Chapter 14
Functional Significance of Insect Gut Bacteria and Their Role in Host Insect Processes, Development, and Crop Production

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14.1 Introduction

The existence of an intimate relationship between many insect species and large numbers of microorganisms has been known for over a century. The success of insects has created myriad of opportunities for bacteria to occupy niches created by insects. The guts of insects offer such niches where the insects are able to take advantage of the products of bacterial metabolism and the adaptability of the prokaryotes. These insect–host and microbe associations form a dynamic spectrum in relation to the necessity as well as physiological impact toward those involved. The association of insects with a wide variety of bacteria includes beneficial endosymbionts, occasional disease causing entomopathogens, and harmless transient cuticle contaminants acquired from the environment (Wernegreen 2002). Endosymbiotic bacteria may be extracellular or intracellular

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D.K. Maheshwari (ed.), *Bacteria in Agrobiology: Plant Growth Responses*,
DOI 10.1007/978-3-642-20332-9_14,

and can be found in the digestive system, hemocoel, reproductive tract, or localized in specialized cells called mycetome. The interplay between insects and the microorganisms inhabiting the gut was recognized as early as 1929. In this year, Wigglesworth (1929) suggested that blood sucking triatomines, which feed solely on blood during its whole life cycle, would have symbionts to provide the insects with complex B vitamins. Bacterial symbionts have also been implicated in furnishing nutrients to sap feeders (aphids, psyllids, whiteflies, mealybugs, etc.). In aphids, the bacterium *Buchnera* was found to utilize nonessential amino acids present in the phloem sap to produce essential amino acids, completing the dietary needs of aphids (Shigenobu et al. 2000; Moran et al. 2003; Wilcox et al. 2003).

Insects living on nutrient-poor diets, where amino acids are very scarce like wood and humus, benefit from symbiotic bacteria that are able to fix atmospheric nitrogen and synthesize amino acids (Nardi et al. 2002). Nevertheless, gut microbes are not limited to insects with unbalanced diets, but could also be involved in insect temperature resistance (Montllor et al. 2002), host plant detoxification (Lauzon et al. 2003), and parasite protection (Olivier et al. 2003). Most of the arthropod symbioses involve highly specific interactions, often between one bacterial species and one insect (Kikuchi et al. 2005). There are a few examples of multispecies communities, such as the associates of the hindgut of termites. Degradation of complex carbohydrates and fixation of nitrogen form the metabolic foundation for the community's function (Nakashima et al. 2002; Bakalidou et al. 2002). The intricacies of managing the carbon and nitrogen economy of the hindgut provide evidence for interdependence and coevolution among the members of the community. The termite system offers one of the most powerful existing models for studying nutritional interactions among community members.

Evidence for a more interactive relationship between microbiota and host was assessed by many researchers (Dillon and Dillon 2004; Genta et al. 2006). However, studies have often been restricted to a catalogue of species present (Sittenfeld et al. 2002; Broderick et al. 2004; Xiang et al. 2006). This might be due to the culture-independent method of analysis of such interrelationships. In order to evaluate the functional significance of the gut bacteria to the host insect, the bacterial isolate should be cultivable under in vitro condition (Mohr and Tebbe 2007).

Thus, the interactions between insects and their microbial symbionts have provided the bases for many principles in symbiosis and have revealed unexpected mechanisms that illustrate how organisms cooperate to perform life functions and gain a competitive edge.

This chapter describes some of the bacteria associated with the class *Insecta* and their functional role in insect host nutrition and development. Furthermore, insight is given into the possible use of insect gut bacteria as potential plant-growth-promoting bacteria (PGPB) for crop production.

14.2 Functional Significance of Insect Gut Bacteria

Numerous studies in the past have described the microbial gut community using classical techniques, but little information was provided on its biological role. A number of reviewers commented on the lack of information on the biological role of the insect microbiota. Brooks (1963) stated that further isolation and characterization of insect intestinal biota were pointless unless they were correlative with either the ability of the host to control its biota or the effect of the biota on the host's physiology. The microbial midgut inhabitants seem likely to benefit the insect host, based on the results of studies conducted with different insect orders by various researchers (Takatsuka and Kunimi 1998, 2000; Lauzon et al. 2003; Dillon and Dillon 2004; Behar et al. 2005; Xiang et al. 2006; Indiragandhi et al. 2007a, 2008a; Figs. 14.1 and 14.2). Gradually, literature related to the biological roles of gut bacteria to the host insect has been increasing. Some of the reported examples of benefits/biological roles of gut bacteria include its assistance in host insect morphogenesis (Zacharuk 1976a, b; Iverson et al. 1984) and food digestion (Borkott and Insam 1990), detoxification of plant allelochemicals and xenobiotics (both synthetic and biopesticides), disease resistance by providing antimicrobial metabolites, and modification of midgut pH by their own metabolism, thereby conferring protection against indigenous and invading pathogenic microorganisms.

14.2.1 Role of Chitinase Produced by Gut Bacteria in Host Insect Processes

Chitin is one of the most important biopolymers in nature. In insects, it supports the cuticles of the epidermis and trachea as well as the peritrophic membrane (PM) lining the gut epithelium. An invertebrate–unique structure, the PM, is irreversibly permeable to secreted digestive enzymes and to the products of digestion (Chapman 1985). Insect growth and morphogenesis are strictly dependent on the capability to remodel chitin containing structures and food materials. For this purpose, insects repeatedly produce chitin synthases and chitinolytic enzymes in different tissues. In the context of morphogenesis, chitinolytic activity of the gut bacteria helps in the emergence of an adult from puparium (Iverson et al. 1984). Similar observations were reported in certain coleopterans in which gut bacteria were found to secrete enzymes capable of digesting portions of the pupal case, thereby weakening the wall and facilitating emergence. Winicur and Mitchell (1974) demonstrated that chitinase was present in the molting fluid of *Drosophila melanogaster* and described about the digestion of endocuticular chitin before larval emergence. It seems that a low level of chitinase activity imparted by the symbiotic bacteria is essential for the host insect throughout its life cycle (Iverson et al. 1984). It has been anticipated that gut microbial symbionts in the intestinal tract can be situated in the gut lumen or associated with the gut epithelium or PM (Buchner 1965). Likewise,

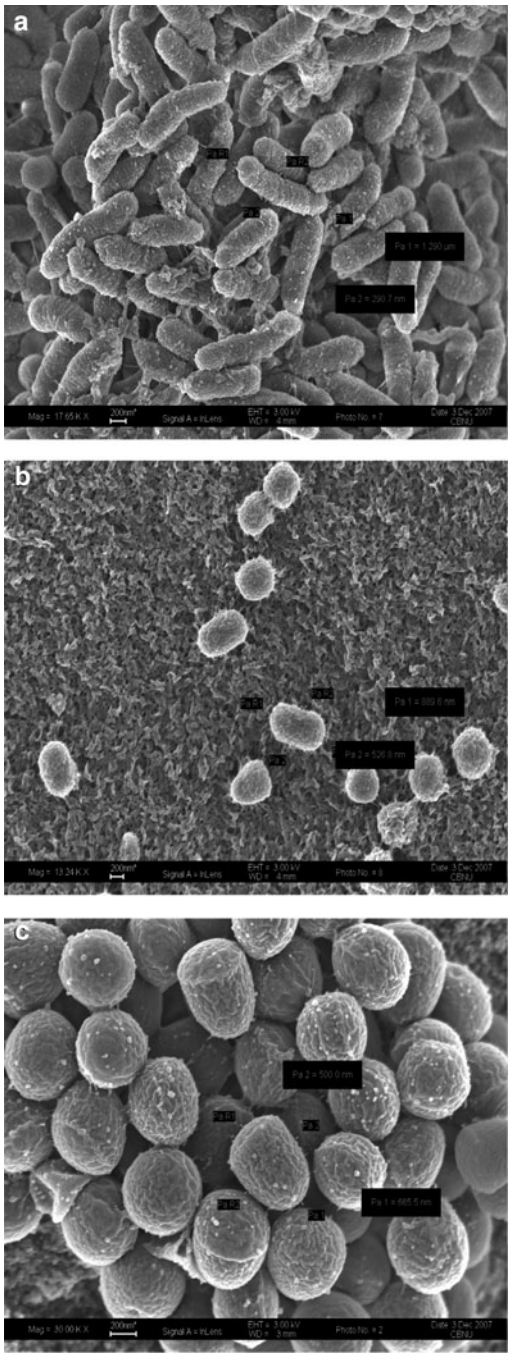

Fig. 14.1 Scanning electron micrograph of *P. xylostella* gut bacterial strains (**a**) *Pseudomonas* sp. PRGB06, (**b**) *Stenotrophomoas* sp. PRGB08, and (**c**) *Brachybacterium* sp. PSGB10

