

## Fungal, bacterial, and archaeal diversity in the digestive tract of several beetle larvae (coleoptera)

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### Abstract

© Copyright 2018 Elvira E. Ziganshina et al. Interpretation of how partnerships between fungi, bacteria, archaea, and insects are maintained through the life of the hosts is a big challenge within the framework of symbiosis research. The main goal of this work was to characterize the gut microbiota in larvae of several Coleoptera species using sequencing of the bacterial and archaeal 16S rRNA genes and fungal internal transcribed spacer (ITS) region. Thus, larvae with various food preferences, including *Amphimallon solstitiale*, *Oryctes nasicornis*, *Cucujus cinnaberinus*, *Schizotus pectinicornis*, *Rhagium mordax*, and *Rhagium inquisitor*, were thoroughly investigated in this work. We revealed an association of these beetle species mainly with four bacterial phyla, Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes, as well as with three fungal phyla, Ascomycota, Zygomycota, and Basidiomycota, but microbial communities varied depending on the beetle host, individual organism, and surrounding environment. Moreover, archaea within the phyla Euryarchaeota and Crenarchaeota in the hindgut content of *O. nasicornis* and *A. solstitiale* were additionally detected. The identified microbial communities suggest their potential role in the exploitation of various resources, providing nutritional needs for the host organism. These microorganisms can also represent a valuable source of novel metabolic capacities for their application in different biotechnologies.

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### References

- [1] J. A. Ceja-Navarro, N. H. Nguyen, U. Karaoz et al., "Compartmentalized microbial composition, oxygen gradients and nitrogen fixation in the gut of *Odontotaenius disjunctus*," *The ISME Journal*, vol. 8, no. 1, pp. 6-18, 2014.
- [2] A.-A. Durand, A. Bergeron, P. Constant, J.-P. Buffet, E. Deziel, and C. Guertin, "Surveying the endomicobiome and ectomicobiome of bark beetles: The case of *Dendroctonus simplex*," *Scientific Reports*, vol. 5, Article ID 17190, 2015.
- [3] S. Grunwald, M. Pilhofer, and W. Holl, "Microbial associations in gut systems of wood- and bark-inhabiting longhorned beetles [Coleoptera: Cerambycidae]," *Systematic and Applied Microbiology*, vol. 33, no. 1, pp. 25-34, 2010.
- [4] V. Lopez-Martinez, O. R. Vargas, I. Alia-Tejacal et al., "Xylophagous beetles (Coleoptera: Buprestidae and Cerambycidae) from *Ficus carica* L. (Moraceae) in Morelos, Mexico," *The Coleopterists Bulletin*, vol. 69, no. 4, pp. 780-788, 2015.
- [5] S. Huang, P. Sheng, and H. Zhang, "Isolation and identification of cellulolytic bacteria from the gut of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae)," *International Journal of Molecular Sciences*, vol. 13, no. 3, pp. 2563-2577, 2012.
- [6] M. Kaltenpoth and S. Steiger, "Unearthing carrion beetles' microbiome: Characterization of bacterial and fungal hindgut communities across the Silphidae," *Molecular Ecology*, vol. 23, no. 6, pp. 1251-1267, 2014

- [7] A. Rizzi, E. Crotti, L. Borruso et al., "Characterization of the bacterial community associated with larvae and adults of *anoplophora chinensis* collected in Italy by culture and culture-independent methods," BioMed Research International, vol. 2013, Article ID 420287, 2013.
- [8] M. Egert, U. Stingl, L. D. Bruun, B. Pommerenke, A. Brune, and M. W. Friedrich, "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, 2005.
- [9] A. Brune, "Methanogenesis in the digestive tracts of insects," in Handbook of Hydrocarbon and Lipid Microbiology, K. N. Timmis, Ed., pp. 707-728, Springer, 2010.
- [10] E. E. Ziganshina, D. E. Belostotskiy, R. V. Shushlyayev, V. A. Miluykov, P. Y. Vankov, and A. M. Ziganshin, "Microbial community diversity in anaerobic reactors digesting turkey, chicken, and swine wastes," Journal of Microbiology and Biotechnology, vol. 24, no. 11, pp. 1464-1472, 2014.
