Review Article

Beyond *Bt*: New Bacterial Resources for Insect Biocontrol

Andreas Leclerque, Institute for Microbiology and Biochemistry, Geisenheim University, Von-Lade-Strasse 1, 65366 Geisenheim, Germany

ABSTRACT

Leclerque, A. 2018. Beyond *Bt*: new bacterial resources for insect biocontrol. Tunisian Journal of Plant Protection 13 (si): 1-9.

The Gram-positive bacterium *Bacillus thuringiensis* (*Bt*) is the economically most important entomopathogen for insect biocontrol, and for excellent reason a large part of the effort invested in the development of microbial insecticides has concentrated on *Bt* strains and their *cry* genes. Hundreds of *cry* gene sequences have been determined from nature, and molecular modeling has been used to create artificial recombinant Cry proteins with potentially new properties. However, alternative insect biocontrol agents are increasingly solicited. More recently, research efforts have concentrated upon bacterial insect-pathogens as potential biocontrol resources as, e.g., non-*cry* toxins of *Bt* and other bacteria of the family *Bacillaceae* as well as the bacterium *Saccharopolyspora spinosa* (*Actinobacteria*). Moreover, several different γ-proteobacterial entomopathogens belonging to the species *Serratia entomophila*, *Yersinia entomophaga* or *Pseudomonas entomophila* as well as to the genera *Providencia* or *Rickettsiella* are currently being evaluated for their biocontrol potential. The present literature review gives a brief update on these entomopathogens and toxins.

Keywords: *Bacillus thuringiensis*, microbial biocontrol, *Providencia*, *Pseudomonas entomophila*, *Rickettsiella*, Spinosad, TcABC toxin

Introduction.

Insects are associated with bacteria in relationships ranging from obligate endosymbiosis to pathogenicity. Among the numerous entomopathogens that infect and kill the respective host by a diversity of mechanisms, the Gram-positive rod-shaped bacterium *Bacillus cereus* subsp. *thuringiensis* that is commonly referred to as "*Bacillus thuringiensis*" or "*Bt*", is by far the most studied, best understood and most widely used for biocontrol (Roh et al. 2007; Sanchis 2011; Schnepf et al. 1998; Vega and Kaya 2012). Insecticidal Cry protein toxins produced by *Bt* during the stationary growth phase are localized in parasporal bodies and as a rule display toxicity for a narrow host spectrum (De Maagd et al. 2003). Hundreds of toxin encoding *cry* gene sequences have been described (http://www.lifesci.sussex.ac.uk /home/Neil_Crickmore/Bt/), the general mode of action has been elucidated in molecular detail (Bravo et al. 2007), and insect resistant transgenic crops carrying *cry* sequences have been generated successfully. However, emerging insect resistance to Cry toxins has triggered both a debate on Cry-based integrated pest management (IPM) practices (Bravo and Soberón 2008; Pardo-López et al. 2013) and increased efforts to explore

Corresponding author: Andreas Leclerque
Email: andreas.leclerque@hs-gm.de

Accepted for publication 24 January 2018

alternative biocontrol agents. The present article reviews some of the principal advances in research on alternative bacterial insect biocontrol resources.

Non-Cry *Bacillus thuringiensis* toxins.

Besides Cry toxins, *B. thuringiensis* strains produce three main types of further insecticidal protein toxins designated as Cyt, Vip, and Sip proteins (Palma et al. 2014).

Predominantly dipteran specific "cytotoxic" Cyt proteins display a general cytolytic activity *in vitro* (Butko 2003). They have been grouped into three families, termed Cyt1 through Cyt3. Importantly, besides being themselves entomotoxins, Cyt proteins synergistically increase the insecticidal effects of Cry or Vip3 proteins and thereby hold potential to counteract insect resistance development against Cry proteins (Federici and Bauer 1998; Yu et al. 2012).

The terms "vegetative insecticidal" (Vip) and "secreted insecticidal" (Sip) proteins denote two different classes of insecticidal proteins that are both secreted into the medium by *B. thuringiensis* during the vegetative growth phase (Palma et al. 2014). Vip1/2 forms a binary toxin with insecticidal activity against beetles (Coleoptera) and aphids (Sattar and Maiti 2011). Vip1 functions as a receptor-binding domain that facilitates entry of the Vip2 toxin into the host cell's cytoplasm where it blocks actin polymerization (Barth et al. 2004). Single-chain Vip3 proteins display insecticidal activity against a wide spectrum of Lepidoptera (Estruch et al. 1996; MacIntosh et al. 1990). As for Cry toxins, formation of pores in midgut epithelial cell membranes appears to be a crucial step in Vip3 protein toxicity (Yu et al. 1997). However, the mechanisms of pore formation are different for both toxin classes (De Maagd et al. 2003), and insect resistances against Cry and Vip3 are largely independent from each other (Bommireddy et al. 2007). Vip3 proteins have therefore been proposed as complements to Cry proteins in resistance management programs (Mike et al. 2006). *B. thuringiensis* Sip protein is toxic to coleopteran insects as, e.g., the Colorado potato beetle, *Leptinotarsa decemlineata*, by a yet not deciphered mechanism (Donovan et al. 2006).