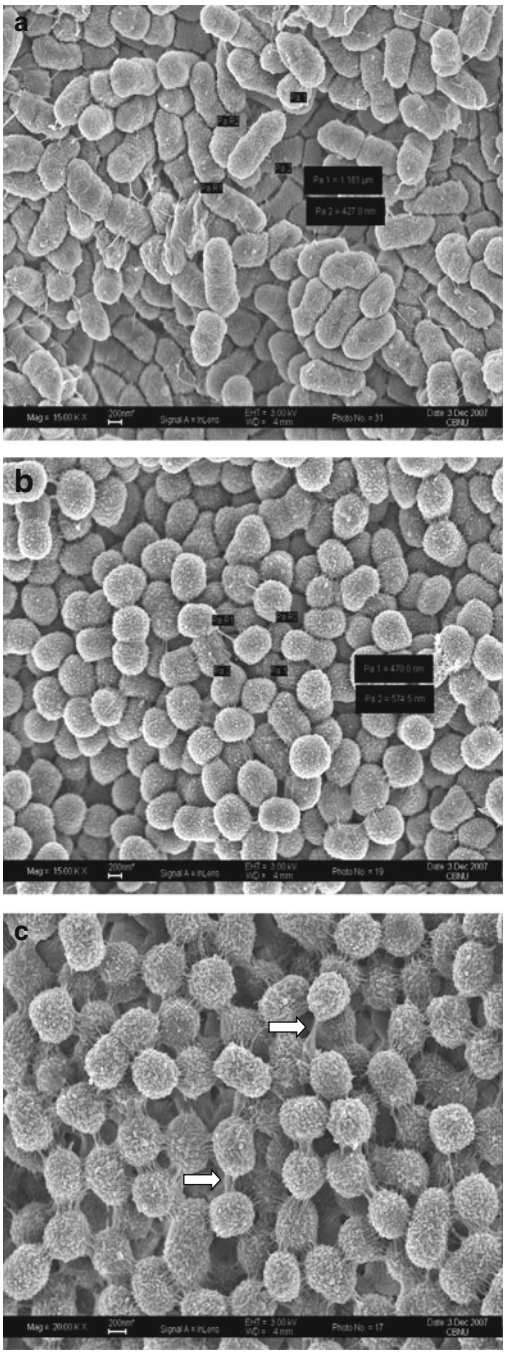

Fig. 14.2 Scanning electron micrograph of *P. xylostella* gut bacterial strains (**a**) *Serratia* sp. PRGB11, (**b**) *Acinetobacter* sp. PSGB03, and (**c**) *arrows* indicates the interlinking pigments among cells of *Acinetobacter* sp. PSGB03

studies on the house cricket (*Acheta domestica*) indicated that part of the bacterial gut microbiota is attached to the PM in the midgut, the gut wall, and cuticular bristles in the hindgut. The ability of insects to digest the chitin containing food resource is rather limited and is often dependent on symbiotic microorganisms. For instance, Brendelberger (1997) observed that the chitinase activity was parallel with the reduction in bacterial numbers in the host gut after antibiotic treatment. It indicates that chitobiase was not synthesized by the host but was taken up by bacteria from the food. This bacterial origin of chitinolytic enzymes was also shown by Genta et al. (2006) for the digestion of cell wall of wheat bran in yellow mealworm, *Tenebrio moliter* (Tenebrionidae), and in some other insects, in the digestion of their own exuvia (Mira 2000).

One more example for the evidence of chitinase produced by gut bacteria on host insect process is with the tsetse fly and its gut bacteria, *Sodalis glossinidius*. *S. glossinidius* has been shown to play a role in potentiating its host's susceptibility to trypanosome infection by influencing the efficacy of the tsetse immune system (Welburn and Maudlin 1991, 1999). In the tsetse fly, it is known that trypanosomes can only be established in the fly midgut if these parasites can successfully evade the action of an *N*-acetyl-D-glucosamine (GlcNAc), a specific trypanocidal lectin which is secreted during feeding (Maudlin and Welburn 1987). *Sodalis* is thought to influence lectin activity through the production of lectin-inhibitory sugar (GlcNAc), which is known to accumulate during pupal development as a result of this bacterium's chitinolytic activity (Welburn et al. 1993). More recently, it has been shown that the chitinase-producing gut bacteria (Fig. 14.1) isolated from *P. xylostella* increased the host insect consumption index (CI), relative growth rate (RGR), approximate digestibility (AD), efficiency of conversion of ingested food (ECI), and efficiency of conversion of digested food (ECD) (Table 14.1) (Indiragandhi et al. 2008a).

14.2.2 Gut Bacteria Mediated Detoxification of Allelochemical and Xenobiotics

Miyazaki et al. (1968) documented the significant role of gut bacteria *Rhagoletis* to detoxify harmful plant compounds, thereby protecting the host fly. Bacterial culture of *Pseudomonas* spp. could break down a variety of pesticides, apparently through the hydrolytic action of strong esterases, and might synthesize amino acids essential for growth of the maggot. Furthermore, gut microbes are reported for their detoxification of allelochemicals such as flavanoids, tannins, and alkaloids (Lauzon et al. 2003). There has been a report on bacterial modification of plant phenolics leading to the production of volatile phenolics in the locust *Schistocerca gregaria* to give guaiacol, a component of the locust aggregation pheromone (Dillon and Dillon 2004). *Pseudomonas aerugenosa* was reported for its detoxification of the plant secondary metabolite polyketide known as pederin in *Paederus* beetle (Piel 2002). Participation of gut bacteria *Pantoea* (=*Enterobacter*) *agglomerans* (a common

Table 14.1 Quantitative food-use efficiency measures for third instar larvae of *Plutella xylostella* after treatment with chitinase-producing *Serratia marcescens* PSGB12 and FLGB16

	Milligram of ingested food mg^{-1} avg. larval weight day^{-1}				
Treatment	CI	RGR	AD (%)	ECI (%)	ECD (%)
A. Cell pellet + buffer	0.62 ± 0.029^{c}	0.068 ± 0.007^{d}	23.17 ± 1.56^{cb}	3.71 ± 0.98^{d}	2.42 ± 0.23^{d}
B. Cell pellet + crude enzyme	1.63 ± 0.226^{b}	0.283 ± 0.062^{b}	30.88 ± 0.91^{abc}	8.45 ± 0.34^{a}	12.83 ± 1.08^{c}
C. Crude enzyme	0.61 ± 0.066^{c}	0.032 ± 0.004^{d}	24.66 ± 0.26^{bc}	0.81 ± 0.03^{f}	1.86 ± 0.37^{de}
D. Cell pellet + buffer	0.34 ± 0.036^{d}	0.179 ± 0.007^{c}	32.53 ± 0.73^{ab}	4.72 ± 0.37^{c}	40.0 ± 0.75^{b}
E. Cell pellet + crude enzyme	3.06 ± 0.036^{a}	0.579 ± 0.066^{a}	46.8 ± 22.72^{a}	5.6 ± 0.45^{b}	65.05 ± 1.96^{a}
F. Crude enzyme	0.22 ± 0.022^{de}	0.177 ± 0.015^{c}	13.8 ± 1.80^{cb}	2.95 ± 0.32^{e}	1.58 ± 0.25^{de}
G. Buffer	0.06 ± 0.015^{e}	0.016 ± 0.003^{d}	11.5 ± 1.32^{c}	0.97 ± 0.16^{f}	0.47 ± 0.073^{e}
LSD ($P \geq 0.05$)	0.18	0.06	20.0	0.74	1.62

