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RESEARCH ARTICLE

BACILLUS THURINGIENSIS AS BIO PESTICIDE IN INSECT OF TEAK

¹Prabhat Kumar Mishra, ¹Harish Rao and ²Kamal Uddin Zaidi

¹Scientist, Director People's University, Bhanpur, Bhopal

²Biotechnology and Pharmacology Lab, Centre for Scientific Research & Development, People's University, Bhanpur, Bhopal – 462037

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ABSTRACT

Bacillus thuringiensis (Bt) was a positive bacteria. It is forming spore, it was crystalline protein which was called cry protein. It was toxic against many types of teak insect, cotton insect. This bacterium was toxic against insect. Its toxin was used in bioinsecticide.

INTRODUCTION

Bacillus thuringiensis (Bt) was a well recognized entomopathogenic bacterium used as a biopesticide for many years. Its insecticidal properties of Bt were mainly due to cry toxins forming a protein with the spore. After intake of susceptible insect larvae, protein induced a favorable atmosphere for spore germination. The bacteria increased in the insect and made mutual behavior using signaling molecules present for quorum sensing. Microorganisms and microbial products with potential insecticidal activity can play an important role in controlling diseases by interrupting transmission mechanisms by killing insect vectors at community level (Patil *et al.*, 2012). Worldwide efforts to screen effective entomopathogenic microorganisms for control of agriculturally and medically important insect pests have yielded many Bacillus thuringiensis (Bt) isolates with various insecticidal properties (Feitelson *et al.*, 1992). The Gram-positive bacterium *Bacillus thuringiensis* is well known for its ability to form spores and crystal proteins with insecticidal activity against a wide variety of lepidopteran, coleopteran, and dipteran insects (Schnepf *et al.*, 1998). *Bacillus thuringiensis* is a gram positive, spore-forming bacterium that produces parasporal inclusions during the sporulation phase. These inclusions are composed of proteins (Cry proteins) or endotoxins which are highly toxic to a wide variety of insect pests and some invertebrates (Chattopadhyay *et al.*, 2004 and Vilas Boas, 2007).

MATERIALS AND METHODS

Bacillus thuringiensis from soil: a total of 50 soil samples were collected from Bhopal different regions. Samples were collected

from 2 to 5 cm below the surface with a shovel. Each soil sample was collected in an autoclave plastic bag at ambient temperature. *Bacillus thuringiensis* strains were isolated from soil samples using Luria broth and agar media (Travers, 1987).

RESULTS

We have identified 50 bacterial strains from larvae of five families of Coleoptera that had been feeding on decaying wood in tropical wet forests of teak. 50 isolated from *Bacillus thuringiensis*. Cotton leaves had Lepidoptera. The genus *Bacillus thuringiensis* was the most abundant; it was the only one associated with all five families of Coleoptera and also present.

DISCUSSION

We collected larvae of five families of wood-feeding Coleoptera in teak forest. Prediction of availability of Bt in insect. With the aim of estimating the species composition of cultivable fungi and bacteria inhabiting their guts and to identify microorganisms with relevant lignocellulolytic activities. The main limitation of this study is that the cultivation-dependent approach, based on artificial media, covers only a small proportion of the total microbial diversity present in this particular niche. The positive trade-off of this approach was the identification of several isolates with lignocellulose-degrading capabilities, which can be further used for the respective enzyme characterization, for direct degradation assays on residues from agriculture and forestry, for the treatment of industrial effluents, and for bioprospecting novel metabolites with other biotechnological applications. Despite the inherent bias of the isolation method, our results suggest that gut microbiota of wood-feeding tropical beetles

*Corresponding author: Prabhat Kumar Mishra
Scientist, People's University, Bhanpur, Bhopal.