- [11] A. M. Ziganshin, E. E. Ziganshina, S. Kleinsteuber, and M. Nikolausz, "Comparative analysis of methanogenic communities in different laboratory-scale anaerobic digesters," Archaea, vol. 2016, article no. 3401272, 2016.
- [12] E. E. Ziganshina, E. M. Ibragimov, O. N. Ilinskaya, and A. M. Ziganshin, "Bacterial communities inhabiting toxic industrial wastewater generated during nitrocellulose production," Biologia (Poland), vol. 71, no. 1, pp. 70-78, 2016.
- [13] P. Liu, X.-H. Wang, J.-G. Li et al., "Pyrosequencing reveals fungal communities in the rhizosphere of Xinjiang jujube," BioMed Research International, vol. 2015, Article ID 972481, 2015.
- [14] W. Song, L. Li, H. Huang et al., "The Gut Microbial Community of Antarctic Fish Detected by 16S rRNA Gene Sequence Analysis," BioMed Research International, vol. 2016, Article ID 3241529, 2016.
- [15] C. Bayon and J. Mathelin, "Carbohydrate fermentation and byproduct absorption studied with labelled cellulose in *Oryctes nasicornis* larvae (Coleoptera:Scarabaeidae)," Journal of Insect Physiology, vol. 26, no. 12, pp. 833-840, 1980.
- [16] E. Arias-Cordero, L. Ping, K. Reichwald, H. Delb, M. Platzer, and W. Boland, "Comparative Evaluation of the Gut Microbiota Associated with the Below- and Above-Ground Life Stages (Larvae and Beetles) of the Forest Cockchafer, *Melolontha hippocastani*," PLoS ONE, vol. 7, no. 12, Article ID e51557, 2012.
- [17] M. K. Sears, "Mandibular structure and feeding habits of three morphologically similar coleopterous larvae: *Cucujus clavipes* (Cucujidae), *Dendroides canadensis* (Pyrochroidae), and *Pytho depressus* (Salpingidae)," The Canadian Entomologist, vol. 114, no. 2, pp. 173-175, 1982.
- [18] W. E. Hillis, Heartwood and tree exudates, Springer Science and Business Media, 2012.
- [19] C. S. L. Vicente, F. X. Nascimento, M. Espada et al., "Characterization of bacterial communities associated with the pine sawyer beetle *Monochamus galloprovincialis*, the insect vector of the pinewood nematode *Bursaphelenchus xylophilus*," FEMS Microbiology Letters, vol. 347, no. 2, pp. 130-139, 2013.
- [20] J. M. Kim, M.-Y. Choi, J.-W. Kim et al., "Effects of diet type, developmental stage, and gut compartment in the gut bacterial communities of two Cerambycidae species (Coleoptera)," Journal of Microbiology, vol. 55, no. 1, pp. 21-30, 2017.
- [21] A. E. Douglas, "Multiorganismal insects: Diversity and function of resident microorganisms," Annual Review of Entomology, vol. 60, pp. 17-34, 2015.
- [22] S. Kumar, G. Stecher, and K. Tamura, "MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets," Molecular Biology and Evolution, vol. 33, no. 7, pp. 1870-1874, 2016.
- [23] D. P. R. Herlemann, M. Labrenz, K. Jurgens, S. Bertilsson, J. J. Waniek, and A. F. Andersson, "Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea," The ISME Journal, vol. 5, no. 10, pp. 1571-1579, 2011.
- [24] K. Takai and K. Horikoshi, "Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes," Applied and Environmental Microbiology, vol. 66, no. 11, pp. 5066-5072, 2000.
- [25] H. Toju, A. S. Tanabe, S. Yamamoto, and H. Sato, "Highcoverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples," PLoS ONE, vol. 7, no. 7, Article ID e40863, 2012.
- [26] E. E. Ziganshina, E. M. Ibragimov, P. Y. Vankov, V. A. Miluykov, and A. M. Ziganshin, "Comparison of anaerobic digestion strategies of nitrogen-rich substrates: performance of anaerobic reactors and microbial community diversity," Waste Management, vol. 59, pp. 160-171, 2016.