Treatment details: A–C cell pellet, crude enzyme of PSGB12; D–F cell pellet, crude enzyme of FLGB16; G – buffer alone (0.05 M phosphate buffer); CFU ml^{-1} for PSGB12 $= 2 \times 10^{9}$ and for FLGB16 $= 5.75 \times 10^{9}$; 1 ml of supernatant gave 5.03 and 5.01 mU for PSGB12 and FLGB16, respectively. *CI* consumption index, *RGR* relative growth rate, *AD* approximate digestibility, *ECI* efficiency of conversion of ingested food, and *ECD* efficiency of conversion of digested food. Values are mean of $\pm$ SE of five replications per treatment. Means followed by the same letter are not significantly different from each other at 5% (LSD) (Indiragandhi et al. 2008a)

inhabitant of fruit fly and its host plant apple) in biochemical transformation of plant allelochemical dihydrochalcone phloridzin has been explored for *Rhagoletis pomonella*. Since the extent to which insects catabolize harmful compounds in their digestive tracts or on their host plant is of considerable importance to their survival (Lauzon et al. 2003), it would be possible that when insects feed on plant materials that contain plant-produced defensive toxins or/are exposed to insecticides or other pesticides. Therefore, it is likely that the gut bacteria are also exposed to these toxins and may actually contribute to detoxification of these compounds (Davidson et al. 2000; Genta et al. 2006), since gut microbes can adapt rapidly to changes in the diet of the insect by changing the population profiles and induction of requisite enzymes (Santo-Domingo et al. 1998). Therefore, the ability of microbes to modify or detoxify plant allelochemicals is an additional property that may be important in certain insect–microbe relationships (Dillon and Dillon 2004).

Most insects detoxify chemicals by converting lipophilic compounds to more water-soluble compounds that are more easily excreted. A well-known enzyme responsible for this detoxification action is glutathione-S-transferase (GST), which is widely distributed in all living organisms. So far, reports indicate that the action of GST in the insect gut is the well-explored enzyme in the detoxification of insecticides sprayed against the insects (Mohan and Gujar 2003a). Some studies reported that the bacterially produced enzymes were responsible for the detoxification of toxic substances in host insects. For instance, field-collected strains of *Spodoptera frugiperda* (J.E. Smith) showed high activity of detoxification enzymes, namely, microsomal oxidases, glutathione S-transferases, and hydrolase (Yu 1992). Interestingly, the enzyme phosphotriesterase (PTE) from microorganisms in soil

seems to be associated with the detoxification of organophosphorous pesticides and organophosphate resistance mechanism in insects. PTE was first detected in the soil microoganisms, *Pseudomonas diminuta* and *Flavobacterium* sp., which are capable of hydrolyzing paraoxon and parathion at a high catalytic rate (Munnecke and Hsieh 1974). Gut isolates of *P. xylostella* which are resistant to prothiofos were shown to have increased activity of GST than that of the same but susceptible field population (Indiragandhi and Sa, unpublished data). The high reliance to insecticides for the control of insect pests and the repeated use of the same insecticides may result in significant reduction of their biological efficacy. This loss of efficacy might be attributed to the rapid microbial degradation of insecticides by a gut symbiotic microflora as reported in soil applied nematicides (Karpouzas et al. 2005). Therefore, the enzymes of gut bacteria might contribute to the detoxification of insecticides in insects, consequently giving rise to resistance development.

14.2.3 Gut Bacteria Mediated Disease Resistance in Host Insect

The insect intestinal tract is generally inhospitable to fungi and infection rarely occurs via the gut. This is surprising as both foregut and hindgut are lined with unsclerotized cuticle, which is actively invaded, for example, in locust by an adapted fungal pathogen. It was generally thought that anaerobiosis, digestive enzymes, adverse pH, speed of food throughput, and protection from the peritrophic membrane are primarily responsible for the gut barrier to fungi (Dillon and Charnley 1991). However, none of these factors can account for the failure of the conidia of *Metarhizium anisopliae* to germinate in the guts of locusts (Dillon and Charnley 1991). Since the guts of germfree locusts do not have such inhibitory properties, it was concluded that an antifungal toxin was produced by the gut microbiota of the locust. Subsequently, antimicrobial phenolic compounds were purified from the gut and fecal pellets of conventionally reared locusts. Three phenolics were identified using GC-MS, namely, hydroxyquinone, 3,4-dihydroxybenzoic, and 3,5-dihydroxybenzoic acids. These compounds were not detected in fractions from germfree insects. A solution of authentic phenols inhibited germination of *M. anisopliae* at concentrations estimated to occur in the fecal pellets. When *Pantoea agglomerans*, a prominent member of the microbial population in the locust gut, was introduced into germfree insects the antifungal compounds reappeared in the feces in proportion to the size of the gut bacterial population (Dillon and Charnley 1995, 1996). In Hymenopteran social insect honeybees, in addition to the genetically determined hygienic behavior (uncapping of cells and removal of diseased and dead larvae) toward the Chalkbrood disease caused by *Ascosphaera apis*, gut bacteria of worker bees such as *Paenibacillus alvei*, *Bacillus circulans*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, and *B. subtilis* were reported for the host insect resistance to disease (Gilliam 1997). The phenolics produced by the gut microbiota are toxic to a wide range of insect and plant

Table 14.2 Antagonistic activities of *P. xylostella* gut bacterium *Pseudomonas* sp. PRGB06 against phytopathogenic fungi

Phytopathogenic fungi	Host	Disease	Inhibition zone diameter (cm)
Botrytis cinerea	Tomato	Gray mold	1.8 ± 0.4^{bc}
Colletotrichum acutatum KACC 41932	Strawberry	Leaf spot	–
C. capsici KACC 40978	Red pepper	Anthracnose	–
C. coccodes KACC 40008	Red pepper	Anthracnose	1.2 ± 0.1^{c}
C. gloespoiroides KACC 40690	Red pepper	Anthracnose	3.6 ± 0.1^{a}
Fusarium oxysporum f. sp. *niveum* KACC 40902	Muskmelon	Wilt	–
Rhizoctonia solani KACC 40106	Rice	Sheath blight	3.0 ± 0.4^{a}
Sclerotia sclerotiorum	Ginseng	Wilt	2.3 ± 0.2^{b}
Phytophthora capsici KACC 40483*	Red pepper	Anthracnose	–
LSD ($P \geq 0.05$)			0.7

– Absence of antagonism

Values are the mean ± SE of three replications. In the same column, significant differences according to the LSD at 0.05% levels are indicated by different letters (Indiragandhi et al. 2008a)

*Growth of *Phytophthora capsici* was inhibited by *Serratia* sp. PRGB11 (1.4 ± 0.3), *Acinetobacter* sp. strains PRGB15 (1.8 ± 0.6), PRGB16 (1.6 ± 0.4), PSGB03 (1.6 ± 0.5), PSGB04 (1.6 ± 0.2), and PSGB05 (1.6 ± 0.3); plus values in parentheses following strain names indicate diameter of inhibition zone (cm)

pathogenic fungi and insect pathogenic bacteria (Dillon and Charnley 2002). Antifungal activity of gut bacteria isolated from *P. xylostella* was reported for entomo- and phytopathogenic fungi under in vitro condition (Table 14.2 and Fig. 14.3) (Indiragandhi et al. 2007a, 2008a). The gut bacterial isolates showed significant activity of biocontrol determinants such as siderophore (Fig. 14.4), chitinase, and β-1,3 glucanase (Tables 14.3 and 14.4). Consequently, the antifungal activity of gut bacteria toward entomopathogenic fungi might be due to the action of individual or combination of all the biocontrol determinants. For the former case, it could be explained that the antibiotic activity might be due to the siderophore and its cross-utilization of siderophores produced by the fungal pathogens (Indiragandhi et al. 2008a, b) (Table 14.4).

