, Cana-Roca JF, Osuna A, Vilchez S. Selection of a *Bacillus pumilus* strain highly active against *Ceratitis capitata* (Wiedemann) larvae. Appl. Environ. Microbiol. 2010; 76:1320-1327.
13. Warren GW. Vegetative insecticidal proteins: Novel proteins for control of corn pests. In: Taylor & Francis (ed.), *Advances in Insect Control*. London, UK. 1997, 109-121.
14. Zubasheva MV, Ganushkina LA, Smirnova TA, Azizbekyan RR. Larvicidal activity of crystal-forming strains of Brevibacillus laterosporus. Appl. Biochem. Microbiol. 2010; 46:755-762.
15. Buchner. Insect pathology. In: (E. A. Steinhaus) (ed.) Non sporulating bacterial pathogens. New York. 1963, 117-147.
16. Lysenko O, Kucera M. The mechanism of pathogenicity of *Pseudomonas aeruginosa.* Folia Microbiol. Prague. 1971; 13:259-299.
17. Pimenta DLA, Martino DP, Bouder LE, Hulen C, Blight MA. *In vitro* identification of two adherence factors required for *in vivo* virulence of *Pseudomonas fluorescens*. Microbiol. Infecta. 2003; 5:1177-1187.
18. Sezen K, Demir Y, Kati H, Demirbag Z. Investigations on bacteria as a potential biological control agent of summer chafer, Amphimallon solstitiale L. (Coleoptera: Scarabaeidae). J Microbiol. 2004; 43(5):463-468.
19. Sezen K, Demir Y, Demirbag Z. Identification and pathogenicity of entomopathogenic bacteria from common cockchafer, *Melolontha melolontha* (Coleoptera: Scarabaeidae). N. Z. J. Crop Hort. Sci. 2007; 35(1):79-85.
20. Meca A, Sepulveda B, Ogona JC, Grados N, Moret A, Morgan M, *et al.* In vitro pathogenicity of northern Peru native bacteria on *Phyllocnistis citrella* Stainton (Gracillariidae: Phyllocnistinae), on predator insects (*Hippodamia convergens* and *Chrisoperna externa),* on *Citrus aurantifolia* Swingle and white rats. *Span.* J Agric. Res. 2009; 7(1):137-145.
21. Mohandkaci HO, Khemili S, Benzena F, Halouane F. Isolation and identification of entomopathogenic bacteria from Algerian desert soil and their effects against migratory locust, *Locusta migratoria* (L.). Egyptian J Biol. Pest Control. 2015; 25(3):739-746.
22. Gopal M, Gupta A, Sathiamma B. Microbial pathogens of the coconut pest *Oryctes rhinoceros*: influence of weather factors on their infectivity and study of their coincidental ecology in Kerala, India. Wld J Microbiol. Biotech. 2002; 18:417.
23. Aswathy V. Management of epilachna beetle, *Henosepilachna vigintioctopunctata* (Fab.) with phylloplane and pathogenic microorganisms. M. Sc. (Ag) thesis, Kerala Agricultural University, Thrissur. 2015, 56.
24. Johnson VW, Pearson JF, Jackson TA. Formulation of *Serratia entomophila* for biological control of grass grub. N.Z. Pl. Prot. 2001; 54:125-127.
25. Hurst MRH. Plasmid-located pathogenecity determinants of *Serratia entomophila*, the causal agent of amber disease of grass grub, show similarity to the insecticidal toxins of *Photorhabdus luminescens*. J Bacteriol. 2000; 182(18):5127-5138.
26. Lauzon CR, Bussert TG, Sjogren RE, Prokopy RJ. *Serratia marcescens* as a bacteria pathogen of *Rhagoletis pomonella* flies (Diptera: Tephritidae). *Eur.* J Entomol. 2003; 100:87-92.
27. Patel CD, Patel SV, Salunke BK, Salunkhe RB. Prodigiosin produced by *Serratia marcescens* NMCC 46 as a mosquito larvicidal agent against *Aedes aegypti* and *Anopheles stephensi.* Parasitol. Res. 2011; 109(4):1179-1187.
28. Pu YC, Hou YM. Isolation and identification of bacterial strains with insecticidal activities from *Rhynchophorus ferrugineus* Oliver (Coleoptera: Curculionidae). J Appl. Entomol. 2016; 140(8):617-626.
29. Briceno M. Biological control of fruit flies: *Anastrepha fraterculus* and *Ceratitis capitata*. Pest Manag Sci. 2004; 63:394-398.
30. Meca A, Sepulveda B, Ogona JC, Grados N, Moret A, Morgan M, *et al.* In vitro pathogenicity of northern Peru native bacteria on *Phyllocnistis citrella* Stainton (Gracillariidae: Phyllocnistinae), on predator insects (*Hippodamia convergens* and *Chrisoperna externa),* on *Citrus aurantifolia* Swingle and white rats. *Span.* J Agric. Res. 2009; 7(1):137-145.
