Detection of a new bacterium of the family Holosporaceae (Alphaproteobacteria: Holosporales) associated with the oribatid mite *Achipteria coleoprata*

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Abstract

We detected an unknown bacterium in *Achipteria coleoprata* (Acari: Oribatida). Its 16S rDNA gene sequence showed 89% identity to the endosymbiont “*Candidatus Nucleicultrix amoebiphila*” from amoebae and “*Candidatus Gortzia* sp.” from ciliates. Phylogenetic analysis revealed that the microorganism is a member of the family Holosporaceae, order Holosporales of Alphaproteobacteria. Its occurrence in Oribatida is enigmatic. It cannot be excluded that it is a symbiont of Oribatida as well as it is an endosymbiont of a smaller, even unicellular, organisms living inside the mite. The issue of the occurrence of this microorganism is interesting and further research is needed to gain the knowledge of its role and the nature of bacterium-host interaction.

Keywords Endosymbiotic bacterium · Oribatida · 16S rDNA · Phylogenetic analysis

Introduction

The microbiome of a eukaryotic organism is usually highly diverse, complex and profoundly affects the host biology. Its composition and role have become a dramatically growing area of research in recent years due to the explosion of metagenomics (Louca et al. 2016; Nayfach and Pollard 2016). Despite that, the data on invertebrate microbiology have gaps. The vast majority of living animals are invertebrates (Petersen and Osvatic 2018) and among them, Arthropoda are the largest group of terrestrial animals adapted to all environments. Although there is a number of works concerning the issue of the microbiome of invertebrates (e.g.: Glowska et al. 2015; Konecka and Olszanowski 2015; Larsen et al. 2016; Laport et al. 2018; Mioduchowska et al. 2018; Selkrig et al. 2018; Konecka and Olszanowski 2019a,

2019b; Ma et al. 2019), this topic has not been exhausted yet and compared to studies on the microbiome of vertebrates, including humans, relatively few studies focus on of the microbiomes of arthropods (Scopus database). Not only the species composition of the invertebrate microbiome, but also the interrelations between the bacteria of different taxonomic groups present in one host are extremely interesting, especially that there may be a high degree of variation in bacterial taxa in the same individual host (Jones et al. 2008).

The knowledge on intracellular microorganisms, their distribution and role are particularly appealing because relations between some of these bacteria and the host may have intimate character, like in the case of endosymbionts from *Paramecium* sp. that can replicate directly in the cytoplasm or within organelles of the host cells (Boscaro et al. 2013; Serra et al. 2016). In arthropods, symbiotic bacteria were observed within the mitochondria and multiply therein (Sassera et al. 2006). Some of them infect nucleus which is rich in proteins, nucleic acids and nucleoside triphosphates required for bacterial DNA replication and transcription so it seems to be an attractive place for intracellular bacteria. The endosymbionts, represented by different species, like “*Candidatus Nucleicultrix amoebiphila*” (Schulz et al. 2014), “*Candidatus Gortzia infectiva*” (Boscaro et al. 2013) or “*Candidatus Gortzia shahrazadis*” (Serra et al. 2016), use the nucleus of eukaryotic cells as a niche. Indeed, they are

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macronuclear symbionts (Boscaro et al. 2013) and have been found, for example, in free-living amoebae (Schulz et al. 2014) and ciliates (Boscaro et al. 2013; Serra et al. 2016). Generally, these intracellular symbionts are regarded to have neutral effect on the host with no killing traits (Boscaro et al. 2013). However, the parasitological activity of macronuclear bacteria has been noted (Lohse et al. 2006) and they have been found to help their hosts to adapt in challenging environmental conditions like changing temperature (Hori and Fujishima 2003). “*Candidatus Nucleicultrix amoebiphila*” and “*Candidatus Gortzia* sp.” represent Caedimonadaceae and Holosporaceae family, respectively. The families together with Paraceadibacteriaceae and Hepatincolaceae belong to the order Holosporales of Alphaproteobacteria (Szokoli et al. 2016a; Schrallhammer et al. 2018). Bacteria of Holosporales are phylogenetically closely related with Rickettsiales. Both orders comprise mainly intracellular (only one extracellular species was found by Castelli et al. 2018a) bacteria associated with protists and invertebrates (Castelli et al. 2016).