14.2.4 Gut Bacteria Mediated Protection Against Biopesticides

The soil bacterium, *Bacillus thuringiensis* (*Bt*), is an important component of insect pest management program all over the world. Insecticidal proteins of *Bt* are effective for controlling many insect species, but insect resistance to *Bt* threatens the long-term effectiveness of these toxins (Oppert et al. 1997; Mohan and Gujar 2003b). The salient feature of this species is the accumulation of crystalline parasporal inclusions during sporulation. These inclusions are composed of one or more protoxins, known as δ-endotoxins, each of which is specific primarily at the level of insect orders, particularly Lepidoptera, Diptera, and Coleoptera. In

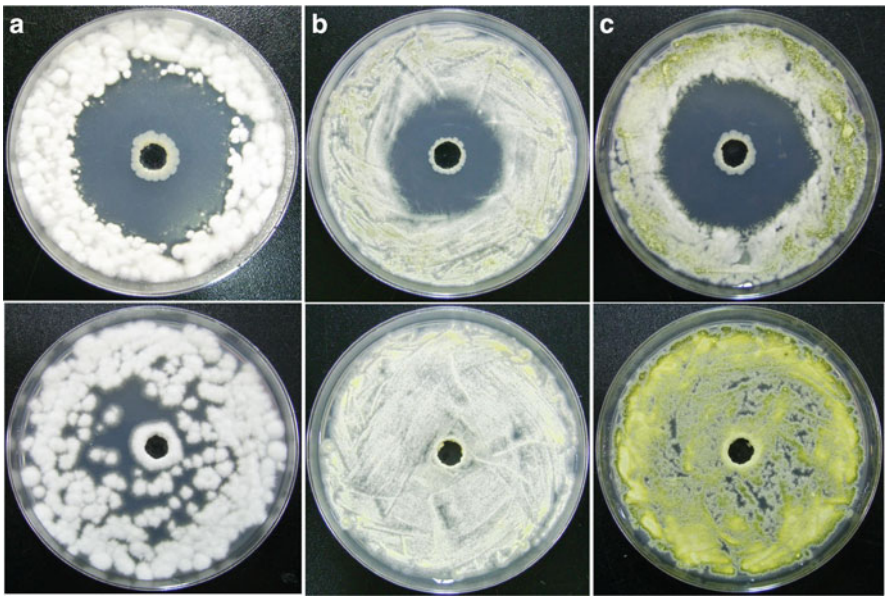

Fig. 14.3 Antagonistic activity of *P. xylostella* gut associated *Pseudomonas* sp. PRGB06 against the entomopathogens (**a**) *Beauveria bassiana* KACC 40039, (**b**) *Metarhizium* sp. KACC 400217, (**c**) *Metarhizium anisopliae* KACC 40029; their respective controls were shown at bottom panel

Lepidoptera, specificity is in part due to the extremely alkaline midgut environment that is required to solubilize the protoxin into the active form (Broderick et al. 2004). In vivo, protoxins of 120 kDa undergo proteolytic conversion to active toxins of 60–65 kDa through the action of gut protease activity at high pH in the larval midgut. Several mechanisms of insect resistance to *Bt* toxin have been proposed, such as reduced binding of toxin to larval brush border membrane vesicle which results in increased resistance in the Indian meal moth – *Plodia interpunctella*, tobacco budworm, *Heliothis virescens*, and beet armyworm, *Spodoptera litura* (Oppert et al. 1997). However, in some insect species the development of resistance to *Bt* has been linked to altered gut protease activity that interacts with *Bt* toxins. Possibly, these protease enzymes in the gut juice would originate from the gut bacterial genera present in the host insect. Few studies have indicated the inhabitation of the lepidopteran insect gut by different phylotypes of bacteria and their role in determining the insecticidal activity of *Bt* (Takatsuka and Kunimi 2000; Broderick et al. 2004). Midgut pH is important from the perspective of both the microbes and the insect. A predominant member of the midgut microbial community, *Enterobacter faecalis*, is commonly found at higher pH (8–9) and acidifies its environment through its metabolism (Manero and Blanch 1999). This could confer some advantage to the insect host, as well-known microbial toxins of Lepidoptera such as *Bt* toxin are activated only in alkaline conditions (Wilson and Benoit 1993). Thus, *E. faecalis* might protect the insect from *Bt* toxin by decreasing the midgut pH as evidenced by the smaller population of *E. faecalis* in the larvae of

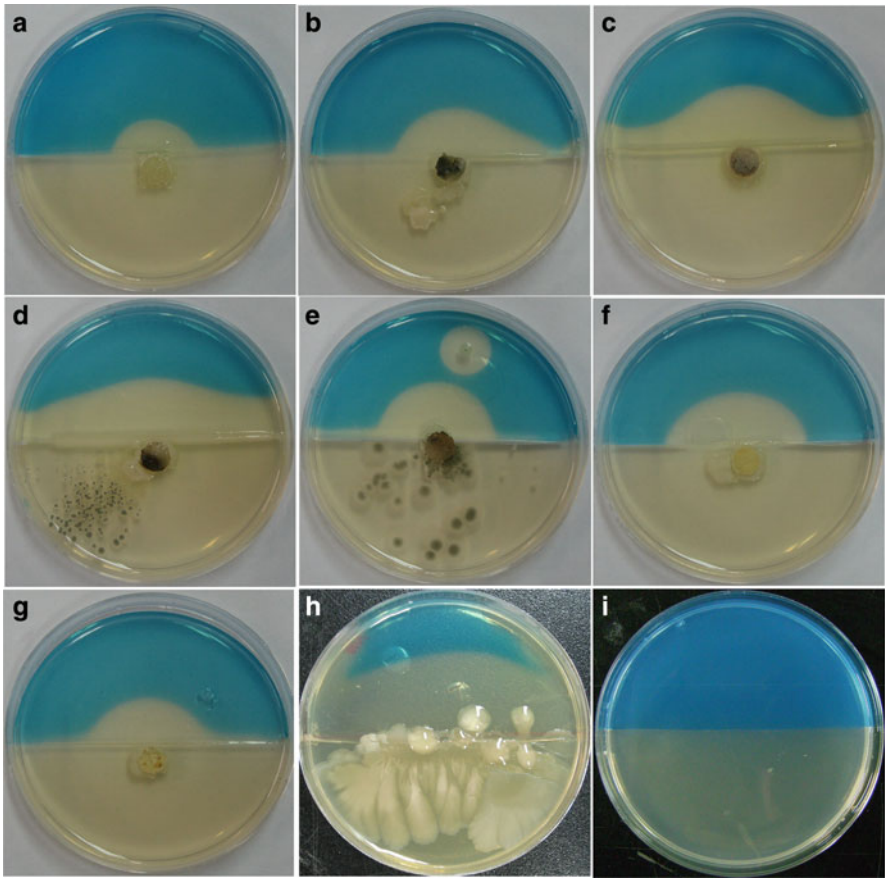

Fig. 14.4 Modified CAS agar plate assay for testing siderophore production by bacterial and fungal entomopathogens. Agar plate containing CAS-blue agar (*top half*) and MEA for fungi (**a**–**g**) and NA for *Bt* (**h**) media (*bottom half*) inoculated with (**a**) *Beauveria bassiana* KACC 40227, (**b**) *Hirsutella thompsoni* KACC 40023, (**c**) *Metarhizium anisopliae* KACC 40230, (**d**) *Metarhizium* sp. KACC 40229, (**e**) *Metarhizium* sp. KACC 40217, (**f**) *Paecilomyces tenuipes* KACC 40503, (**g**) *Paecilomyces* sp., (**h**) *Bacillus thuringiensis* KACC 10168, and (**i**) control (Indiragandhi et al. 2008a)