Recently, the *Bt* zinc metalloprotease InhA has been shown to specifically hydrolyze antimicrobial peptides that contribute to the humoral response of Lepidopteran insect; combined application of InhA and Cry proteins leads to a mutual increase in toxicity (Dammak et al. 2015).

Insecticidal toxins from further *Bacillaceae* bacteria.

Insecticidal toxins often, but not always, homologous to the different classes of *Bt* toxins have been described from further bacteria belonging to the taxonomic family *Bacillaceae* as, e.g., to the species *Brevibacillus laterosporus*, *Lysinibacillus sphaericus*, and *Paenibacillus popilliae* (Ruiu 2015). The latter species is best known as the causative agent of "milky disease" of scarabaeid larvae and is considered a potential biocontrol agent for these agricultural pests. *P. popilliae* expresses a parasporal Cry-homologous protein of still uncertain significance for pathogenicity (Zhang et al. 1997).

B. laterosporus is both notable as a versatile insect pathogen, infecting and killing Coleoptera, Lepidoptera, and Diptera, and as a producer of both antibacterial and antifungal secondary metabolites (Ruiu 2013). Mosquiticidal activity of certain *B. laterosporus* strains has been demonstrated to depend on the

presence of parasporal inclusion bodies similar to those found in *Bt (*Zubasheva et al. 2010). However, a strain lacking crystal proteins has been found highly virulent to the house fly, *Musca domestica*, and has been developed into a commercially available biocontrol agent (Ruiu et al. 2007; 2012). Moreover, a different group of *B. laterosporus* strains infecting larvae of coleopteran pests as, e.g., corn rootworms, secretes binary insecticidal proteins termed ISPs that are related by primary amino acid sequence to the Vip1/2 toxins of *Bt* (Warren 1997).

Actinobacterial macrolids as entomotoxins: avermectins and spinosyns.

Among the large variety of secondary metabolites produced by different *Streptomyces* spp., several compounds have been demonstrated to possess insecticidal activity (Craveri and Giolitti 1957; Kido and Spyhalski 1950; Oishi et al. 1970). Probably best studied is the class of "avermectins", i.e. large macrocyclic lactones produced by the *S. avermitilis* that have been demonstrated to impair neurotransmission in the insect peripheral nervous system by gamma-aminobutyric acid (GABA) receptor binding (Turner and Schaeffer 1989).

Binding to neuronal receptors has been proposed as the main mechanism of action of the better studied members, namely spinosyn A and D (Kirst et al. 1991), of a complex class of polyketide compounds, collectively referred to as "spinosyns" (Kirst 2010; Salgado and Sparks 2005), that confer broad-spectrum insecticidal activity to actinobacteria belonging to the species *Saccharopolyspora spinosa* (Mertz and Yao 1990; Waldron et al. 2000) and *S. pogona* (Lewer et al. 2009). Mixtures of - both natural and semisynthetic - spinosyn derivatives, marketed under the collective name "spinosad", display activity against a wide variety of Lepidoptera and Diptera, but are considered safe for non-target organisms, particularly for vertebrates (Sparks et al. 2001).

Insecticidal TcABC toxin complexes.

A heterogeneous group of Gram-negative bacterial entomopathogens carries large "TcABC" toxin complexes: component A displays the entomotoxic activity that is under certain conditions potentiated by the action of auxiliary components B and C (ffrench-Constant and Waterfield 2006). TcABC toxins have been identified in *Serratia entomophila* (termed SepABC), i.e. the causative agent of "amber disease" of *Costelytra zealandica* grubs in New Zealand, in *Yersinia entomophaga* from the same geographic and host origin, in a further isolate of *S. entomophila* found associated with larvae of the scarabaeid *Phyllophaga* spp., an important pest of maize plants in Mexico (Nunez-Valdez et al. 2008), and in the nematode symbiotic bacteria *Photorhabdus* and *Xenorhabdus* (Blackburn et al. 1998; Bowen and Ensign 1998; Bowen et al. 1998). Moreover, PCR-based screening has led to the identification of a homologous *tcABC* gene cluster in *B. thuringiensis* (Blackburn et al. 2011). Toxin encoding *tcABC* gene clusters are localized on large plasmids. Whereas the action of SepABC from *S. entomophila* is highly specific for the natural host, *C. zealandica* (Hurst et al. 2007; Jackson et al. 2001), further Tc toxin complexes have been described as considerably less specific (Hurst et al. 2011a; Nunez-Valdez et al. 2008). *Y. entomophaga*, for instance, has been shown to be highly virulent for further Coleoptera as well as Lepidoptera and Orthoptera (Hurst et al. 2011b).