presents a relatively high diversity in terms of microbial richness, phylogenetic composition, and lignocellulolytic activities. This white-rot fungus is a known model for studying degradation of lignin in free-living conditions and in this work reported in its association with the gut microbiota of wood-feeding insects (Borokhov, 2000 and Abadulla *et al.*, 2000). It is difficult to know whether these fungal isolates are truly endosymbionts of the intestinal tracts of the coleopteran larvae or are transitory inhabitants associated with host feeding habits. Hence, it is also possible that some of these microorganisms could be commensals, parasites, and facultative endosymbionts. They might even be using the insect as a dispersal mechanism (Morales-Jiménez *et al.*, 2012 and Dreaden *et al.*, 2014). It is clear, however, that the overall taxonomic composition of the gut-inhabiting microbes and the proportion of lignocellulolytic-positive fungi seem to be particular to the larval microenvironment. The structure of this endosymbiotic community is distinguished from the fungal composition observed in other wood-related microhabitats such as the fungal populations in living plant tissues. They are also dominated by members of Ascomycetes, but they present a different abundance distribution of fungal families (Suryanarayanan *et al.*, 2002 and Arnold, 2007); decaying logs are dominated mainly by Basidiomycetes (Heilmann-Clausen, 2005 and Prewitt, 2014). The analysis of the taxonomic composition of the bacterial isolates showed the presence of seven major phylogenetic classes, codominated by γ -Proteobacteria and Firmicutes.

This finding is consistent with results obtained in similar studies (Morales-Jiménez *et al.*, 2009; Schloss *et al.*, 2006; Vasanthakumar *et al.*, 2008; Geib *et al.*, 2008 and Reid *et al.*, 2011). Within the γ -Proteobacteria, the most abundant genera were *Enterobacter*, *Serratia*, *Acinetobacter*, and *Pseudomonas*. Interestingly, *Serratia* and *Pseudomonas* were isolated from all five coleopteran families studied; *Enterobacter* and *Acinetobacter* were present in four out of the five insect families, and they exhibited positive results in the lignocellulolytic assays, except for lignin degradation. Similar characteristics related to the degradation of lignocellulose and to fermentative metabolism were observed in *Bacillus*, the most abundant genus within Firmicutes (Egert *et al.*, 2003 and Lee *et al.*, 2008). Together, these results support the notion that some species of fungi and bacteria, such as *Trichoderma*, *Serratia*, *Pseudomonas*, and *Bacillus*, can be common gut inhabitants of wood-feeding larvae in tropical forests, suggesting that certain affinities might have developed between the beetle host and its microbiota (Vargas-Asensio *et al.*, 2014, Rhoads *et al.*, 1995, Nagy *et al.*, 2002, Weslien *et al.*, 2011 and Jones *et al.*, 2013). When comparing the fungal and bacterial species composition among the beetle families, the plots of the Bray-Curtis distances and canonical correspondence analyses produced biologically meaningful clusters to group the environments that share similar microbial compositions. The first fungal cluster relates the microbiota associated with the guts of Cerambycidae, Passalidae, and Scarabaeidae. This is consistent with the observation of a high diversity of isolates from Cerambycidae that shared members of the fungal phyla Basidiomycota and Ascomycota with Passalidae and members of Zygomycota and Ascomycota with Scarabaeidae. The cluster formed by Tenebrionidae-Elateridae shared, in a lower proportion, members of the Basidiomycota and Ascomycota. The bacterial microbiota associated with Passalidae and Scarabaeidae also formed a cluster, sharing members of five major bacterial clades; microbiota of Cerambycidae, Elateridae,

and Tenebrionidae shared members only of γ -Proteobacteria and Firmicutes. efficacy of Cry1Ab can depend on additional factors (Schnepf *et al.*, 1998). The clustering analyses revealed that Cerambycidae presented a high diversity of fungi but not of bacteria, while Passalidae and Elateridae exhibited a high diversity of bacteria and moderate diversity of fungi. Scarabaeidae and Tenebrionidae contained a similar composition of both. These results suggest that the nature of the beetle host has an important effect on the phylogenetic diversity of its associated microbiota and that many factors can influence its configuration. These factors may include the biology of the host, the physical and chemical characteristics of the gut compartments, the feeding habits of the insects, and the microbial diversity associated with the environment in which the insect is living (Egert *et al.*, 2005, Ceja-Navarro *et al.*, 2014, Lemke *et al.*, 2003 and Geib *et al.*, 2009). Our results consistently showed that both the fungal and bacterial populations associated with the guts of beetle larvae are highly diverse in terms of the number of species obtained and in their phylogenetic composition. These microbial inhabitants could be forming complex consortia that would be acting synergistically to provide many of the nutritional needs of the beetle host. Some of these functions include the degradation and fermentation of lignocellulosic materials, as shown by the high percentage of fungal and bacterial genera that presented positive activities or by the production of proteins and other metabolites necessary for the development of the insect (Scott *et al.*, 2008, Geib *et al.*, 2008, Cazemier *et al.*, 2003, Genta *et al.*, 2006 and Oh *et al.*, 2009). Certain affinities for substrates can be expected according to the nature of the gut inhabitant. Members of the Basidiomycota could possibly degrade larger polymeric molecules, the Ascomycota deplete diverse lignocellulosic constituents, bacteria degrade and ferment the smaller monomeric and dimeric hexoses and pentoses produced by the fungal counterparts. The bacteria also likely use these sugars to produce other nutrients and metabolites. Concerning the existence of microbial consortia acting synergistically to give the nutritional needs of the hosts, the nature of the ecological and evolutionary processes it contribute to ensure the fitness of the insect, and the mechanisms that rule the interactions among the fungi, the bacteria, and the beetle host.

