Short Communication

Burkholderia Gut Symbionts Associated with European and Japanese Populations of the Dock Bug Coreus marginatus (Coreoidea: Coreidae)

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Insects of the heteropteran superfamilies Coreoidea and Lygaeoidea are consistently associated with symbionts of a specific group of the genus Burkholderia, called the “stinkbug-associated beneficial and environmental (SBE)” group. The symbiosis is maintained by the environmental transmission of symbionts. We investigated European and Japanese populations of the dock bug Coreus marginatus (Coreoidea: Coreidae). High nymphal mortality in reared aposymbiotic insects suggested an obligate host-symbiont association in this species. Molecular phylogenetic analyses based on 16S rRNA gene sequences revealed that all 173 individuals investigated were colonized by Burkholderia, which were further assigned to different subgroups of the SBE in a region-dependent pattern.

Key words: Burkholderia, stinkbug, obligate gut symbiosis, region-dependent symbionts

The suborder Heteroptera is a diverse taxonomic group in insects and consists of 42,300 described species (9). Phytophagous members commonly possess symbiotic bacteria inside their bodies (1, 15, 19). While some heteropteran species harbor symbionts intracellularly (10, 20–22, 24), the majority of phytophagous species possess symbiotic bacteria extracellularly in the lumen of sac-like tissues, called “crypts”, in the posterior midgut (2, 8, 25). Members of the superfamily Pentatomomidea harbor specific bacterial symbionts belonging to Gammaproteobacteria (19). These symbionts are essential for host growth and reproduction and are vertically transmitted from mother to offspring. In contrast, most members of the superfamilies Lygaeoidea and Coreoidea are associated with betaproteobacterial symbionts of a specific clade in the genus Burkholderia, called the “stinkbug-associated beneficial and environmental (SBE)” group (7, 16, 30). The coreoid and lygaeoid species not vertically transmit Burkholderia symbionts, but they acquire them from environmental soil every generation (14, 17). At this stage, the biological effects of the Burkholderia symbiont have only been reported in the bean bug Riptortus pedestris (superfamily Coreoidea: family Alydidae), in which the symbiont is not essential, but significantly enhances the growth rate, body size, and fecundity of the bean bug host (14, 18).

We previously investigated 22 species of Coreoidea and Lygaeoidea, all of which were collected in Japan and harbored the SBE group Burkholderia (13, 16). Six species of American Coreoidea and Lygaeoidea were also examined and the symbiotic organs of these species were also dominated by SBE-group Burkholderia (1, 7, 26), although other groups of Burkholderia were also detected in some cases (1). A recent study on European and Japanese species of the spurge bug, Dicranochalpus spp. (superfamily Coreoidea: family Stenocephalidae), revealed that while the Japanese species are consistently associated with the SBE group Burkholderia, European species are more likely to harbor a distinct lineage of Burkholderia, tentatively named “Stenocephalidae-clade” Burkholderia (23). This finding suggests the geographical divergence of the stinkbug-Burkholderia association. However, it currently remains unclear whether the case of the spurge bug is exceptional.

The dock bug Coreus marginatus (superfamily Coreoidea: family Coreidae) (Fig. 1A) is broadly distributed in the Northern Hemisphere, from Europe over central Asia to Japan (11, 12). It feeds on the leaves and seeds of Rumex plants (Fig. 1B), and is a serious pest of Rumex herbs, such as sorrel (11). In the present study, we investigated the symbiotic bacteria of C. marginatus, which belong to the SBE, and examined their fitness effects on the host insect. We further clarified whether a region-dependent divergence of symbionts exists between European and Japanese host populations.