more susceptible insects to *Bt* toxin (Broderick et al. 2003). The bacteria may also contribute to their own survival by managing midgut pH to enhance their own growth or exclude competing microorganisms. This was demonstrated in the reduction of *Bt* growth to about 20 times lesser than that of larvae without true gut bacteria *Staphylococcus* sp. and *Streptococcus* sp. of lepidopteran oriental tea tortrix, *Homona magnanima* (Takatsuka and Kunimi 2000). On the other hand, it was shown that the siderophore produced by the gut bacteria cross-utilizes the siderophore produced by other bacteria and fungi (i.e., entomopathogenic fungi and bacteria), such as *Bt* through their outer membrane receptor protein (OMRP) (Table 14.4 and Fig. 14.5) (Indiragandhi et al. 2008a).

Table 14.3 Plant-growth-promoting characteristics of bacterial strains isolated from larval guts of *P. xylostella*

	Direct plant-growth promotion through							Indirect plant-growth promotion through		
	Mineral solubilization/oxidation			Production of enzymes and phytohormones				Production of biocontrol determinants		
Bacterial strains[†]	Soluble P (μg^{-1} ml^{-1})	Zn SE (%)	Sulfate content (μg^{-1} ml^{-1})	ARA (nmol ethylene/h/mg protein)	ACCD (nmol α-KB/min/mg protein)	IAA (μg^{-1} ml^{-1})	Salicylic acid (μg^{-1} ml^{-1})	Siderophore	Chitinase	β-1,3-glucanase (μg glucose/min/mg protein)
Acinetobacter sp. PSGB03	458 ± 15^{b}	286 ± 5^{d}	7.7 ± 1.3^{ab}	2.7 ± 0.2^{bc}	60.4 ± 0.6^{d}	7.9 ± 0.2^{e}	5.8 ± 0.5^{d}	+	+	1.3 ± 0.1^{c}
Acinetobacter sp. PSGB04	468 ± 6^{b}	300 ± 3^{c}	2.4 ± 0.9^{c}	2.0 ± 0.4^{e}	572 ± 1.2^{a}	8.3 ± 0^{dc}	4.7 ± 0.3^{e}	+	–	0.7 ± 0.1^{d}
Acinetobacter sp. PSGB05	479 ± 35^{b}	271 ± 2^{e}	6.5 ± 0.6^{b}	2.8 ± 0.1^{bc}	133 ± 1.4^{b}	8.0 ± 0.1^{e}	6.0 ± 0.3^{d}	+	–	–
Acinetobacter sp. PRGB15	540 ± 20^{a}	300 ± 4^{c}	–	2.8 ± 0.2^{ab}	86.5 ± 1.2^{c}	8.2 ± 0^{d}	3.1 ± 0.3^{f}	+	+	–
Acinetobacter sp. PRGB16	547 ± 59^{a}	314 ± 10^{b}	–	2.2 ± 0.2^{de}	–	8.2 ± 0^{dc}	7.2 ± 0.6^{c}	+	–	2.4 ± 0.2^{b}
Pseudomonas sp. PRGB06	250 ± 35^{c}	171 ± 1^{g}	10.0 ± 3.4^{a}	3.0 ± 0.4^{ab}	–	10.0 ± 0.1^{a}	6.8 ± 0.4^{c}	+	–	8.0 ± 0.4^{a}
Serratia sp. PRGB11	216 ± 5^{c}	250 ± 3^{f}	8.0 ± 1.2^{ab}	2.4 ± 0.3^{cd}	–	8.7 ± 0^{b}	8.0 ± 0.4^{b}	+	+	–
Serratia sp. PSGB13	120 ± 18^{d}	514 ± 3^{a}	2.4 ± 0.9^{c}	3.3 ± 0.4^{a}	–	8.4 ± 0.1^{c}	10.1 ± 0.7^{a}	+	+	–
LSD ($P \geq 0.05$)	43	7.0	3.3	0.4	3.5	0.2	0.7			0.4

ARA acetylene reduction assay, *IAA* indole-3-acetic acid, *ACCD* 1-aminocyclopropane-1-carboxylate deaminase, *α-KB* α-ketobutyrate, + presence of trait, – absence of trait. The values indicate the mean ± SE of three replications. In the same column, significant differences according to the LSD at 0.05% levels are indicated by different letters (Indiragandhi et al. 2008b)

[†]All the strains were positive for ammonia production and negative for HCN and pectinase production

Table 14.4 Cross-utilization of homologous and heterologous siderophores by *P. xylostella* gut bacteria

Bacterial strain[a]	MIC[b] (μM)	Gut bacterial isolates whose siderophores (homologous) were utilized	Entomopathogenic bacteria and fungi, and phytopathogenic fungi (heterologous) whose siderophores were utilized[c]
Brachybacterium sp. PSGB10	200	A, B, F, G	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17
Pseudomonas sp. PRGB06	500	B, C, E, F	1, 2, 4, 5, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17
Serratia marcescens FLGB16	500	A, B, E, F	1, 3, 8, 11, 12, 13, 14

[a]*Acinetobacter* sp. PRGB16 (MIC-1,000 μM), *Stenotrophomonas* sp. PRGB08 (500 μM), and *Serratia* sp. PRGB11 (500 μM) did not cross-feed either homologous or heterologous siderophores
[b]Minimum inhibitory concentration of 2,2′-dipyridyl (DPD)
[c](1) *Bacillus thuringiensis* KACC 10168, (2) *Beauveria bassiana* KACC 40218, (3) *B. bassiana* KACC 40224, (4) *B. bassiana* KACC 40039, (5) *Cordyceps millitans* KACC 40665, (6) *Metarhizium* sp. KACC 40217, (7) *Metarhizium* sp. KACC 40230, (8) *M. anisopliae* KACC 40029, (9) *Hirsutella thompsoni* KACC 40023, (10) *Paecilomyces amarosiensis* KACC 41779, (11) *P. tenuipes* KACC 40503, (12) *Botrytis cinerea*, (13) *Colletotrichum acutatum* KACC 40218, (14) *C. coccodes* KACC 40218, (15) *C. gleosporoides* KACC 40218, (16) *Fusarium oxysporum* f. sp. *niveum* KACC 40902, and (17) *Phytophthora capsici* KACC 40483 (Indiragandhi et al. 2008a)