REFERENCES

- Abadulla, E. Tzanov, T. Costa, S. Robra, K.-H. Cavaco-Paulo, A. and Gubitz, G. M. 2000. "Decolorization and detoxification of textile dyes with a laccase from *Trametes hirsuta*," *Applied and Environmental Microbiology*, vol. 66, no. 8, pp. 3357–3362, View at Publisher, View at Google Scholar • View at Scopus
- Arnold, A. E. and Lutzoni, F. 2007. "Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots?" *Ecology*, vol. 88, no. 3, pp. 541–549, View at Publisher, View at Google Scholar, View at Scopus
- Borokhov, O. and S. Rothenburger, "Rapid dye decolorization method for screening potential wood preservatives," *Applied and Environmental Microbiology*, vol. 66, no. 12, pp. 5457–5459, 2000. View at Publisher, View at Google Scholar, View at Scopus
- Cazemier, A. E. Verdoes, J. C. Reubsat, F. A. G. Hackstein, J. H. P. van der Drift, C. and Op den Camp, H. J. M. 2003. "Promicromonospora pachnodae sp. nov., a member of the (hemi) cellulolytic hindgut flora of larvae of the scarab beetle *Pachnoda marginata*," *Antonie van Leeuwenhoek*,

- vol. 83, no. 2, pp. 135–148, View at Publisher • View at Google Scholar • View at Scopus
- Ceja-Navarro, J. A. Nguyen, N. H. Karaoz U. *et al.*, 2014. “Compartmentalized microbial composition, oxygen gradients and nitrogen fixation in the gut of *Odontotenus disjunctus*,” *The ISME Journal*, vol. 8, no. 1, pp. 6–18, 2014. View at Publisher • View at Google Scholar • View at Scopus
- Chattopadhyay A, Bhatnagar NB, Bhatnagar R. 2004. Bacterial Insecticidal toxins. *Crit Rev Microbiol*30:33-54.
- Dreaden, T. J. Davis, J. M. de Beer Z. W. *et al.*, 2014. “Phylogeny of ambrosia beetle symbionts in the genus *Raffaelea*,” *Fungal Biology*, vol. 118, no. 12, pp. 970–978, View at Publisher • View at Google Scholar • View at Scopus
- Egert, M. Stingl, U. Bruun, L. D. Pommerenke, B. Brune, A. and Friedrich, M. W. 2005. “Structure and topology of microbial communities in the major gut compartments of *Melolontha melolontha* larvae (Coleoptera: Scarabaeidae),” *Applied and Environmental Microbiology*, vol. 71, no. 8, pp. 4556–4566, View at Publisher • View at Google Scholar • View at Scopus
- Egert, M. Wagner, B. Lemke, T. Brune, A. and Friedrich, M. W. 2003. “Microbial community structure in midgut and hindgut of the humus-feeding larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae),” *Applied and Environmental Microbiology*, vol. 69, no. 11, pp. 6659–6668, 2003. View at Publisher • View at Google Scholar • View at Scopus
- Feitelson, J. S. Payne, J. and Kim, L. 1992. “*Bacillus thuringiensis*: insects and beyond,” *Nature Biotechnology*, vol. 10, no. 3, pp. 271–275, View at Publisher • View at Google Scholar •
- Geib, S. M. Filley, T. R. Hatcher P. G. *et al.*, 2008. “Lignin degradation in wood-feeding insects,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 35, pp. 12932–12937, View at Publisher • View at Google Scholar • View at Scopus
- Geib, S. M. Filley, T. R. Hatcher P. G. *et al.*, 2008. “Lignin degradation in wood-feeding insects,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 35, pp. 12932–12937. View at Publisher • View at Google Scholar • View at Scopus
- Geib, S. M. Jimenez-Gasco, M. D. M. Carlson, J. E. Tien, M. and Hoover, K. 2009. “Effect of host tree species on cellulase activity and bacterial community composition in the gut of larval Asian longhorned beetle,” *Environmental Entomology*, vol. 38, no. 3, pp. 686–699, View at Publisher • View at Google Scholar • View at Scopus
- Genta, F. A. Dillon, R. J. Terra, W. R. and Ferreira, C. 2006. “Potential role for gut microbiota in cell wall digestion and glucoside detoxification in *Tenebrio molitor* larvae,” *Journal of Insect Physiology*, vol. 52, no. 6, pp. 593–601, 2006. View at Publisher • View at Google Scholar • View at Scopus