Although some endosymbiotic microorganisms have been found in oribatid mites (Acari: Oribatida) (Perrot-Minnot and Norton 1997; Cordaux et al. 2001; Weeks et al. 2003; Liana and Witaliński 2010; Konecka and Olszanowski 2015, 2019a, b), still the knowledge of the microbiome diversity in Oribatida is insufficient. Additionally, the number of parthenogenetic species within oribatid mites is relatively high (ca. 10%). As some intracellular bacteria, e.g. *Wolbachia*, could induce parthenogenesis in arthropods (Kajtoch and Kotásková 2018), we undertook the studies on the occurrence of microbial symbionts in the Oribatida and we detected an unknown bacterium of Holosporaceae in oribatid mite *Achipteria coleoptrata* (Linnaeus, 1758) by using molecular approach. We conducted phylogenetic analysis based on 16S rDNA sequences of the bacterium from *A. coleoptrata* and endosymbionts of other host that showed sequence identity by BLASTn. Additional sequence data were included in the analysis according to literature data (Boscaro et al. 2013; Schulz et al. 2014; Hess et al. 2016; Lanzoni et al. 2016; Szokoli et al. 2016a, b; Castelli et al. 2018b; Potekhin et al. 2018; Schrallhammer et al. 2018; Tashyreva et al. 2018).

Materials and methods

Isolation of *Achipteria coleoptrata*

Achipteria coleoptrata (Acari: Oribatida) was isolated from a sample of mosses collected in the mixed forest near Ostrowiec Świętokrzyski, Świętokrzyskie voivodeship in Poland (50°59' N, 21°20' E). Mites were extracted by using high-gradient Tullgren funnels, segregated intravitaly and immediately placed directly in 96% ethanol for genetic analysis.

Some comparative specimens were conserved in 70% ethanol and then determined by using the key of Weigmann (2006).

DNA extraction, polymerase chain reaction (PCR), and sequencing

Total DNA of mite individuals preserved in 96% ethyl alcohol was extracted with Genomic Mini kit (A&A Biotechnology). Amplifications were performed in a 10 µl mixtures containing: 4 µl DNA, 1 µl 10× PCR DreamTaq Buffer (Thermo Scientific), 0.4 µl 5 mM dNTP (Novazym), 0.6 µM each primer (Oligo.pl), 0.8 U DreamTaq Hot Start DNA Polymerase (Thermo Scientific) and sterile bidistilled water to a total volume of 10 µl. Negative controls with no template DNA were included in each reaction. In searching for *Wolbachia* and other bacteria of Anaplasmataceae, we used a pair of specific primers: EHR16SD and EHR16SR (Brown et al. 2001; Hornok et al. 2008) in PCR method to identify approximately 350 bp of 16S rDNA of bacterial symbionts. Only one PCR product was obtained. The amplicon was electrophoresed, directly sequenced with BigDye Terminator v3.1 in an ABI Prism 3130XL Analyzer (Applied Biosystems) and analyzed with BLASTn.

For larger part of the 16S rDNA gene, two additional PCR reactions were conducted: (1) with the specific primer EHR16SD (Brown et al. 2001) and universal eubacterial primer 1513R (Weisburg et al. 1991), and (2) with the specific primer EHR16SR (Brown et al. 2001) and universal eubacterial primer 27F (Weisburg et al. 1991). The PCR annealing temperature was 55 °C. In each of the two PCR reactions, a single product was obtained. The amplicons were electrophoresed, directly sequenced with BigDye Terminator v3.1 in an ABI Prism 3130XL Analyzer (Applied Biosystems) and analyzed with BLASTn. These two sequences were assembled and the 1268-bp-long 16S rDNA sequence from the bacterium associated with *A. coleoptrata* was deposited in GenBank under accession no. MH838020.