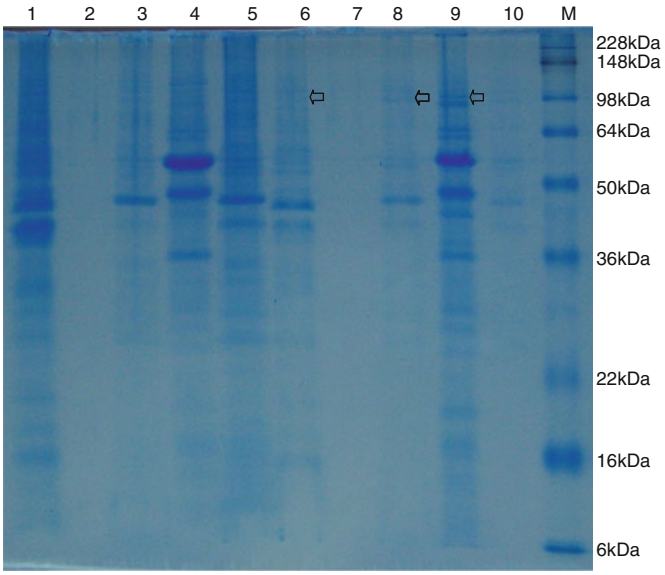

Fig. 14.5 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12%) (SDS-PAGE) analysis of OMRP fraction isolated from bacterial cells of *Pseudomonas* sp. PRGB06, *Stenotrophomonas* sp. PRGB08, *Brachybacterium* sp. PSGB10, *Serratia marcescens* FLGB16, and *Serratia* sp. PRGB11 grown under normal and iron-deficient conditions (NA + MIC of 2,2′-DPD for respective strain), shown in the order of lanes 1–5 and 6–10, respectively. 10 μg of protein was loaded in each lane. Standard marker is shown in lane M. The *arrowhead* shows 96–98 kDa outer membrane protein (Indiragandhi et al. 2008)

14.2.5 Gut Bacteria Mediated Nitrogen Fixation in Insects

Nitrogen, although abundant in the atmosphere, is paradoxically a limited resource for multicellular organisms. In the class Insecta, biological nitrogen fixation has been greatly demonstrated in termites. Even though termite gut cells are now known to be capable of digesting lignocellulose without direct assistance from gut symbionts (Bignell 2000), no cells of arthropod guts are competent to fix nitrogen or to convert carbon dioxide to acetate. These two anaerobic activities are the sole province of the gut microbes. Microbial acetogenesis in termite guts has been demonstrated to provide at least one third of the energy requirements of termites (Breznak 2000). Anaerobic prokaryotes provide their arthropod hosts with important nutritional benefits that cannot be provided by eukaryotic cells alone. Behar et al. (2005) found that all individuals of field-collected mediterranean fruit flies, *Ceratitis capitata*, harbor large diazotrophic enterobacterial populations that express dinitrogen reductase in the gut. Moreover, nitrogen fixation was demonstrated in isolated guts and in live flies. This may significantly contribute to the fly's nitrogen intake. The presence of similar bacterial consortia/activity in additional insect orders such as Lepidoptera (Table 14.3) (Hameeda et al. 2006b; Indiragandhi et al. 2008b) suggests that nitrogen fixation occurs in vast pools of terrestrial insects. On a large scale, this phenomenon may have a considerable impact on the nitrogen cycle (Behar et al. 2005). Insect gut bacteria associated with various orders of insects and their functional role in insects are summarized in Table 14.5.

14.3 Potential of Antibiotic Therapy for Insect Pest Management

The literatures reviewed under the various topics highlighted the importance of gut bacteria to the host insect species. The loss of gut bacteria often results in detrimental fitness costs for the host, such as growth impairment, shortened life span, and sterility, while their presence contributes to insect morphogenesis, digestion, nutrition, protection against toxic substances, pheromone production, and even reproduction (Brand et al. 1975; Buchner 1965; Nolte 1977; O'Neill et al. 1992). Evidently, the reduction or elimination of the gut bacteria in insects by antibiotic therapy would certainly cause deleterious effects on the host insect. For instance, Costa et al. (1997) evaluated the effect of different substances on the growth and development of whiteflies. They found significant negative effects on growth and development of whiteflies due to the bacterial protein synthesis affected by the test substances such as tetracycline and rifampicin. Likewise, the removal of *Buchnera* with antibiotics severely debilitates aphid performance and fecundity (Koga et al. 2007). Broderick et al. (2000) observed that lepidopterous larvae fed on antibiotics in various combinations display numerous signs of reduced health. For example,

Table 14.5 Gut bacterial phylotypes identified from different orders of class Insecta

Sl. no.	Insect pests	Gut bacterial phylotypes	Functional significance	References
Coleoptera				
1	Pine bark beetle *Ips paraconfusus* (Scolytidae)	*Bacillus cereus*	Pheromone production	Brand et al. (1975)
2	Rice weevil *Sitophilus* spp. (Dryopthoridae)	*Aeromonas haywardensis*, *Erwinia carotovora*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Providencia stuartii*, *Serratia liquefaciens*, *Escherichia coli*, *Paracoccus* sp., *P. fluorescens*	Not determined	Lefévre et al. (2004)
Diptera				
3	Crucifer root maggot *Delia radicum* (Anthomyiidae)	*Erwinia amylovora*, *E. carotovora*, *K. pneumoniae*, *E. coli*, *Helicobacter canadiensis*, *Mycobacterium avium*, *Pseudomonas aeroginosa*, *P. cichorii*, *Rhodothermus marinus*, *Serratia ficaria*, *Serratia marcescens*	Nutritional role	Lukwinski et al. (2006)
4	Mediterranian fruit fly *Ceratitis capitata*	*Enterobacter* spp., *Klebsiella* spp.	Nitrogen fixation	Behar et al. (2005)
5	Apple fruit fly *Rhagoletis pomonella* (Tephritidae)	*Pseudomonas* spp., *Enterobacter* sp., *Micrococcus* sp., *Flavobacterium* sp., *Bacillus* sp.	Dihydrochalcone phloridzin degradation, detoxification of Azinphosmethyl	Lauzon et al. (1994, 2003)
6	Mexican fruit fly *Anastrepha ludens* (Tephritidae)	*Enterobacter*, *Listeria*, *Povidencia*, *Serratia*, *Staphylococcus* spp. *Citrobacter*, *Streptococcus*, *Aerococcus*, *Listeria*	Not determined	Kuzina et al. (2001)
7	Queensland fruit fly *Bactrocera tryoni* (Tephritidae)	*Klebsiella oxytoca*, *E. cloacae*	Nitrogen fixation	Murphy et al. (1994)
8	Sugar beet root maggot *Tetanops myopaeformis* (Otitidae)	*Acinetobacter calcoaceticus*, *S. liquefaciens*, *S. marcescens*, *P. maltophilia*, *P. fluorescens*	Morphogenesis	Iverson et al. (1984)

(*continued*)

Table 14.5 (continued)

Sl. no.	Insect pests	Gut bacterial phylotypes	Functional significance	References
Hemiptera				
9	Pentatomid bug *Leptocorisa chinensis*, *Riptortus clavatus*, *R. linearis* (Alydidae)	*Burkholderia*	Not determined	Kikuchi et al. (2005)
10	Pink sugarcane mealybug *Saccharicoccus sacchari* (Pseudococcidae)	*Gluconacetobacter sacchari*	Not determined	Franke et al. (1999)
11	Whitefly *Bemisia argentifolii*, *B. tabaci* (Aleyrodidae)	*Acinetobacter lwoffsii*, *A. baumannii*, *Agrobacterium* sp., *Bacillus megaterium*, *B. amyloliquefasciens*, *B. licheniformis*, *Cellulomonas turbata*, *Citrobacter* sp., *Enterobacter cloacae*, *Flavomonas oryzihabitans*, *Staphylococcus aureus*, *S. epidermidis*	Honey dew production	Davidson et al. (2000)
12	Cotton bollworm *Helicoverpa armigera* (Noctuidae)	*Acinetobacter anitratus*, *A. venetianus*, *Aquamonas fontana*, *Flavobacterium*, *Enterobacter avium*, *E. casseliflavus*, *E. gallinarum*, *Leuconostoc citreum*, *Lactococcus lactis*, *Pseudomonas mevalonii*, *Sphingomonas* sp., *Stenotrophomonas maltophilia*	Not determined	Xiang et al. (2006)
13	Pink bollworm (PBW) *Pectinophora gossypiella* (Gelechidae)	*Aeromonas salmonicida* sp. *salmonicida*, *Chromobacterium violaceum*, *Citrobacter koseri*, *Enterococcus faecium*, *Enterobacter gergoviae*, *Klebsiella oxytoca*	Not determined	Kuzina et al. (2002)
14	Automeris *Automeris zugana* (Saturnidae)	*Bacillus* sp., *B. thuringiensis*, *Corynebacterium durum*, *C. pyogenes*, *Citrobacter* spp., *Curtobacterium* spp., *Enterobacter gergoviae*, *E. aerogenes*, *E. agglomerans*, *K. pneumoniae*, *Listeria monocytogenes*, *Micrococcus* spp., *P. diminuta*, *Serratia marcescens*, *S. liquefaciens*, *Staphylococcus* spp.	Not determined	Sittenfeld et al. (2002)