- [27] O. Folmer, M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek, "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates," Molecular Marine Biology and Biotechnology, vol. 3, no. 5, pp. 294-299, 1994.
- [28] M. J. Raupach, J. J. Astrin, K. Hannig, M. K. Peters, M. Y. Stoeckle, and J.-W. Wagele, "Molecular species identification of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes," Frontiers in Zoology, vol. 7, article no. 26, 2010.
- [29] J. G. Caporaso, J. Kuczynski, J. Stombaugh et al., "QIIME allows analysis of high-throughput community sequencing data," Nature Methods, vol. 7, no. 5, pp. 335-336, 2010.

- [30] D. McDonald, M. N. Price, J. Goodrich et al., "An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea," *The ISME Journal*, vol. 6, no. 3, pp. 610-618, 2012.
- [31] Q. Wang, G. M. Garrity, J. M. Tiedje, and J. R. Cole, "Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy," *Applied and Environmental Microbiology*, vol. 73, no. 16, pp. 5261-5267, 2007.
- [32] K. Abarenkov, R. H. Nilsson, K.-H. Larsson et al., "The UNITE database for molecular identification of fungi - recent updates and future perspectives," *New Phytologist*, vol. 186, no. 2, pp. 281-285, 2010.
- [33] J. Oksanen, *Multivariate Analysis of Ecological Communities in R: Vegan Tutorial*, vol. 83, University of Oulu Computer Services Centre, 2011.
- [34] E. E. Ziganshina, V. S. Mohammed, N. V. Shulaev et al., "Features of bacterial community of intestines of some larvae of xylophagous beetles (Cerambycidae)," *Bulletin of the Technological University*, vol. 20, pp. 120-124, 2017.
- [35] L. V. Hooper, T. Midwedt, and J. I. Gordon, "How hostmicrobial interactions shape the nutrient environment of the mammalian intestine," *Annual Review of Nutrition*, vol. 22, pp. 283-307, 2002.
- [36] A. Grabowski, B. J. Tindall, V. Bardin, D. Blanchet, and C. Jeanthon, "Petricimonas sulfuriphila gen. nov., sp. nov., a mesophilic fermentative bacterium isolated from a biodegraded oil reservoir," *International Journal of Systematic and Evolutionary Microbiology*, vol. 55, no. 3, pp. 1113-1121, 2005.
- [37] M. Morrison and J. Miron, "Adhesion to cellulose by *Ruminococcus albus*: A combination of cellulosomes and Pil-proteins?" *FEMS Microbiology Letters*, vol. 185, no. 2, pp. 109-115, 2000.
- [38] P. Sheng, Y. Li, S. D. G. Marshall, and H. Zhang, "High genetic diversity of microbial cellulase and hemicellulase genes in the hindgut of *Holotrichia parallela* larvae," *International Journal of Molecular Sciences*, vol. 16, no. 7, pp. 16545-16559, 2015.
- [39] S. L. A. Sari, A. Pangstuti, A. Susilowati et al., "Cellulolytic and hemicellulolytic bacteria from the gut of *Oryctes rhinoceros* larvae," *Biodiversitas*, vol. 17, no. 1, pp. 78-83, 2016.
- [40] T. L. Miller and C. Lin, "Description of *Methanobrevibacter gottschalkii* sp. nov., *Methanobrevibacter thaueri* sp. nov., *Methanobrevibacter woesei* sp. nov. and *Methanobrevibacter wolinii* sp. nov," *International Journal of Systematic and Evolutionary Microbiology*, vol. 52, no. 3, pp. 819-822, 2002.
- [41] A. Spang, A. Poehlein, P. Offre et al., "The genome of the ammonia-oxidizing *Candidatus Nitrososphaera gargensis*: insights into metabolic versatility and environmental adaptations," *Environmental Microbiology*, vol. 14, no. 12, pp. 3122-3145, 2012.
- [42] R. C. Preece and D. R. Bridgland, *Late Quaternary Environmental Change in North-west Europe: Excavations at Holywell Coombe, South-east England*, Springer Netherlands, London, UK, 1998.
- [43] J. Morales-Jimenez, G. Zuniga, H. C. Ramirez-Saad, and C. Hernandez-Rodriguez, "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, 2012.