The dock bug possessed numerous crypts in the posterior region of the midgut. These crypts were white and arranged in two rows (Fig. 1C). To investigate the prevalence of Burkholderia in this species, wild populations collected in diverse locations of Europe and Japan were assessed by diagnostic PCR with a Burkholderia-specific primer set (29). The insects examined in the present study are listed in Table S1. The crypt region was dissected out by forceps under a binocular, and the symbiotic organ (M4 in Fig. 1C) was sub

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The Burkholderia symbiont of the dock bug was successfully isolated from the midgut crypts of an insect collected in Crèche Belle-Image, the CNRS campus, Gif-sur-Yvette, France on 24th May 2017 by culturing the crypt content on a YG (yeast-glucose) agar plate, as previously described (16). A green fluorescence protein (GFP)-expressing derivative, constructed from this isolate as previously described (18) and fed to second instar nymphs that descended from wild insects collected at the same location (Crèche Belle-Image, CNRS-campus, Gif-sur-Yvette, France in 2017), showed a specific localization in the midgut crypts (Fig. 1D and E), confirming the gut symbiotic association between Burkholderia and the dock bug. Using this cultured strain, the fitness effects of the Burkholderia symbiont were investigated. Second instar nymphs were fed cultured Burkholderia 6 d after hatching and maintained in a clean plastic cup at 25°C under a long day regimen (16 h light, 8 h dark) by feeding on roasted pistachio and peanut seeds (Pistacia vera and Arachis hypogaea, respectively) and distilled water containing 0.05% ascorbic acid. While uninfected insects showed a survival rate of only 7.7% (survived/total=1/13), insect survival significantly improved to 52.5% (21/40) in infected insects (Fig. 1F), strongly suggesting an obligate host-symbiont relationship in the dock bug. In the case of the bean bug R. pedestris, the Burkholderia association is facultative: the symbiont does not strongly affect host survival, but does influence the growth and fecundity of the insect host (14, 18). Although the biological function of the Burkholderia symbiont remains unclear, metabolic dependency on the symbiont appears to differ between stinkbug species that feed on different host plants.

To clarify the phylogenetic placement of Burkholderia symbions associated with dock bugs, selected individuals from the European and Japanese populations were subjected to a clone library analysis of a 1.5-kb fragment of the bacterial 16S rRNA gene, as previously described (16). Ten and four insects representing ten European and two Japanese populations, respectively, were investigated (Table S1). A total of 110 clones were sequenced and subjected to a BLAST search. The top BLAST hits of all sequences were the 16S rRNA gene sequences of Burkholderia species. The 110 sequences were classified into five OTUs (Table S2 and S3) based on the UCLUST clustering method with a 99% sequence identity threshold in QIIME (3). These results indicated that (i) 11 and three individuals were infected with single and multiple Burkholderia OTUs, respectively, and (ii) OTU3 was the most frequently detected and present in all European individuals and two out of four Japanese specimens (Table S2). Although the clone library analysis demonstrated that the Burkholderia composition is simple in the dock bug, this result needs to be confirmed in a more comprehensive analysis using deep sequencing of the bacterial content in midgut crypts.

The genus Burkholderia is grouped into three phylogenetically and ecologically distinct clades (6, 32). The first clade consists of many human, animal, and plant pathogens, including B. cepacia, B. pseudomallei, and B. mallei, designated as the “Burkholderia cepacia complex (BCC)” group. The second clade includes a number of plant growth-promoting rhizobac-
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The phylogenetic placement of the Burkhodera OTUs detected from the dock bug is shown in Fig. 2. OTU1 and OTU2, detected in two Japanese populations and one French population of the dock bug, were placed in SBE group α, in which OTUs were clustered with Burkhodera detected from Japanese and American coreooid and lygaeoid stinkbugs (Fig. 2, Table S2). The three other OTUs, including OTU3 detected in most European dock bug populations, were placed in SBE group β (Fig. 2). It is important to note that all of the ten insects investigated in seven European countries (France, Germany, Belgium, Italy, Hungary, Denmark, and Ukraine) were almost exclusively associated with Burkhodera of SBE group β (Table S2). Based on our previous findings on spurge bugs (23), it is plausible that coreoid stinkbugs inhabiting Europe are consistently associated with this specific clade of Burkhodera. Recent worldwide surveys revealed a “region-dependent pattern” of soil microbiota (4, 27), which may affect the region-dependent Burkhodera infection of stinkbugs. To clarify this point, further worldwide surveys on both soils and inhabiting stinkbugs are needed.

The nucleotide sequence data of the 16S rRNA gene obtained in the present study have been deposited in the DDBJ/EMBL/GenBank public databases with the accession...
numbers LC441114–LC441145 and LC455791–LC455869 (summarized in Table S1).