15	Gypsy moth *Lymantria dispar* (Noctuidae)	*Bacillus* sp., *E. faecalis*, *P. putida*, *Staphylococcus xylosus*, *S. cohnii*, *S. lentus*, *Pantoae agglomerans*, *Serratia marcescens*, *Paenibacillus*, *Micrococcus*, *Rhodococcus*	Enhances insecticidal activity of *Bt*	Broderick et al. (2004)
16	Beet armyworm *Spodoptera exigua* (Noctuidae)	*Myroides odoratus*, *Ochrobactrum* sp.	*N*-Acylamino acid production	Spiteller et al. (2000)
17	Cut worm *Agrotis segetum* (Noctuidae)	*Providencia rettigeri*	*N*-Acylamino acid production	Spiteller et al. (2000)
18	Cabbage moth *Mamestra brassicae* (Noctuidae)	*Acinetobacter* sp.	*N*-Acylamino acid production	Spiteller et al. (2000)
19	Oriental tea tortrix *Homona magnanima* (Tortricidae)	*Streptococcus* spp., *Staphylococcus* spp.	Inhibits *Bt* germination in host gut	Takatsuka and Kunimi 2000
20	Leek moth *Acrolepiopsis assectella* (Yponomeutidae)	*Bacillus* spp., *Klebsiella oxytoca*	Kairomone production	Thibout et al. (1995)
21	Diamondback moth *Plutella xylostella* (Yponomeutidae)	*Pseudomonas* sp., *Stenotrophomonas* sp., *Acinetobacter* sp., *Serratia marcescens*, *Brachybacterium* sp. (Figs. 14.1 and 14.2)	Chitinase and siderophore production	Indiragandhi et al. (2008a, b)
Others				
22	Desert locust *Schistocerga gregaria* (Acrididae)	*P. agglomerans*, *K. pneumoniae*, *Enterobacter agglomerans*, *E. cloacea*, *E. casseliflavus*	CR by phenolics and cohesion pheromone production	Dillon et al. (2005)
23	Western flower thrips *Frankliniella occidentalis* (Thripidae)	*Erwinia* spp.	Not determined	de Vries et al. (2001)
24	Formosan subterranean termite *Coptotermes formosanus* (Termitidae)	*Serratia marcescens*, *Enterobacter aerogens*, *E. cloacae*, *C. farmeri*	Scavenge oxygen and create anaerobic condition for anaerobes	Adams and Boopathy (2005)
25	Leaf cutting ants *Atta sexdans*	*Burkholderia* spp.	Antibiotic production	Santos et al. (2004)

a cocktail of antibiotics including tetracycline reduced larval survival by 50% over the first 20 days after hatching, and the surviving larvae weigh one tenth of the untreated control larvae. Tetracycline alone has a similar effect on development, whereas gentamicin, rifampicin, and penicillin have no effect on survival or growth. Another antibiotic, zwittermicin A, dramatically increases larval sensitivity to the insecticidal toxin produced by *Bt* and also alters the population size of more than one member of the gut community. It is possible that a change in the gut microbiota may inhibit larval growth, instar development, and make the larvae more sensitive to pathogens and toxins. Thus, gut bacteria provide a potential target for insect control with systemic antibacterial materials, or transgenic plants that produce antibacterial proteins (Jaynes et al. 1987). With reference to *P. xylostella*, antibiotic-treated leaf-fed larvae showed malformed adult development and failed to successfully develop into pupae (Indiragandhi and Sa, unpublished data).

14.4 Insect Gut Bacteria as Biocontrol Agents and PGPB

Genetic modification of gut isolates which are mildly pathogenic to their host may be exploited as biocontrol agent. For example, *Enterobacter cloacae* WFA73, a mild pathogen to *Bemisia argentifolii*, has the ability to penetrate whitefly gut cells, which suggests that this microorganism could be genetically modified to enhance its effectiveness as a biological control agent. A similar report has been documented for soil microarthropods by Thimm et al. (1998).

Simultaneously, it is possible to exploit the insect gut bacterial isolates for the enhancement of growth and development of crop plants. The transformation of a phyllosphere bacterium, *Bacillus megaterium*, with *Bt* toxin genes for the control of Lepidoptera is an example of such manipulation (Bora et al. 1994). Similar modification of gut bacteria in other insects, to alter their ability to vector plant viruses or other characteristics of these insects, is also an interesting possibility (Richards 1993).

It could be noticeable that there is a tritrophic interaction between plant–insect–bacteria. As well documented, insect guts are known for their microbial population, which is able to fix atmospheric nitrogen, have the ability for mineral solubilization and oxidation and possess hydrolytic enzymes such as chitinase and indole derivatives.

Most reported PGPB are rhizosphere, phyllosphere, and soil isolates, except for a few from milk and cow dung (Nagarajkumar et al. 2004; Swain and Ray 2007). The microbial population in insect guts (4×10^5 to 2×10^{11} ml^{-1} of gut suspension) is greater than that in the phyllosphere (3×10^5 cm^{-2}) (Jacques et al. 1995), representing a well-explored niche for beneficial bacterial isolation. It is assumed that many insect species derive their microbiota from the surrounding environment, for instance, the phylloplane of plants (Dillon and Dillon 2004; Kremer and Souissi 2001). Presumably, the nutrient cycling rates in the gut environment are much higher and the potential disturbances are more dramatic than in soil and plants. Crop plants generally immobilize the nutrients in their leaves. Insects also have well-developed

mouth parts that are used to grind and shred organic substances, making insects more accessible for microbial colonization. Insect gut bacterial strains are also more metabolically versatile than isolates from elsewhere (Dillon and Dillon 2004). Spiteller et al. (2000) reported that the metabolic products produced by gut bacteria of *Spodoptera exigua*, *Mamestra brassicae*, and *Agrotis segetum* play a role in triggering a range of systemic responses with the result of larval feeding. Synthesis of *N*-acyl amino acid by the gut bacteria of *Spodoptera exigua* attracts the predators of the herbivores, when the host insect regurgitates its oral secretions. Findings of this study indicated that the involvement of bacteria in the biosynthesis of compounds which play a pivotal role in the interaction of plants, herbivores, and their predators adds a new trophic level to this complex network of interactions.