Phylogenetic analysis

The sequence of 16S rDNA from the bacterium associated with *A. coleoptrata* was aligned to those from protist and invertebrate hosts, and from water samples. The alignment of 49 sequences was constructed with the use of CLUSTAL W (Thompson et al. 1994). jModelTest 2 software (Darriba et al. 2012) was used for choosing the optimal model of sequence evolution. The General Time Reversible model with gamma distribution (GTR + G) was selected. Phylogenetic analysis was conducted using MEGA version 6.0 (Tamura et al. 2013). The maximum likelihood bootstrap support was determined by using 1000 bootstrap replicates.

Results and discussion

The analysis of the 16S rDNA of the bacterium associated with *A. coleoprata* by using BLASTn showed the highest sequence identity of $\geq 91\%$ to uncultured microbes discovered in the samples of seawater (accession no. JQ194949) and coal seam groundwater (accession no. AB294318). The sequence also exhibited the high identity of 89% to the sequence of endosymbiotic microbes “*Candidatus Nucleicultrix amoebiphila*” and “*Candidatus Gortzia sp.*” from the family Caedimonadaceae (Schrallhammer et al. 2018) and Holosporaceae (Boscaro et al. 2013), respectively: “*Candidatus Nucleicultrix amoebiphila*” was found in *Hartmannella sp.* (accession no. KF697195), “*Candidatus Gortzia infectiva*” in *Paramecium jenningsi* (accession no. HE797907), “*Candidatus Gortzia infectiva*” in *P. quadecaurelia* (accession nos.: HE797908, HE797909, and HE797910), and “*Candidatus Gortzia shahrazadis*” in *P. multimicronucleatum* (accession no. LT549002). The amplicon also revealed 88% identity to the sequence of endosymbiont of *Xestospongia muta* (Schmidt, 1870) (accession no. JN596597). The references associated with the accession numbers of sequences mentioned above are: Shimizu et al. (2007), Montalvo and Hill (2011), Boscaro et al. (2013), Schulz et al. (2014), Serra et al. (2016).

In phylogenetic analysis, the bacteria from water samples (accession nos. JQ194949 and AB294318) were used as a comparative material because the 16S rDNA sequences of these microbes were most similar to the bacterium of *A. coleoprata* by BLASTn. We expanded the list of comparative sequences by 16S rDNA of the sequences of “*Candidatus N. amoebiphila*”, “*Candidatus G. infectiva*”, and “*Candidatus G. shahrazadis*”. The bacterial families represented by the endosymbionts (Holosporaceae, Caedimonadaceae) belong to the order Holosporales of Alphaproteobacteria. In order to solve the phylogenetic position of the newly characterized bacterium from Oribatida, the selected sequences from these two families and from other Holosporales families (sensu Szokoli): (1) Paracaeodibacteraceae (“*Candidatus Odysella sp.*”, “*Candidatus Paracaeodibacter sp.*”), (2) Holosporaceae (*Paraholospora sp.*, “*Candidatus Bealeia sp.*”, “*Candidatus Hepatobacter sp.*”, *Holospora sp.*) and (3) Caedimonadaceae (*Caedimonas sp.*, *Caedibacter sp.*, and endosymbionts of *Acanthamoeba sp.*) were also included in the analysis. The accession numbers of these sequences were published by Loy et al. (1996), Horn et al. (1999), Eschbach et al. (2009), Dohra et al. (2014), Szokoli et al. (2016a), and Schrallhammer et al. (2018). Furthermore, we included the 16S rDNA sequences of endosymbiont of *X. muta* and bacteria noted in other marine hosts: *Porites compressa* Dana, 1846 and *Hippocampus guttulatus* Cuvier, 1829. In order to make the data comprehensive, we applied in the analysis also the

sequences of other bacteria published in similar works (Hess et al. 2016; Lanzoni et al. 2016; Szokoli et al. 2016a, b; Castelli et al. 2018b; Potekhin et al. 2018; Schrallhammer et al. 2018; Tashyreva et al. 2018; Fokin et al. 2019) using Rickettsiales sequences as an outgroup. The bacterial sequences used in the analysis were presented in the Fig. 1. The tree showed that the family Holosporaceae is paraphyletic that may suggest heterogeneous evolutionary rate or long branch attraction in it. The highly accelerated rates of evolution of obligate intracellular Alphaproteobacteria may also be an explanation of the paraphyly.