- Heilmann-Clausen J. and Boddy, L. 2005. “Inhibition and stimulation effects in communities of wood decay fungi: exudates from colonized wood influence growth by other species,” *Microbial Ecology*, vol. 49, no. 3, pp. 399–406, 2005. View at Publisher • View at Google Scholar • View at Scopus
- Jones, R. T. Sanchez, L. G. and Fierer, N. 2013. “A cross-taxon analysis of insect-associated bacterial diversity,” *PLoS ONE*, vol. 8, no. 4, Article ID e61218, 2013. View at Publisher • View at Google Scholar • View at Scopus
- Lee, C. C. Kibblewhite-Accinelli, R. E. Smith, M. R. Wagschal, K. Orts, W. J. and Wong, D. W. S. 2008. “Cloning of *Bacillus licheniformis* xylanase gene and characterization of recombinant enzyme,” *Current Microbiology*, vol. 57, no. 4, pp. 301–305, View at Publisher • View at Google Scholar • View at Scopus.
- Lemke, T. Stingl, U. Egert, M. Friedrich, M. W. and Brune, A. 2003. “Physicochemical conditions and microbial activities in the highly alkaline gut of the humus-feeding larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae),” *Applied and Environmental Microbiology*, vol. 69, no. 11, pp. 6650–6658, 2003. View at Publisher • View at Google Scholar • View at Scopus
- Morales-Jiménez, J. Zúñiga, G. Villa-Tanaca, L. and Hernández-Rodríguez, C. 2009. “Bacterial community and nitrogen fixation in the red turpentine beetle, *Dendroctonus valens* LeConte (Coleoptera: Curculionidae: Scolytinae),” *Microbial Ecology*, vol. 58, no. 4, pp. 879–891, View at Publisher • View at Google Scholar • View at Scopus
- Morales-Jiménez, J. Zúñiga, G. Ramírez-Saad, H. C. and Hernández-Rodríguez, C. 2012. “Gut-associated bacteria throughout the life cycle of the bark beetle *Dendroctonus rhizophagus* Thomas and Bright (Curculionidae: Scolytinae) and their cellulolytic activities,” *Microbial Ecology*, vol. 64, no. 1, pp. 268–278, View at Publisher • View at Google Scholar • View at Scopus
- Nagy, T., Emami, K. Fontes, C. M. G. A. Ferreira, L. M. A. Humphry, D. R. and Gilbert, H. J. 2002. “The membrane-bound α -glucuronidase from *Pseudomonas cellulosa* hydrolyzes 4-O-methyl-D-glucuronoxyloligosaccharides but not 4-O-methyl-D-glucuronoxylan,” *Journal of Bacteriology*, vol. 184, no. 17, pp. 4925–4929, View at Publisher • View at Google Scholar • View at Scopus
- Oh, D.C. Scott, J. J. Currie, C. R. Clardy, and J. 2009. “Mycangimycin, a polyene peroxide from a mutualist *Streptomyces* sp.,” *Organic Letters*, vol. 11, no. 3, pp. 633–636, 2009. View at Publisher • View at Google Scholar • View at Scopus
- Patil, C. D., Patil, S. V., Salunke, B. K. and Salunkhe, R. B. 2012. “Insecticidal potency of bacterial species *Bacillus thuringiensis* SV2 and *Serratia nematodiphila* SV6 against larvae of mosquito species *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*,” *Parasitology Research*, vol. 110, no. 5, pp. 1841–1847.
- Prewitt, L. Kang, Y. Kakumanu, M. L. and Williams, M. 2014. “Fungal and bacterial community succession differs for three wood types during decay in a forest soil,” *Microbial Ecology*, vol. 68, no. 2, pp. 212–221, View at Publisher • View at Google Scholar • View at Scopus
- Reid, N. M. Addison, S. L. Macdonald, L. J. and Lloyd-Jones, G. 2011. “Biodiversity of active and inactive bacteria in the gut flora of wood-feeding Huhu beetle larvae (*Prionoplus reticularis*),” *Applied and Environmental Microbiology*, vol. 77, no. 19, pp. 7000–7006, View at Publisher • View at Google Scholar • View at Scopus
- Rhoads, T. L. Mikell Jr., A. T. and Eley, M. H. “Investigation of the lignin-degrading activity of *Serratia marcescens*: biochemical screening and ultrastructural evidence,” *Canadian Journal of Microbiology*, vol. 41, no. 7, pp. 592–600, 1995. View at Publisher • View at Google Scholar • View at Scopus
- Schloss, P. D. Delalibera Jr., I. Handelsman, J. and Raffa, K. F. 2006. “Bacteria associated with the guts of two wood-boring beetles: *anoplophora glabripennis* and *Saperda vestita* (Cerambycidae),” *Environmental Entomology*, vol.