The gut passage may also enhance rates of decomposition by inoculating the organic material with bacteria, which might continue to grow outside the gut in the feces. After getting into the soil, the bacteria in the feces can establish in the rhizosphere. Living plant roots exude organic compounds, e.g., water-soluble sugars, amino acids, and organic acids into the rhizosphere, which in turn utilize the products of bacterial metabolism for plant growth. Bacterial features such as the ability to produce ACC (1-aminocyclopropane-1-carboxylate) deaminase, indole derivatives (IAA), and salicylic acid (SA) could benefit plants by restraining the inhibition of root elongation by reducing the level of stress ethylene (Fig. 14.6). SA also acts as (1) a signaling compound in inducing systemic resistance against insect and disease attack and (2) antimicrobial agent (Indiragandhi et al. 2007b). By using the sugar compound as growth substrate, bacterial strains could produce some organic acids, which lead to solubilization of insoluble nutrients such as P and Zn and make them available to crop plants. This may be the reason behind the hypothesis that the insect herbivores can accelerate nutrient mobilization in soil. A number of bacterial isolates colonizing insect gut area have been reported for its potential for plant-growth promotion. Bacterial isolates from the macrofauna and *P. xylostella* were reported for their antagonistic activity against various phytopathogenic fungi, solubilization of rock phosphate by the gluconic acid production, siderophores, ACC deaminase, and hydrolytic enzyme production (Hameeda et al. 2006a, b; Indiragandhi et al. 2008b). Siderophores and indole derivatives synthesized by the insect gut bacterial strains were documented for their antagonistic activity against soil-borne pathogen (Ciche et al. 2003). Bacterial strains of *P. xylostella* significantly inhibit the phytopathogenic fungal growth and enhanced the seedling growth and vigor in economically important crops such as canola, tomato (Indiragandhi et al. 2008b) (Table 14.6), rice, chilli, and maize (Indiragandhi and Sa, unpublished data).

14.5 Future Thrust

This review of earlier studies is indicative of the possibility of utilizing the insect gut bacterial strains as biocontrol agent and PGPB. Therefore, isolation, characterization, and exploitation of microbes from such specialized dynamic environments

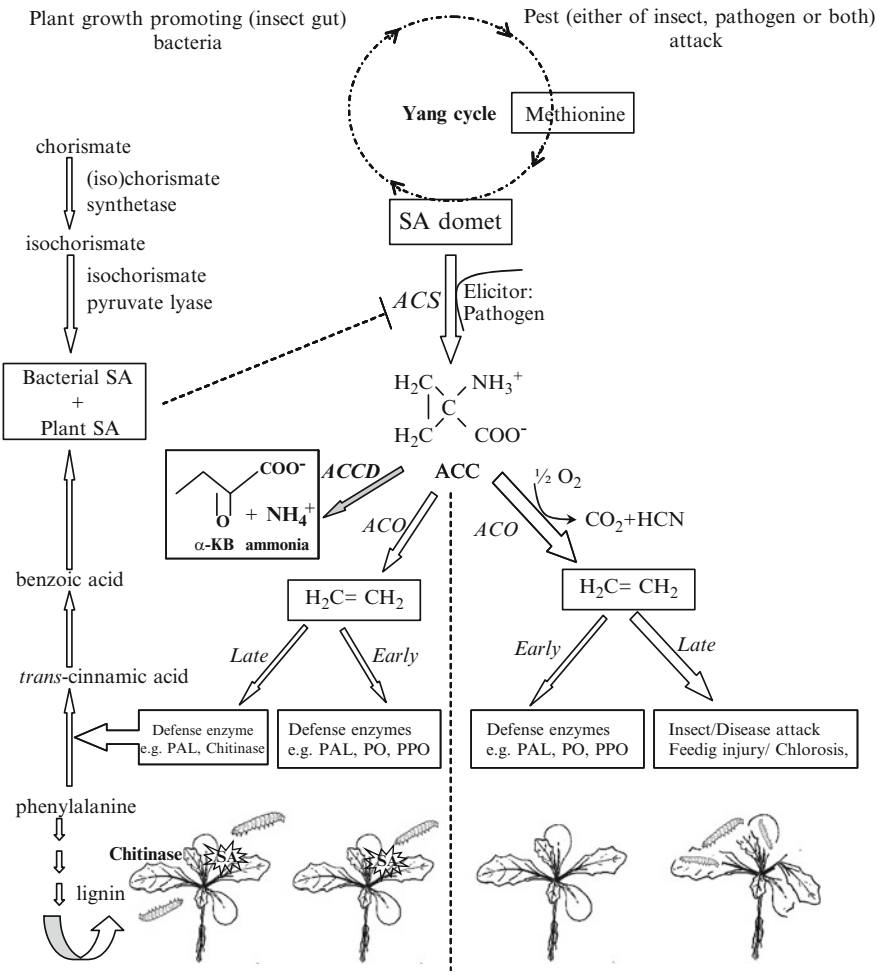

Fig. 14.6 A proposed model for plant-growth-promoting gut bacteria. The eventual increase in defense enzymes activity is a consequence of induction of systemic resistance against insect pests. *SAdomet* S-adenosylmethionine, *SA* salicylic acid, *ACS* 1-aminocyclopropane-1-carboxylate synthase, *ACC* 1-aminocyclopropane-1-carboxylate, *ACO* 1-aminocyclopropane-1-carboxylic acid oxidase, *ACCD* 1-aminocyclopropane-1-carboxylate deaminase, *α-KB* α-ketobutyrate. *Perpendicular* symbol shows the inhibition of ACS by salicylic acid (SA) (Indiragandhi et al. 2007a)

represent a step forward in the development of bioinoculants for increased crop production through effective crop protection.

Acknowledgments The authors wish to thank the financial support given by a BK21 Research Fellowship through a doctoral fellowship to PI to carry out the works pertaining to Diamond Back moth in Department of Agricultural Chemistry at Chungbuk National University, South Korea. The facility provided by TamilNadu Agricultural University, Tamil Nadu, India, to complete this review work is greatly acknowledged.

Table 14.6 Effect of DBM larval gut bacterial strain seed treatment on early growth of canola and tomato under gnotobiotic conditions

	Root length (cm)		Seedling vigor		Dry biomass (mg)	
Treatment	Canola	Tomato	Canola	Tomato	Canola	Tomato
Acinetobacter sp. PSGB03	8.0 ± 0.2^{a} (39)	5.7 ± 0.3^{c} (9)	$1{,}048 \pm 4^{a}$	892 ± 2^{c}	136 ± 6^{bc} (17)	96 ± 0.5^{b} (22)
Acinetobacter sp. PSGB04	8.0 ± 0^{a} (41)	5.5 ± 0.3^{d} (3)	$1{,}007 \pm 4^{b}$	835 ± 9^{d}	151 ± 2^{a} (30)	100 ± 2.8^{a} (26)
Acinetobacter sp. PSGB05	7.7 ± 0^{ab} (35)	6.4 ± 0.1^{a} (21)	957 ± 6^{d}	937 ± 9^{b}	150 ± 1^{a} (30)	83 ± 1.8^{c} (5)
Acinetobacter sp. PRGB15	7.9 ± 0.6^{a} (38)	5.6 ± 0.2^{dc} (7)	995 ± 9^{c}	819 ± 5^{e}	128 ± 1^{c} (11)	89 ± 1.4^{c} (13)
Pseudomonas sp. PRGB06	7.2 ± 0.1^{b} (27)	6.5 ± 0.1^{a} (23)	881 ± 4^{g}	982 ± 2^{a}	136 ± 7^{bca} (17)	100 ± 1.0^{a} (26)
Serratia sp. PRGB11	8.0 ± 0.1^{a} (41)	6.1 ± 0.2^{ba} (16)	930 ± 7^{e}	934 ± 7^{b}	138 ± 3^{b} (19)	91 ± 1.0^{c} (15)
Control	5.7 ± 0.5^{c}	5.3 ± 0.6^{d}	920 ± 6^{f}	811 ± 8^{f}	116 ± 11^{d}	79 ± 1.7^{c}
LSD ($P \geq 0.05$)	0.6	0.4	5.0	7.0	9.0	2.0

The values indicate the mean ± SE of three replications. In the same column, significant differences according to the LSD at 0.05% levels are indicated by different letters. Values in parentheses indicate the percentage increase over the control (Indiragandhi et al. 2008b)

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