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References

1. Boucias, D.G., A. Garcia-Maruniak, R. Cherry, H. Lu, J.E. Maruniak, and V.U. Lietze. 2012. Detection and characterization of bacterial symbionts in the Heteropteran, Blissus insulatus. FEMS Microbiol. Ecol. 82:629–641.
2. Buchner, P. 1965. Endosymbiosis of Animals with Plant Microorganisms. Interscience, New York.
3. Caporaso, J.G., J. Kuczynski, J. Stombaugh, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7:335.
4. Delgado-Baquerizo, M., A.M. Oliverio, T.E. Brewer, A. Benavent-González, D.J. Eldridge, R.D. Bardgett, F.T. Maestre, B.K. Singh, and N. Fierer. 2018. A global atlas of the dominant bacteria found in soil. Science 359:320–325.
5. Dobrissa, A.P., and M. Samadpour. 2016. Transfer of eleven species of the genus Burkholderia to the genus Paraburkholderia and proposal of Caballeronia gen. nov. to accommodate twelve species of the genera Burkholderia and Paraburkholderia. Int. J. Syst. Evol. Microbiol. 66:2836–2846.
6. Estrada-de los Santos, P., F.U. Rojas-Rojas, E.Y. Tapia-Garcia, M.S. Vásquez-Murrieta, and A.M. Hirsch. 2016. To split or not to split: an opinion on dividing the genus Burkholderia. Ann. Microbiol. 66:1303–1314.
7. Garcia, J.R., A.M. Laughton, Z. Malik, B.J. Parker, C. Trincot, S.S.L. Chiang, E. Chung, and N.M. Gerardo. 2014. Partner associations across sympatric broad-headed bug species and their environmentally acquired bacterial symbionts. Mol. Ecol. 23:1303–1314.
8. Glasgow, H. 1914. The gastric caeca and the caecal bacteria of the Heteroptera. Biol. Bull. 26:101–171.
9. Henry, T.J. 2009. Biodiversity of Heteroptera, p. 223–263. In R.G. Footitt, and P.H. Adler (ed.), Insect Biodiversity: Science and Society. Wiley-Blackwell, Oxford.
10. Hosokawa, T., Y. Kikuchi, X.Y. Meng, and T. Fukatsu. 2010. Wolbachia as a bacteriocyte-associated nutritional mutualist. Proc. Natl. Acad. Sci. U.S.A. 107:769–774.
11. Hrušková, M., A. Honěk, and S. Pekár. 2005. Heteroptera. Biol. Bull. 26:101–171.
12. Itoh, H., M. Aita, A. Nagayama, X. Meng, Y. Kamagata, R. Navarro, T. Horii, S. Ohgiya, and Y. Kikuchi 2014. Evidence of environmental and vertical transmission of Burkholderia symbionts in the oriental chinch bug, Cavelerius saccharivorus (Heteroptera: Blissidae). Appl. Environ. Microbiol. 80:5974–5983.
13. Kikuchi, Y., T. Hosokawa, and T. Fukatsu. 2007. Insect-microbe mutualism without vertical transmission: a stinkbug acquires a beneficial gut symbiont from the environment every generation. Appl. Environ. Microbiol. 73:4308–4316.
14. Kikuchi, Y., T. Hosokawa, and T. Fukatsu. 2009. Endosymbiotic bacteria in insects: their diversity and culturability. Microbes Environ. 24:195–204.
15. Kikuchi, Y., T. Hosokawa, and T. Fukatsu. 2011. An ancient but promiscuous host-symbiont association between Burkholderia gut symbionts and their heteropteran hosts. ISME J. 5:446–460.
16. Kikuchi, Y., T. Hosokawa, and T. Fukatsu. 2011. Specific developmental window for establishment of an insect-microbe gut symbiosis. Appl. Environ. Microbiol. 77:4075–4081.
17. Kikuchi, Y., T. Hosokawa, and T. Fukatsu. 2014. Live imaging of symbiosis: spatiotemporal infection dynamics of a GFP-labelled Burkholderia symbiont in the bean bug Riptortus pedestris. Mol. Ecol. 23:1445–1456.