31. Chattopadhyay P, Chatterjee S, Gorthi S, Sen SK. Exploring agricultural potentiality of *Serratia entomophila* AB2: dual property of biopesticide and biofertilizer. British Biotechnol. J. 2012; 2:1-12.
32. Nicolas L, Hamon S, Frachon E, Sebald M, De Barjac H. Partial inactivation of the mosquitocidal activity of *Clostridium bifermentans* serovar malaysia by extracellular proteinases. Applied microbiol biotechnology. 1990; 34(1):36-41.
33. Kil YJ, Seo MJ, Kang DK, Cho HS, Youn YN, Yasunaga CY. Effects of Enterobacteria (*Burkholderia* sp) on development of *Riptortus pedestris.* J Fac. Agric. 2014; 59:77-84.
34. Kreylos C, Fernandez LE, Koivunen M, Yang A, Flor L, Marrone PG. Isolation and characterization of *Burkholderia rinojensis* sp, a non-*Burkholderia cepacia* complex soil bacterium with insecticidal and miticidal activities. Appl. Environ. Microbiol. 2013; 79:7669-7678.
35. Martin PAW, Gundersen RD, Blackburn M, Buyer J. *Chromobacterium subtsugae* sp. nov., a betaproteobacterium toxic to Colorado potato beetle and other insect pests. Int. J of Syst. Evolutionary Microbiol. 2007; 57:993-999.
36. Josephrajkumar A, Prathibha PS, Babu M, Mohan C, Hedge V, Krishnakumar V, *et al. Red plam weevil in Coconut- Knack to crack Trajectory*. ICAR-CPCRI, Regional Station, Kayamkulam. 2017, 28.
37. Bowen D, Rocheleau TA, Blackburn M, Andreev O, Golubeva E, Bhartia R, *et al.* Insecticidal toxins from the bacterium Photorhabdus luminescens. Sci. 1998; 280:2129-2132.
38. Waterfield NR, Dowling A. Insecticidal toxins from *Photorhabdus* bacteria and their potential use in

agriculture. Toxicon. 2007; 9:436-451.
39. Andrews H. Biological control in phyllosphere. Ann. Rev. Phytopath. 1992; 30:603-635.
40. Glick BR. The enhancement of plant growth by free-living bacteria. Can. J Microbiol. 1995; 41:109-117.
41. Bostock RM, Karban R, Thaler JF, Weyman PD, Gilchrist D. Signal interactions in induced resistance to pathogens and insect herbivores. *Eur.* J Plant Pathol. 2001; 107:103-111.
42. VanLoon C, Bakker PA, Pieterse CMJ. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 1998; 36:453-83.
43. Schroth MN, Hancock JG. Disease-suppressive soil and root-colonizing bacteria. Sci. 1982; 216:1376-81.
44. Kloepper JW, Schroth MN. Plant growth-promoting rhizobacteria on radishes. In: Proceedings of the fourth International Conference on Plant Pathogenic Bacteria Vol. 2, Station de Pathologie Vegetale et Phytobacteriologie. 1980; 879-882.
45. Tomczyk A. Increasing resistance to cucumber mite by biotic plant inducers. Biological Lett. 2006; 43(2):381-387.
46. Hanafi A, Traore M, Woithe SM. Induced resistance of tomato to whiteflies and *Pythium* with PGPR *Bacillus subtilis* in a soilless crop grown under green house condition. Acta Hort. 2007; 74:315-323.
47. Siddiqui AZ, Baghel G, Akhtar MS. Biocontrol of *Meloidogyne javanica* by Rhizobium and plant growth-promoting rhizobacteria on lentil. World J Microbiol. Biotechnol. 2007; 23(3):435-441.
48. Bong CFJ, Sikorowski PP. Effects of cytoplasmic polyhedrosis virus and bacterial contamination on growth and development of the corn earworm, *Helicoverpa zea*. J Invertebr. Pathol. 1991; 57:406-412.
49. Qingwen Z, Ping L, Gang W, Qingnian C. On the biochemical mechanism of induced resistance of cotton to cotton bollworm by cutting of young seedling at plumular axis. Acta. Phytophylacica. Sin. 1998; 25:209-212.
50. Saravanakumar D, Lavanya N, Muthumeena B, Raguchander T, Suresh S, Samiyappan R. *Pseudomonas fluorescens* enhances resistance and natural enemy population in rice plant against leaf folder. J Appl. Entomol. 2007; 132:469-479.
51. Melvin JM, Muthukumaran N. Role of certain elicitors on the chemical induction of resistance in tomato against the leaf caterpillar, Spodoptera litura. Not. Bot. Hort. Agrobot. Cluj. 2008; 36(2):71-75.