- 35, no. 3, pp. 625–629, View at Publisher • View at Google Scholar • View at Scopus
- Schnepf E, Crickmore N, Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH. 1998 Bacillus thuringiensis and its pesticidal crystal proteins. *Microbiol Mol Biol Rev.* 62(3):775–806.
- Schnepf, E. Crickmore, N. Van Rie, J. *et al.*, 1998. “Bacillus thuringiensis and its pesticidal crystal proteins,” *Microbiology and Molecular Biology Reviews*, vol. 62, no. 3, pp. 775–806.
- Scott, J. J. Oh, D.-C. Yuceer, M. C. Klepzig, K. D. Clardy, J. and Currie, C. R. 2008. “Bacterial protection of beetle-fungus mutualism,” *Science*, vol. 322, no. 5898, p. 63. View at Publisher • View at Google Scholar • View at Scopus
- Suryanarayanan, T. S., Murali, T. S. and Venkatesan, G. 2002. “Occurrence and distribution of fungal endophytes in tropical forests across a rainfall gradient,” *Canadian Journal of Botany*, vol. 80, no. 8, pp. 818–826, View at Publisher • View at Google Scholar • View at Scopus
- Travers RS, Martin In PAW, Reichelderfer CF 1987. Selective Isolation of Soil Bacillus spp. *Appl Environ Microbiol* 53:1263-1266.
- Vargas-Asensio, G. Pinto-Tomas, A. Rivera B. *et al.*, 2014. “Uncovering the cultivable microbial diversity of costarican beetles and its ability to break down plant cell wall components,” *PLoS ONE*, vol. 9, no. 11, Article ID e113303, 2014. View at Publisher • View at Google Scholar • View at Scopus
- Vasanthakumar, A. Handelsman, J. O. Schloss, P. D. Bauer, L. S. and Raffa, K. F. 2008. “Gut microbiota of an invasive subcortical beetle, *Agrilus planipennis* Fairmaire, across various life stages,” *Environmental Entomology*, vol. 37, no. 5, pp. 1344–1353, 2008. View at Publisher • View at Google Scholar • View at Scopus
- Vilas Boas Gt, Peruca APS, Arantes OMN 2007. Biology and taxonomy of *Bacillus cereus*, *Bacillus anthracis* and *Bacillus thuringiensis*. *Can J Microbiol* 53:673-687.
- Weslien, J. Djupström, L. B. Schroeder, M. and Widenfalk, O. 2011. “Long-term priority effects among insects and fungi colonizing decaying wood,” *The Journal of Animal Ecology*, vol. 80, no. 6, pp. 1155–1162, View at Publisher • View at Google Scholar • View at Scopus