Proteobacterial fruit fly pathogens: *Pseudomonas entomophila* and *Providencia* spp.

The gamma-Proteobacterium *Pseudomonas entomophila* (Mulet et al. 2012) perorally infects and rapidly kills larvae and adults of *D. melanogaster* (Vodovar et al. 2005) and has been employed to develop an infection model for bacterial fruit fly pathogens (Liehl et al. 2006). *P. entomophila* has been shown to produce both hydrogen cyanide HCN (Ryall et al. 2009) and a novel ß-pore-forming toxin termed "monalysin" (Blemont et al. 2013; Leone et al. 2015; Opota et al. 2011). Whole genome sequencing of the specific type strain L48 (Vodovar et al. 2006) has led to the development of a PCR-based diagnostic approach for *P. entomophila* employed in a broad screening of olive fly populations around the Mediterranean (Papagiannoulis et al. 2009).

Enterobacteria of the genus *Providencia* are mainly of clinical importance as opportunistic pathogens typically causing dysentery (Galac and Lazzaro 2012). However, *Providencia* bacteria have been found associated with wild-caught *D. melanogaster* and the Mexican fruit fly, *Anastrepha ludens* (Kuzina et al. 2001), and several species as, e.g., *Providencia sneebia* and *P. alcalifaciens* display pronounced virulence to the common vinegar fly (Juneja and Lazzaro 2009). The species *P. vermicola* appears to be the insecticidal agent carried by certain entomo-pathogenic nematodes, and isolated *P. vermicola* bacteria cause elevated mortality in Lepidoptera (Park et al. 2011; Somvanshi et al. 2006). Moreover, *Providencia* bacteria have been demonstrated to attract both male and female fruit flies from several *Bactrocera* species potentially facilitating infection in IPM measures (Hadapad et al. 2016).

Insect-associated *Rickettsiella* bacteria.

Bacteria of the taxonomic genus *Rickettsiella* have been described as intra-cellular pathogens of a wide range of arthropods (Fournier and Raoult 2005) including - besides both crustaceans and arachnids - insects of agricultural or medical importance as, e.g., scarabaeid grubs (Leclerque and Kleespies 2008; Leclerque et al. 2012), wireworms (Leclerque et al. 2011; Schuster et al. 2013) or ticks (Kurtti et al. 2002; Leclerque and Kleespies 2012). Moreover, a mutualistic relationship has been described for bacteria of the species `*Candidatus* Rickettsiella vidridis´ and their host, the green pea aphid, *Acyrthosiphon pisum*, with the *Rickettsiella* endosymbiont being involved in host pigmentation (Tsuchida et al. 2010; 2014) and providing protection against aphid-pathogenic fungi (Lukasik et al. 2013). Despite the misleading genus name, *Rickettsiella* are not closely related to bacteria of the taxonomic genus *Rickettsia* (*Alphaproteobacteria*), but instead belong to the gamma-proteobacterial order *Legionellales* (Fournier and Raoult 2005). However, morpho- and cytological development inside the host and histopathology of infection resemble those of both *Rickettsia* and *Chlamydia*. In a generalized picture, *Rickettsiella* bacteria typically multiply in cytoplasmic vesicles within fat body cells or hemocytes, and multiplication is typically accompanied by the formation of crystal proteins of presumably lysogenic properties (Jurat-Fuentes and Jackson 2012 and references therein; Kleespies et al. 2014). Currently, *Rickettsiella* bacteria are under intensive evaluation as a new source of insect biocontrol agents.

Conclusion.

Past research has indicated several promising approaches for the solicited complementation, not substitution of *Bt*-based products by other microbial insecticides for biological control and integrated pest management. Present and future intensified and systematic screening for new bacterial entomopathogens will likely reveal further novel biocontrol resources. However, development of these microbial resources into innovative bio-insecticides in most cases is still well ahead.

RESUME

Leclerque A. 2018. Au-delà de *Bt*: Nouvelles ressources bacteriennes pour la lutte biologique contres les insectes. Tunisian Journal of Plant Protection 13 (si): 1-9.

La bactérie Gram-positive *Bacillus thuringiensis* (*Bt*) est l'agent entomopathogène le plus économiquement important pour la lutte biologique contre les insectes et pour une excellente raison, une large partie de l'effort investi dans le développement d'insecticides microbiens s'est concentrée sur les souches de *Bt* et leurs gènes *cry*. Des centaines de séquences de gènes *cry* ont été déterminées à partir de la nature, et la modélisation moléculaire a été utilisée pour créer des protéines recombinantes Cry artificielles avec des propriétés potentiellement nouvelles. Toutefois, des agents alternatifs pour la lutte biologique contre les insectes sont de plus en plus sollicités. Plus récemment, les efforts de la recherche se sont concentrés sur les agents pathogènes bactériens des insectes comme ressources potentielles d'agents de lutte biologique telles que les toxines non-*cry* de *Bt* et d'autres bactéries de la famille des *Bacillaceae* ainsi que la bactérie *Saccharopolyspora spinosa* (*Actinobacteria*). De plus, divers entomopathogènes γ-protéobactériens appartenant aux espèces *Serratia entomophila, Yersinia entomophaga* ou *Pseudomonas entomophila* ainsi que les genres *Providencia* ou *Rickettsiella* sont actuellement en cours d'évaluation pour leur potentiel de lutte biologique. La présente revue de littérature donne une brève mise à jour sur ces agents entomopathogènes et ses toxines.