52. Maria PT, Denny J, Esther F, Marcella D, Henkels K, and Donahue M. Molecular analysis of a novel gene cluster encoding an insect toxin in plant-associated strains of Pseudomonas fluorescens. Environ. Microbiol. 2008; 9(2):3-19.
53. Zehnder G, Kloepper JW, Tuzun S, Yao C, Wei G. Induction of systemic resistance in cucumber against cucumber beetle (Coleoptera : Chrysomelidae) by plant growth promoting rhizobacteria. J Econ. Entomol. 1997; 90(2):391-396.
54. Stout MJ, Zehnder GW, Baur ME. Potential for the use of elicitors of plant defense in arthropod management programs. Arch. Insect Biochem. 2002; 51(4):222-235.
55. Kavitha SJ. Management of major sucking pests in cowpea *Vigna unguiculata* (L.) Walp. with entomopathogenic and plant defense inducing rhizobacteria. M. Sc. (Ag) thesis, Kerala Agricultural University, Thrissur. 2010, 104.
56. Prischmann AD, Lehman RM, Christie AA, Dashiell KE. Characterization of bacteria isolated from maize roots: Emphasis on *Serratia* and infestation with corn rootworms (Chrysomelidae: *Diabrotica*). Appl. Soil Ecol. 2008; 40(3):417-431.
57. Sturz AV, Christie BR, Nowak J. Bacterial endophytes: Potential role in developing sustainable systems of crop production. Critical Rev. Plant Sci. 2000; 19:1-30.
58. Azevedo JL, Macheroni JW, Pereira JO, Araújo WL. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electron J Biotechnol. 2000; 3:40-65.
59. Thuler RB, Barros R, Mariano RLR, Vendramim JD. Effect of plant growth promoting bacteria in developing *Plutella xylostella* (L.) (Lepidoptera: plutellidae) in cabbage. Sci. 2006; 34(2):217-212.
60. Rajendran L, Ramanathan A, Durairaj C, Samiyappan R. Endophytic *Bacillus subtilis* enriched with chitin offer induced systemic resistance in cotton against aphid infestation. Archives Phytopath Pl. Prot. 2011; 44(14):1375-1389.
61. Praca LB. Interactions between *Bacillus thuringiensis* strains and hybrids of cabbage for the control of *Plutella xylostella* and plant growth promotion. M. Sc. thesis, Faculty of Agronomy and veterinary medicine, University of Brasilia, Brasilia. 2012, 141.
62. Fahey JW, Dimock MB, Tomasino SF, Taylor JM, Carlson PS. Genetically engineered endophytes as biocontrol agents: A case study in industry. Microbial Ecol. 1991; 12(3):402-411
63. Gaofu G, Zhang XF, Zhao X. Endophytic *Bacillus subtilis* WH2 containing Pinellia ternata agglutinin showed insecticidal activity against white backed planthopper Sogatella furcifera. Biocontrol. 2012; 58:233-246.
64. Beattie GA, Lindow SE. The secret life of foliar bacterial pathogens on leaves. Ann. Rev. Phytopathol. 1995; 33:145-172.
65. Aggarwal C, Paul S, Tripathi V, Paul B, Khan A. Chitinase producing *Serratia marcescens* for biocontrol of *Spodoptera litura* (Fab). and studies on its chitinolytic activities. Ann. Agric. Res. 2015; 36(2):132-137.
66. Smith RA, Couche GA. Cellular organization with ferritin uptake in the midgut epithelium of the moth Ephestia kuehniella. Appl. Environ. Microbiol. 1991; 57:311-315.
67. Meadows MP. *Bacillus thurigiensis, an Environmental Biopesticise: Theory and Practice.* John Wiley and Sons, New York. 1993, 220.
68. Otsu Y, Matsuda Y, Shimizu H, Ueki H, Fujwara K, Nakajima T, *et al.* Biological control of phytophagous ladybird beetle *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae) by chitinolytic phylloplane bacteria *Alcaligenes paradoxus* entrapped in alginate beads. J Appl. Ent. 2003; 127(8):441-446.
69. Otsu Y, Matsuda H, Mori H, Ueki T, Nakajiama K, Fujiwara M, *et al.* Stable phylloplane colonization by entamopathogenic bacteria *Pseudomonas fluorescens* KPM-018P and biological control of phytophagous ladybird beetle *Epilachna vigintioctopunctata* (Coleoptera:Coccinellidae). Biocontrol Sci. Techn. 2004; 14(5):427-439.
70. 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. Microbial Ecol. 2002; 44(2):144-153.

71. Gonzalez-cabrera J, Molla O. Efficacy of *Bacillus thuringiensis* in controlling tomato miner, *Tutu absoluta.* Biocontrol. 2011; 56:71-80.