Phylogenetic analysis showed that the newly characterized microorganism clusters within Holosporaceae with closest relation to a bacterium “*Candidatus Hepatobacter penaei*”, a microbe of finger coral *P. compressa* and an endosymbiont of giant barrel sponge *X. muta* (Fig. 1). *H. penaei* is an intracellular bacterial pathogen that infects the hepatopancreas of *Penaeus vannamei* (Boone, 1931) shrimp (Loy et al. 1996). The bacterium is a member of Holosporaceae (Szokoli et al. 2016a). No information is available about the microbes of the coral and sponge, except from their 16S rRNA gene sequences deposited in GenBank and the report on the bacteria discovery in *X. muta* (Montalvo and Hill 2011). Earlier, the symbionts were not assigned to any taxonomic group. Our analysis revealed that they are the members of Holosporaceae.

The next close branching lineage in the tree was formed by bacteria from water samples. One of them (accession no. AB294318) was found in deep coal seam groundwater (Shimizu et al. 2007) and assigned to the family Holosporaceae (Szokoli et al. 2016a). No information is available about the other water microbe (accession no. JQ194949), except from its 16S rRNA gene sequences deposited in GenBank. Our analysis showed it belongs to Holosporaceae. In our opinion, it could not be excluded that the bacteria found in water were in fact endosymbionts of small, unicellular hosts collected together with the water sample.

The bacterium associated with *A. coleoprata* showed 89% identity of 16S rDNA to the symbiotic microbe *N. amoebiphila* of free-living amoebae *Hartmannella sp.* that interacts with chromatin during host cell division and initiates replication (Schulz et al. 2014). The same identity of the 16S rDNA sequence (89%) was also observed between the bacterium of *A. coleoprata* and “*Candidatus Gortzia sp.*” from ciliates *Paramecium sp.* Additionally, phylogenetic analysis revealed relatively close relationship of these microorganisms with *Holospora sp.*, *Paraholospora nucleivisitans* and the bacterium found in an insect *Oropsylla hirsuta* Baker, 1895 (a flea collected from black-tailed prairie dog) by Jones et al. (2008). The “*Candidatus G. infectiva*” and “*Candidatus G. shahrazadis*” have been reported to infect the macronuclei and shown no killer effect on the hosts (Boscaro et al. 2013; Serra et al. 2016). Strains of *Holospora sp.* were also noted in *Paramecium sp.* and they inhabited the

Fig. 1 The 16S rRNA-based relationship of the bacterium from *Achipteria coleoptrata* (Acari: Oribatida), endosymbionts of other hosts and bacteria from water. NCBI accession numbers for sequences used for

phylogenetic analysis are presented after the names of microorganisms. Bootstrap values based on 1000 replicates are shown on the branches

macro- and micronucleus of the host (Fokin et al. 2004; Hori et al. 2008), however the range of their functions was much broader, e.g. *H. undulata* increased host resistance to osmotic stress (Duncan et al. 2010) and tolerance to heat-shock (Fujishima et al. 2005). In contrast to *Holospora* sp., *P. nucleivisitans* resided also in the cytoplasm of *Paramecium* sp. and the role of the bacteria is unknown apart from that *P. nucleivisitans* had no killing activity towards ciliates (Eschbach et al. 2009).

In conclusion, we report the occurrence of a new bacterium in the moss mite *A. coleoptrata*. The bacterium was classified within the family Holosporaceae, order Holosporales of Alphaproteobacteria. As it was also phylogenetically closely related to microbes that were found in sponge, coral, ciliates, shrimps, and flea insect, it cannot be excluded that it is an intracellular symbiont of mites or an endosymbiont of a smaller, even unicellular, organisms living inside the arachnid. In our opinion, regardless of what is the actual host of the

bacterium, the issue of the occurrence of these unnamed microorganism in oribatid mite is extremely interesting and further research is needed to gain the knowledge on the nature of bacterium-host interaction and role of this microbe.

Author's contributions Edyta Konecka designed research, conducted detection and phylogenetic analysis of the bacterium, analyzed data, wrote the manuscript; Ziemowit Olszanowski conducted isolation and identification of the mite, contributed to the writing the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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