- [44] M. D. Collins and H. N. Shah, "Reclassification of *Bacteroides termitidis* Sebald (Holdeman and Moore) in a new genus *Sebaldella*, as *Sebaldella termitidis* comb. nov," *International Journal of Systematic Bacteriology*, vol. 36, no. 2, pp. 349-350, 1986.
- [45] M. J. Taherzadeh and K. Karimi, "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review," *International Journal of Molecular Sciences*, vol. 9, no. 9, pp. 1621-1651, 2008.
- [46] Y. Ding, J. Wang, Y. Liu, and S. Chen, "Isolation and identification of nitrogen-fixing bacilli from plant rhizospheres in Beijing region," *Journal of Applied Microbiology*, vol. 99, no. 5, pp. 1271-1281, 2005.
- [47] P. Menna and M. Hungria, "Phylogeny of nodulation and nitrogen-fixation genes in *Bradyrhizobium*: Supporting evidence for the theory of monophyletic origin, and spread and maintenance by both horizontal and vertical transfer," *International Journal of Systematic and Evolutionary Microbiology*, vol. 61, no. 12, pp. 3052-3067, 2011.
- [48] W.-M. Chen, L. Moulin, C. Bontemps, P. Vandamme, G. Bena, and C. Boivin-Masson, "Legume Symbiotic Nitrogen Fixation by -Proteobacteria Is Widespread in Nature," *Journal of Bacteriology*, vol. 185, no. 24, pp. 7266-7272, 2003.
- [49] E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, *The Prokaryotes*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2014.
- [50] R. Nalin, P. Simonet, T. M. Vogel, and P. Normand, "Rhodanobacter lindaniclasticus gen. nov., sp. nov., a lindanedegrading bacterium," *International Journal of Systematic Bacteriology*, vol. 49, no. 1, pp. 19-23, 1999.
- [51] H. N. Prasanna, G. Ramanjaneyulu, and B. Rajasekhar Reddy, "Optimization of cellulase production by *Penicillium* sp," *3 Biotech*, vol. 6, no. 2, article no. 162, 2016.
- [52] J. Yang, H. Yuan, H. Wang, and W. Chen, "Purification and characterization of lignin peroxidases from *Penicillium decumbens* P6," *World Journal of Microbiology and Biotechnology*, vol. 21, no. 4, pp. 435-440, 2005.

- [53] A. V. Rice and R. S. Currah, "Two new species of *Pseudogymnoascus* with *Geomycetes* anamorphs and their phylogenetic relationship with *Gymnstellatospora*, " *Mycologia*, vol. 98, no. 2, pp. 307-318, 2006.
- [54] G. Wibbelt, A. Kurth, D. Hellmann et al., "White-nose syndrome fungus (*Geomycetes destructans*) in bats, Europe, " *Emerging Infectious Diseases*, vol. 16, no. 8, pp. 1237-1242, 2010.
- [55] J. Klimaszewski, M.-J. Morency, P. Labrie et al., "Molecular and microscopic analysis of the gut contents of abundant rove beetle species (Coleoptera, staphylinidae) in the boreal balsam fir forest of Quebec, Canada, " *ZooKeys*, vol. 353, pp. 1-24, 2013.
- [56] X. Hu, M. Li, and H. Chen, "Community structure of gut fungi during different developmental stages of the Chinese white pine beetle (*Dendroctonus armandi*), " *Scientific Reports*, vol. 5, p. 8411, 2015.
- [57] I. Delalibera Jr., J. Handelsman, and K. F. Raffa, "Contrasts in cellulolytic activities of gut microorganisms between the wood borer, *Saperda vestita* (Coleoptera: Cerambycidae), and the bark beetles, *Ips pini* and *Dendroctonus frontalis* (Coleoptera: Curculionidae), " *Environmental Entomology*, vol. 34, no. 3, pp. 541-547, 2005.
- [58] H. Chen, "Biotechnology of lignocellulose: Theory and practice, " *Biotechnology of Lignocellulose: Theory and Practice*, pp. 1-511, 2014.