Mots clés: *Bacillus thuringiensis*, lutte microbiologique, *Providencia, Pseudomonas entomophila, Rickettsiella,* Spinosad, toxine TcABC

ملخص

لوكليرك، أندرياس. 2018. ما بعد *Bt*: موارد بكتيرية جديدة للمكافحة البيو لوجية ضد الحشرات.

Tunisian Journal of Plant Protection 13 (si): 1-9.

إن البكتيريا غرام (+) من نوع *Bacillus thuringiensis* (*Bt*) هي العامل الممرض للحشرات الأهم اقتصاديا في المكافحة البيولوجية ضد الحشرات ولسبب ممتاز، جزء كبير من الجهد استُثمر في تطوير مبيدات ميكروبيولوجية للحشرات تكثّف حول سلالات *Bt* ومورّثاتها *cry*. حُددت مائات التتابعات للمورثات *cry* واستُعمل التصميم الجزيئي لانتاج البروتينات المؤتلفة Cry اصطناعيا ذات خصوصيات محتملة جديدة. إلا أن عوامل بديلة للمكافحة البيولوجية ضد الحشرات أصبحت مطلوبة أكثر فأكثر. في الآونة الأخيرة، تكثفت جهود البحث على عوامل ممرضة بكتيرية للحشرات كموارد محتملة لعوامل مكافحة بيولوجية مثل توكسينات لا-*cry* للبكتيريا *Bt* وبكتيريات أخرى من فصيلة *Bacillaceae* وكذلك البكتيريا *Saccharopolyspora spinosa* (*Actinobacteria*). أيضا، يتم حاليا تقييم إمكانية المكافحة البيولوجية لمختلف الممرضات الحشرية من طائفة γ-بروتيوبكتيريات التي تنتمي إلى الأنواع *Serratia entomophila* و *Yersinia entomophaga* أو *Pseudomonas entomophila* وكذلك الجنسان *Providencia* و *Rickettsiella*. إن هذه المقالة التوليفية تقدم تحيين مقتضب لهذه العوامل الممرضة للحشرات وتكسيناتها.

كلمات مفتاحية: توكسينات TcABC، سبينوراد، مكافحة ميكروبيولوجية، *Bacillus thuringiensis*، *Providencia*، *Pseudomonas entomophila*، *Rickettsiella*

LITERATURE CITED

Barth, H., Aktories, K., Popoff, M.R., and Stiles, B.G. 2004. Binary bacterial toxins: Biochemistry, biology, and applications of common *Clostridium* and *Bacillus* proteins. Microbiology and Molecular Biology Reviews 68: 373-402.

Blackburn, M., Golubeva, E., Bowen, D., and ffrench-Constant, R.H. 1998. A novel insecticidal toxin from *Photorhabdus luminescens*, toxin complex a (Tca), and its histopathological effects on the midgut of *Manduca sexta*. Applied and Environmental Microbiology 64: 3036-3041.

Blackburn, M.B., Martin, P.A.W., Kuhar, D., Farrar, Jr., R.R., and Gundersen-Rindal, D.E. 2011. The Occurrence of *Photorhabdus*-Like Toxin Complexes in *Bacillus thuringiensis*. PLoS ONE 6: e18122. doi:10.1371/journal.pone.0018122.

Blemont, M., Vincentelli, R., Kellenberger, C., Opota, O., Lemaitre, B., Roussel, A., and Leone, P. 2013. Crystallization and preliminary X-ray analysis of monalysin, a novel β-pore-forming toxin from the entomopathogen *Pseudomonas entomophila*. Acta Crystallographica Section F Structural Biology and Crystallography Communications 69: 930-933.

Bommireddy, P.L., Leonard, B.R., and Emfinger, K. 2007. Heliothine larval behavior on transgenic cotton expressing a *Bacillus thuringiensis* insecticidal exotoxin, Vip3A. Journal of Cotton Science 11: 199-207.

Bowen, D.J., and Ensign, J.C. 1998. Purification and characterization of a high-molecular-weight insecticidal protein complex produced by the entomopathogenic bacterium *Photorhabdus luminescens*. Applied and Environmental Microbiology 64: 3029-3035.