18. Kikuchi, Y., S.S. Prado, and T.M. Jenkins. 2018. Symbiotic microorganisms associated with Pentatomoida, p. 643–674. In J.E. McPherson (ed.), Invasive Stink Bugs and Related Species (Pentatomoida): Biology, Higher Systemsatics, Semiocentomy, and Management. CRC Press, Boca Raton.
19. Kuechler, S.M., K. Dettner, and S. Kehl. 2010. Molecular characterization and localization of the obligate endosymbiotic bacterium in the bithe catkin bug Kleidocerys resedae (Heteroptera: Lygaeidae, Lechoncharini). FEMS Microbiol. Ecol. 73:408–418.
20. Kuechler, S.M., K. Dettner, and S. Kehl. 2011. Characterization of an obligate intracellular bacterium in the midgut epithelium of the bulrush bug Chilacis typhae (Heteroptera, Lygaeidae, Artheneinae). Appl. Environ. Microbiol. 77:2869–2876.
21. Kuechler, S.M., P. Renz, K. Dettner, and S. Kehl. 2012. Diversity of symbiotic organims and bacterial endosymbions of lygaeid bugs of the families Blissidae and Lygaeidae (Hemiptera: Heteroptera: Lygaeoidea). Appl. Environ. Microbiol. 78:2648–2659.
22. Kuechler, S.M., Y. Matsuura, K. Dettner, and Y. Kikuchi. 2016. Phylogenetically diverse Burkholderia associated with midgut crypts of spurge bugs, Dicranocephalus spp. (Heteroptera: Stenocephalidae). Microbes Environ. 31:145–153.
23. Matsuura, Y., Y. Kikuchi, T. Hosokawa, R. Koga, X.Y. Meng, Y. Kamagata, N. Nikoh, and T. Fukatsu. 2012. Evolution of symbiotic organisms and endosymbions in lygaeid stinkbugs. ISME J. 6:397–409.
24. Miyamoto, S. 1961. Comparative morphology of alimentary organs of Heteroptera, with the phylogenetic consideration. Sieboldia 2:197–259.
25. Olivier-Respejel, S., Z.L. Sabree, K. Noe, and J.X. Becerra. 2011. Gut microbiota in nymph and adults of the giant mesquite bug (Thasus neocalifornicus) (Heteroptera: Coreidae) is dominated by Burkholderia acquired de novo every generation. Environ. Entomol. 40:1102–1110.
26. Ramírez, K.S., C.G. Knight, M. De Hollander, F.Q. Brearley, B. Constantimídes, A. Cotton, S. Creer, T.W. Crowther, J. Davison, and M. Delgado-Baquerizo. 2018. Detecting macroecological patterns in bacterial communities across independent studies of global soils. Nat. Microbiol. 3:189.
27. Savana, A., M. Adeolu, and R.S. Gupta. 2014. Molecular signatures and phylogenomic analysis of the genus Burkholderia: proposal for division of this genus into the emended genus Burkholderia containing pathogenic organisms and a new genus Paraburkholderia gen. nov. harboring environmental species. Front. Genet. 5:429.
28. Tago, K., H. Iioh, Y. Kikuchi, et al. 2014. A fine-scale phylogenetic analysis of free-living Burkholderia species in sugarcane field soil. Microbes Environ. 29:434–437.
29. Takeshita, K., and Y. Kikuchi. 2017. Riptortus pedestris and Burkholderia symbiont: an ideal model system for insect-microbe symbiotic associations. Res. Microbiol. 168:175–187.
30. Takeshita, K., H. Tamaki, T. Ohbayashi, Y.X. Meng, T. Sone, Y. Mitani, C. Peeters, Y. Kikuchi, and P. Vandamme 2018. Burkholderia insecticola sp. nov., a gut symbiotic bacterium of the bean bug Riptortus pedestris. Int. J. Syst. Evol. Microbiol. 68:2370–2374.
31. Vandamme, P., C. Peeters, B. De Smet, et al. 2017. Comparative genomics of Burkholderia singularis sp. nov., a low G+C content, free-living bacterium that defies taxonomic dissection of the genus Burkholderia. Front. Microbiol. 8:1679.