- [59] S. Freeman, M. Sharon, M. Dori-Bachash et al., "Symbiotic association of three fungal species throughout the life cycle of the ambrosia beetle *Euwallacea nr. fornicatus*, " *Symbiosis*, vol. 68, no. 1-3, pp. 115-128, 2016.
- [60] Y. Brotman, J. G. Kapuganti, and A. Viterbo, "Trichoderma, " *Current Biology*, vol. 20, no. 9, pp. R390-R391, 2010.
- [61] K. Rojas-Jimenez and M. Hernandez, "Isolation of fungi and bacteria associated with the guts of tropical wood-feeding coleoptera and determination of their lignocellulolytic activities, " *International Journal of Microbiology*, vol. 2015, Article ID 285018, 2015.
- [62] M. Dashtban, H. Schraft, T. A. Syed, and W. Qin, "Fungal biodegradation and enzymatic modification of lignin, " *International Journal of Biochemistry and Molecular Biology*, vol. 1, no. 1, pp. 36-50, 2010.
- [63] G. E. Harman, "Overview of mechanisms and uses of Trichoderma spp, " *Journal of Phytopathology*, vol. 96, no. 2, pp. 190-194, 2006.
- [64] T. E. Yu, K. N. Egger, and L. R. Peterson, "Ectendomycorrhizal associations - Characteristics and functions, " *Mycorrhiza*, vol. 11, no. 4, pp. 167-177, 2001.
- [65] L. M. Avellaneda-Torres, C. P. G. Pulido, and E. T. Rojas, "Assessment of cellulolytic microorganisms in soils of Nevados park, Colombia, " *Brazilian Journal of Microbiology*, vol. 45, no. 4, pp. 1211-1220, 2014.
- [66] C. Berthelot, C. Leyval, J. Foulon, M. Chalot, and D. Blaudez, "Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metalpolluted poplar phytomanagement sites, " *FEMS Microbiology Ecology*, vol. 92, no. 10, Article ID fiw144, 2016.
- [67] W. A. Untereiner, "Capronia and its anamorphs: Exploring the value of morphological and molecular characters in the systematics of the Herpotrichiellaceae, " *Studies in Mycology*, vol. 2000, no. 45, pp. 141-148, 2000.
- [68] H. T. C. Leung, K. R. Maas, R. C. Wilhelm, and W. W. Mohn, "Long-term effects of timber harvesting on hemicellulolytic microbial populations in coniferous forest soils, " *The ISME Journal*, vol. 10, no. 2, pp. 363-375, 2016.
- [69] O. B. Ryabova, O. M. Chmil, and A. A. Sibirny, "Xylose and cellobiose fermentation to ethanol by the thermotolerant methylotrophic yeast *Hansenula polymorpha*, " *FEMS Yeast Research*, vol. 4, no. 2, pp. 157-164, 2003.
- [70] S.-O. Suh and J. J. Zhou, "Methylotrophic yeasts near *Ogataea (Hansenula) polymorpha*: A proposal of *Ogataea angusta* comb. nov. and *Candida parapolymorpha* sp. nov, " *FEMS Yeast Research*, vol. 10, no. 5, pp. 631-638, 2010.
- [71] M. Groenewald and M. T. Smith, "Re-examination of strains formerly assigned to *Hyphopichia burtonii*, the phylogeny of the genus *Hyphopichia*, and the description of *Hyphopichia pseudoburtonii* sp. nov, " *International Journal of Systematic and Evolutionary Microbiology*, vol. 60, no. 11, pp. 2675-2680, 2010.
- [72] I. V. Khilyas, A. M. Ziganshin, A. J. Pannier, and R. Gerlach, "Effect of ferrihydrite on 2, 4, 6-trinitrotoluene biotransformation by an aerobic yeast, " *Biodegradation*, vol. 24, no. 5, pp. 631-644, 2013.
- [73] A. M. Ziganshin, E. E. Ziganshina, J. Byrne et al., "Fe(III) mineral reduction followed by partial dissolution and reactive oxygen species generation during 2, 4, 6-trinitrotoluene transformation by the aerobic yeast *Yarrowia lipolytica*, " *AMB Express*, vol. 5, no. 1, pp. 1-12, 2015.