Bowen, D., Rocheleau, T.A., Blackburn, M., Andreev, O., Golubeva, E., Bhartia, R., and ffrench-Constant, R.H. 1998. Insecticidal toxins from the bacterium *Photorhabdus luminescens*. Science 280: 2129-2132.

Bravo, A., and Soberón, M. 2008. How to cope with insect resistance to Bt toxins? Trends in Biotechnology 26: 573-579.

Bravo, A., Gill, S.S., and Soberon, M. 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon 49: 423-435.

Butko, P. 2003. Cytolytic toxin Cyt1A and its mechanism of membrane damage: Data and hypotheses. Applied and Environmental Microbiology 69: 2415-2422.

Craveri, R., and Giolitti, G. 1957. An antibiotic with fungicidal and insecticidal activity produced by *Streptomyces*. Nature 179: 1307.

Dammak, I., Dammak, M., and Tounsi, S. 2015. Histopathological and combinatorial effects of the metalloprotease InhA1 and Cry proteins of *Bacillus thuringiensis* against *Spodoptera littoralis*. International Journal of Biological Macromolecules 81: 759-762.

De Maagd, R.A., Bravo, A., Berry, C., Crickmore, N., and Schnepf, H.E. 2003. Structure, diversity, and evolution of protein toxins from spore forming entomopathogenic bacteria. Annual Reviews in Genetics 37: 409-433.

Donovan, W.P., Engleman, J.T., Donovan, J.C., Baum, J.A., Bunkers, G.J., Chi, D.J., Clinton, W.P., English, L., Heck, G.R., Ilagan, O.M., Krasomil-Osterfeld, K.C., Pitkin, J.W., Roberts, J.K., and Walters, M.R. 2006. Discovery and characterization of Sip1A: A novel secreted protein from *Bacillus thuringiensis* with activity against coleopteran larvae. Applied Microbiology and Biotechnology 72: 713-719.

Estruch, J.J., Warren, G.W., Mullins, M.A., Nye, G.J., Craig, J.A., and Koziel, M.G. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. Proceedings of the National Academy of Sciences of the USA 93: 5389-5394.

Federici, B.A., and Bauer, L.S. 1998. Cyt1Aa protein of *Bacillus thuringiensis* is toxic to the cottonwood leaf beetle, *Chrysomela scripta*, and suppresses high levels of resistance to Cry3Aa. Applied and Environmental Microbiology 64: 4368-4371.

ffrench-Constant, R., and Waterfield, N. 2006. An ABC Guide to the Bacterial Toxin Complexes. Advances in Applied Microbiology 58: 169-183.

Fournier, P.-E., and Raoult, D. 2005. Genus II. *Rickettsiella* Philip 1956, 267^{AL}. Pages 241-247. In: Bergey's Manual of Systematic Bacteriology. Garrity, G.M., Brenner, D.J., Krieg, N.R., and Staley, J.T., Eds., 2nd Edition, Vol. 2, part B, Springer, New York.

Galac, M.R., and Lazzaro, B.P. 2012. Comparative genomics of bacteria in the genus *Providencia* isolated from wild *Drosophila melanogaster*. BMC Genomics 13: 612. doi: 10.1186/1471-2164-13-612.

Hadapad, A.B., Prabhakar, C.S., Chandekar, S.C., Tripathi, J., and Hire, R.S. 2016. Diversity of bacterial communities in the midgut of *Bactrocera cucurbitae* (Diptera: Tephritidae) populations and their potential use as attractants. Pest Management Science 72: 1222-1230. doi: 10.1002/ps.4102.

Hurst, M.R.H., Jones, S.M., Tan, B., and Jackson, T.A. 2007. Induced expression of the *Serratia entomophila* Sep proteins shows activity towards the larvae of the New Zealand grass grub *Costelytra zealandica*. FEMS Microbiology Letters 275: 160-167.

Hurst, M.R., Becher, S.A., Young, S.D., Nelson, T.L., and Glare, T.R. 2011a. *Yersinia*

entomophaga sp. nov. isolated from the New Zealand grass grub *Costelytra zealandica*. International Journal of Systematic and Evolutionary Microbiology 61: 844-849.

Hurst, M.R.H., Jones, S.A., Binglin, T., Harper, L.A., Jackson, T.A., and Glare, T.R. 2011b. The main virulence determinant of *Yersinia* entomophaga MH96 is a broad-hostrange toxin complex active against insects. Journal of Bacteriology 193: 1966-1980.

Jackson, T.A., Boucias, D.G., and Thaler, J.O. 2001. Pathobiology of amber disease, caused by *Serratia* spp., in the New Zealand grass grub, *Costelytra zealandica*. Journal of Invertebrate Pathology 78: 232-243.

Juneja, P., and Lazzaro, B.P. 2009. *Providencia sneebia sp. nov.* and *Providencia burhodogranariea sp. nov.*, isolated from wild *Drosophila melanogaster*. International Journal of Systematic and Evolutionary Microbiology 59: 1108-1111.

Jurat-Fuentes, J.L., and Jackson, T.A. 2012. Bacterial Entomopathogens. Pages 265-350. In: Insect Pathology. F. Vega, and H.S. Kaya, Eds., Academic Press, London, UK.

Kido, G.S., and Spyhalski, E. 1950. Antimycin A, an antibiotic with insecticidal and miticidal properties. Science 112: 172-173.

Kirst, H.A. 2010. The spinosyn family of insecticides: Realizing the potential of natural products research. Journal of Antibiotics 63: 101-111.

Kirst, H.A., Michel, K.H., Martin, J.W., Creemer, L.C., Chio, E.H., Yao, R.C., Nakatsukasa, W.M., Boeck, L.D., Occolowitz, J.L., and Paschal, J.W. 1991. A83543A-D, unique fermentation-derived tetracyclic macrolides. Tetrahedron Letters 32: 4839-4842.

Kleespies, R.G., Federici, B.A., and Leclerque, A. 2014. Ultrastructural characterization and multilocus sequence analysis (MLSA) of `*Candidatus* Rickettsiella isopodorum´, a new lineage of intracellular bacteria infecting woodlice (Crustacea: Isopoda). Systematic and Applied Microbiology 37: 351-359.

Kurtti, T.J., Palmer, A.T., and Oliver, J.H. 2002. *Rickettsiella*-like bacteria in *Ixodes woodi* (Acari: Ixodidae). Journal of Medical Entomology 39: 534-540.

Kuzina, L.V., Peloquin, J.J., Vacek, D.C., and Miller, T.A. 2001. Isolation and identification of bacteria associated with adult laboratory Mexican fruit flies, *Anastrepha ludens* (Diptera: Tephritidae). Current Microbiology 42: 290-294.

Leclair, M., Polin, S., Jousseaume, T., Simon, J.C., Sugio, A., Morlière, S., Fukatsu, T., Tsuchida, T., and Outreman, Y. 2017. Consequences of coinfection with protective symbionts on the host phenotype and symbiont titres in the pea aphid system. Insect Science 24: 798-808.

Leclerque, A., and Kleespies, R.G. 2008. 16S ribosomal RNA, GroEL, and MucZ based assessment of the taxonomic position of *Rickettsiella melolonthae* and its implications for the organization of the genus *Rickettsiella*. International Journal of Systematic and Evolutionary Microbiology 58**:** 749-755.

Leclerque, A., and Kleespies, R.G. 2012. A *Rickettsiella* Bacterium from the Hard Tick, *Ixodes woodi*: Molecular Taxonomy Combining Multilocus Sequence Typing (MLST) with Significance Testing. PLoS ONE 7, e38062.

Leclerque, A., Kleespies, R.G., Ritter, C., Schuster, C., and Feiertag, S. 2011. Genetic and electron-microscopic characterization of `*Rickettsiella agriotidis´*, a new *Rickettsiella* pathotype associated with wireworm, *Agriotes* sp. (Coleoptera: Elateridae). Current Microbiology 63: 158-163.

Leclerque, A., Kleespies, R.G., Schuster, C., Richards, N.K., Marshall, S.D.G., and Jackson, T.A. 2012. Multilocus sequence analysis (MLSA) of '*Rickettsiella costelytrae*' and '*Rickettsiella pyronotae*', intracellular bacterial entomopathogens from New Zealand. Journal of Applied Microbiology 113: 1228-1237.

Leone, P., Bebeacua, C., Opota, O., Kellenberger, C., Klaholz, B., Orlov, I., Cambillau, C., Lemaitre, B., and Roussel, A. 2015. X-ray and Cryo-electron Microscopy Structures of Monalysin Pore-forming Toxin Reveal Multimerization of the Pro-form. Journal of Biological Chemistry 290: 13191-13201.

Lewer, P., Hahn, D.R., Karr, L.L., Duebelbeis, D.O., Gilbert, J.R., Crouse, G.D., Worden, T., Sparks, T.C., Edwards, P.M., and Graupner, P.R. 2009. Discovery of the butenyl-spinosyn insecticides: Novel macrolides from the new bacterial strain *Saccharopolyspora pogona*. Bioorganic and Medical Chemistry 17: 4185-4196.

Liehl, P., Blight, M., Vodovar, N., Boccard, F., and Lemaitre, B. 2006. Prevalence of local immune response against oral infection in a *Drosophila/Pseudomonas* infection model. PLoS Pathogens 2: e56.

Lukasik, P., van Asch, M., Guo, H., Ferrari, J., and Godfray, J. 2013. Unrelated facultative endosymbionts protect aphids against a fungal pathogen. Ecology Letters 16: 214-218.

MacIntosh, S.C., Stone, T.B., Sims, S.R., Hunst, P.L., Greenplate, J.T., Marrone, P.G., Perlak, F.J., Fischhoff, D.A., and Fuchs, R.L. 1990. Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. Journal of Invertebrate Pathology 56: 258-266.

Mertz, F.P., and Yao, R.C. 1990. *Saccharopolyspora spinosa sp. nov*. isolated from soil collected in a sugar mill rum still. International Journal of Systematic Bacteriology 40: 34-39.

Mike, C., Ryan, J., Maria, M., Martin, T., Dickerson, D., Negrotto, D., O'Reilly, D., Chen, E., and Lee, M. 2006. Effective IRM with a novel insecticidal protein, Vip3A. Pages 1229-1235. In: Proceedings of the Beltwide Cotton Conference. January 3-6, 2006, San Antonio, TX, USA.

Mulet, M., Gomila, M., Lemaitre, B., Lalucat, J., and García-Valdés, E. 2012. Taxonomic characterization of *Pseudomonas* strain L48 and formal proposal of *Pseudomonas entomophila sp. nov*. Systematic and Applied Microbiology 35: 145-149.

Nunez-Valdez, M.E., Calderon, M.A., Aranda, E., Hernandez, L., Ramirez-Gama, R.M., Lina, L., Rodriguez-Segura, Z., Del, C., Gutierrez, M., and Villalobos, F.J. 2008. Identification of a putative Mexican Strain of *Serratia entomophila* Pathogenic against Root-Damaging Larvae of Scarabaeidae (Coleoptera). Applied and Environmental Microbiology 74: 802-810.

Oishi, H., Sugawa, T., Okutomi, T., Suzuki, K., Hayashi, T., Sawada, M., and Ando, K. 1970. Insecticidal activity of macrotetrolide antibiotics. Journal of Antibiotics 23: 105-106.

Opota, O., Vallet-Gély, I., Vincentelli, R., Kellenberger, C., Iacovache, I., Gonzalez, M.R., Roussel, A., van der Goot, F.G., and Lemaitre, B. 2011. Monalysin, a novel ß-pore-forming toxin from the *Drosophila* pathogen *Pseudomonas entomophila*, contributes to host intestinal damage and lethality. PLoS Pathogens 7: e1002259.

Palma, L., Muñoz, D., Berry, C., Murillo, J., and Caballero, P. 2014. *Bacillus thuringiensis* toxins: an overview of their biocidal activity. Toxins 6: 3296-3325.

Papagiannoulis, A., Mathiopoulos, K.D., and Mossialos, D. 2009. Molecular detection of the entomopathogenic bacterium *Pseudomonas entomophila* using PCR. Letters of Applied Microbiology 50: 241-245.

Pardo-López, L., Soberón, M., and Bravo, A. 2013. *Bacillus thuringiensis* insecticidal three-domain Cry toxins: Mode of action, insect resistance and consequences for crop protection. FEMS Microbiology Reviews 37: 3-22.

Park, H.W., Kim, Y.O., Ha, J.S., Youn, S.H., Kim, H.H., Bilgrami, A.L., and Shin, C.S. 2011. Effects of associated bacteria on the pathogenicity and reproduction of the insect-parasitic nematode *Rhabditis blumi* (Nematoda: Rhabditida). Canadian Journal of Microbiology 57: 750-758.

Roh, J.Y., Choi, J.Y., Li, M.S., Jin, B.R., and Je, Y.H. 2007. *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. Journal of Molecular Biology 17: 547-559.

Ruiu, L. 2013. *Brevibacillus laterosporus*, a pathogen of invertebrates and a broad-spectrum antimicrobial species. Insects 4: 476-492.

Ruiu L. 2015. Insect Pathogenic Bacteria in Integrated Pest Management. Insects 6: 352-367.

Ruiu, L., Floris, I., Satta, A., and Ellar, D.J. 2007. Toxicity of a *Brevibacillus laterosporus* strain lacking parasporal crystals against *Musca domestica* and *Aedes aegypti*. Biological Control 43: 136-143.

Ruiu, L., Satta, A., and Floris, I. 2012. Observations on house fly larvae midgut ultrastructure after *Brevibacillus laterosporus* ingestion. Journal of Invertebrate Pathology 111: 211-216.

Ryall, B., Mitchell, H., Mossialos, D., and Williams, HD. 2009. Cyanogenesis by the entomopathogenic bacterium *Pseudomonas entomophila*. Letters of Applied Microbiology 49: 131-135.

Salgado, V.L., and Sparks, T.C. 2005. The spinosyns: Chemistry, biochemistry, mode of action, and resistance. Pages 137-173. In: Comprehensive Molecular Insect Science. Gilbert, L.J., Iatrou, K., and Gill, S.S., Eds. Elsevier, Oxford, UK.

Sanchis, V. 2011. From microbial sprays to insect-resistant transgenic plants: History of the biospesticide *Bacillus thuringiensis*. A review. Agronomy and Sustainable Development 31: 217-231.

Sattar, S., and Maiti, M.K. 2011. Molecular characterization of a novel vegetative insecticidal protein from *Bacillus thuringiensis* effective against sap-sucking insect pest. Journal of Microbiology and Biotechnology 21: 937-946.

Schnepf, E., Crickmore, N., van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., and Dean, D.H. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews 62: 775-806.

Schuster, C., Kleespies, R.G., Ritter, C., Feiertag, S., and Leclerque, A. 2013. Multilocus sequence analysis (MLSA) of *`Rickettsiella agriotidis´*, an intracellular bacterial pathogen of *Agriotes* wireworms. Current Microbiology 66: 1-9.

Somvanshi, V.S., Lang, E., Sträubler, B., Spröer, C., Schumann, P., Ganguly, S., Saxena, A.K., and Stackebrandt, E. 2006. *Providencia vermicola sp. nov.*, isolated from infective juveniles of the entomopathogenic nematode *Steinernema thermophilum*. International Journal of Systematic and Evolutionary Microbiology 56: 629-633.

Sparks, T.C., Crouse, G.D., and Durst, G. 2001. Natural products as insecticides: The biology, biochemistry and quantitative structure-activity relationships of spinosyns and spinosoids. Pest Management Science 57: 896-905.

Tsuchida, T., Koga, R., Horikawa, M., Tsunoda, T., Maoka, T., Matsumoto, S., Simon, J.-C., and Fukatsu, T. 2010. Symbiotic Bacterium Modifies Aphid Body Color. Science 330: 1102-1104.

Tsuchida, T., Koga, R., Fujiwara, A., and Fukatsu, T. 2014. Phenotypic effect of `*Candidatus* Rickettsiella viridis´, a facultative symbiont of the pea aphid (*Acyrthosiphon pisum*), and its interaction with a coexisting symbiont. Applied and Environmental Microbiology 80: 525-533.

Turner, M.J., and Schaeffer, J.M. 1989. Mode of action of ivermectin. Pages 73-88. In: Ivermectin and Abamectin. Cambell, W.C., Ed.. Springer, New York, USA.

Vega, F.E., and Kaya, H.K. 2012. Insect Pathology, 2nd ed., Elsevier, London, UK, 504 pp.

Vodovar, N., Vinals, M., Liehl, P., Basset, A., Degrouard, J., Spellman, P., Boccard, F., and Lemaitre, B. 2005. *Drosophila* host defense after oral infection by an entomopathogenic *Pseudomonas* species. Proceedings of the National Academy of Sciences of the USA 102: 11414-11419.

Vodovar, N., Vallenet, D., Cruveiller, S., Rouy, Z., Barbe, V., Acosta, C., Cattolico, L., Jubin, C., Lajus, A., Segurens, B., Vacherie, B., Wincker, P., Weissenbach, J., Lemaitre, B., Médigue, C., and Boccard, F. 2006. Complete genome sequence of the entomopathogenic and metabolically versatile soil bacterium *Pseudomonas entomophila*. Nature Biotechnology 24: 673-679.

Waldron, C., Madduri, K., Crawford, K., Merlo, D.J., Treadway, P., Broughton, M.C., and Baltz, R.H. 2000. A cluster of genes for the biosynthesis of spinosyns, novel macrolide insect control agents produced by *Saccharopolyspora spinosa*. Antonie van Leeuwenhoek 78: 385-390.

Warren, G.W. 1997. Vegetative insecticidal proteins: Novel proteins for control of corn pests. Pages 109-121. In: Advances in Insect Control: The Role of Transgenic Plants. Carozzi, N.B., and Koziel, M.G., Eds.. Taylor & Francis, London, UK.

Yu, C.G., Mullins, M.A., Warren, G.W., Koziel, M.G., and Estruch, J.J. 1997. The *Bacillus thuringiensis* vegetative insecticidal protein Vip3A lyses midgut epithelium cells of susceptible insects. Applied and Environmental Microbiology 63: 532-536.

Yu, X., Liu, T., Sun, Z., Guan, P., Zhu, J., Wang, S., Li, S., Deng, Q., Wang, L., Zheng, A., and Li, P. 2012. Co-expression and synergism analysis of Vip3Aa29 and Cyt2Aa3 insecticidal proteins from *Bacillus thuringiensis*. Current Microbiology 64: 326-331.

Zhang, J., Hodgman, T.C., Krieger, L., Schnetter, W., and Schairer, H.U. 1997. Cloning and analysis of the *cry* gene from *Bacillus popilliae*. Journal of Bacteriology 179: 4336-4341.

Zubasheva, M.V., Ganushkina, L.A., Smirnova, T.A., and Azizbekyan, R.R. 2010. Larvicidal activity of crystal-forming strains of *Brevibacillus laterosporus*. Applied Biochemistry and Microbiology 46: 755-762.
