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Cardinium in *Plagiomerus diaspidis* (Hymenoptera: Encyrtidae)

Yiftach Matalon, Nurit Katzir, Yuval Gottlieb, Vitaly Portnoy, Einat Zchori-Fein *

The Agricultural Research Organization (ARO), Department of Vegetable Crops, Newe Ya'ar Research Center, P.O. Box 1021, Ramat Yishay 30095, Israel

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Abstract

The bacterial symbiont *Cardinium* (Bacteroidetes) was previously implicated in the thelytokous reproduction of the parasitoid *Plagiomerus diaspidis* Crawford (Hymenoptera: Encyrtidae). Horizontal transmission of the symbiont among the cactus scale *Diaspis echinocacti* Bouché (Homoptera: Diaspididae) and its hymenopteran parasitoids has been suggested. In this study, the bacteria associated with *D. echinocacti*, its parasitoids *P. diaspidis* and *Aphytis* sp. (Hymenoptera: Aphelinidae), and the hyperparasitoid *Marietta leopardina* Motschulsky (Hymenoptera: Aphelinidae) were characterized using molecular fingerprinting techniques, and the localization of *Cardinium* in *P. diaspidis* was studied using fluorescence *in situ* hybridizations (FISH). *Cardinium* was the only bacterium found in *P. diaspidis*, but it could not be detected in any of the other insects tested. The symbiont was specifically located in the reproductive tissues of its *P. diaspidis* host.

Keywords: Bacteroidetes; *Cardinium*; Diaspididae; *Diaspis echinocacti*; FISH; Horizontal transmission; Parthenogenesis; Symbiont

1. Introduction

Plagiomerus diaspidis Crawford (Hymenoptera: Encyrtidae) is a parthenogenetic, primary, internal parasitoid of the cactus scale *Diaspis echinocacti* Bouché (Homoptera: Diaspididae). It shares the diaspidid host with several other parasitoids, including *Aphytis* spp. (Hymenoptera: Aphelinidae), and with the hyperparasitoid *Marietta* spp. (Hymenoptera: Aphelinidae) which can develop on both hymenopterans. Heat treatments of *P. diaspidis* were found to result in the production of male offspring (Gordh and Lacey, 1976), and the discovery of the arthropod symbiont *Cardinium* in the wasp by Zchori-Fein and Perlman (2004) led them to suggest that it may be the causal agent of *P. diaspidis* parthenogenesis.

Symbiotic microorganisms play a crucial role in the ecology and evolution of their arthropod hosts. These endosymbionts are primarily transferred from mothers to their offspring (vertical transmission), but may also be spread horizontally among individuals and among different taxa. Transfer of symbionts between parasitoid wasps and their hosts has been suggested as a potential mechanism for the horizontal transmission of bacterial symbionts such as *Wolbachia* (e.g. van Meer et al., 1999). Similarly, the presence of closely related *Cardinium* in the armored scale *D. echinocacti*, in its two primary parasitoids, *P. diaspidis* and *Aphytis* sp., and in their hyperparasitoid *Marietta spp.* has led to speculations concerning horizontal transmission in this system (Weeks et al., 2003; Zchori-Fein and Perlman, 2004).

In order to ascertain whether *Cardinium* is the only bacterium found in *P. diaspidis* and to test the possibility that this symbiont is horizontally transmitted among different trophic levels, the bacterial assembly in the scale insect guild was characterized.

2. Material and methods

2.1. Insect origin

All insects were field-collected at Kibbutz Alonim, Israel, from prickly pear, *Opuntia aciculata* Griffiths (Cactaceae)

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* Corresponding author. Fax: +972 4 9836936.
E-mail address: einat@volcani.agri.gov.il (E. Zchori-Fein).

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cactus pads during the winter of 2005. Cactus pads heavily infested with cactus scales were kept in emergence cages for six months, and the emerging *P. diaspidis*, *M. leopardina* Motschulsky, and *Aphytis* sp. were collected frequently and placed live in 96% ethanol. *D. echinocacti* was hand-collected from infested cactus pads into 96% ethanol.

2.2. Identification of bacterial assembly

To determine the diversity and identity of bacteria associated with the cactus scale insect and its parasitoids, each of ten individual adult females was subjected to denaturing gradient gel electrophoresis (DGGE) as described in Gottlieb et al. (2006). Two different clones were sequenced for each bacterium (Macrogen Inc., Korea), and the results were compared by BLAST to the NCBI database.

2.3. Localization of Cardinium in P. diaspidis

Fluorescent *in situ* hybridization (FISH) was used to detect the distribution pattern of *Cardinium* within *P. diaspidis*. Fixation, decolorization and hybridization of adults and ovaries followed the procedure described in Gottlieb et al. (2006). The DNA probe *Cardinium*-Cy5 (5′-Cy5-CAATTGCAGTTCTAGCGTTA-3′) was designed based on the *Cardinium* 16S rRNA sequence. Stained samples were whole mounted and viewed under an IX81Olympus FluoView™500 confocal microscope. Specificity of the detection was confirmed using controls consisting of adult wasps with no probe, ovaries with no probe, and the *Cardinium*-free *M. leopardina*.

3. Results

3.1. Identification of bacterial assembly

The DGGE results showed that *D. echinocacti*, *P. diaspidis*, and *Aphytis* sp. each carried a single symbiont. In contrast, *M. leopardina* did not show a stable infection by a symbiont. PCR products originating from *D. echinocacti* samples were 99% identical to the unclassified symbiont of *D. echinocacti* (Flavobacteria). Those originating from *P. diaspidis* samples were 98–99% identical to *Cardinium* (Sphingobacteria), and samples from *Aphytis* sp. were 98% identical to *Wolbachia* (α-Proteobacteria). Samples from *M. leopardina* showed identity to three different bacterial genera, *Sphingobacterium* (Bacteroidetes), *Bacillus* (Firmicutes) and *Asaia* (α-Proteobacteria).

3.2. Localization of Cardinium in P. diaspidis

FISH analysis showed that the signal specific to *Cardinium* is concentrated only in the ovaries of adult wasps (Fig. 1). Within the ovaries, *Cardinium* is located inside the follicle cells with a high concentration in the germarium, and a lower concentration in the mature oocytes (Fig. 2). None of the negative controls produced similar signals.

4. Discussion

Our results do not support the transfer of symbionts between parasitoids and their hosts as the mechanism for horizontal transmission of arthropod symbionts. Of the four species comprising the scale-parasitoid complex that we studied, only *P. diaspidis* was found to carry *Cardinium*, while its host, *D. echinocacti* carried an unclassified symbiont, and the other primary parasitoid, *Aphytis* sp. carried *Wolbachia*. Although previous studies reported the presence of *Cardinium* and *Wolbachia* in some species of the genus *Marietta* (Weeks et al., 2003; Zchori-Fein and Perlman, 2004), none of the bacteria found in *M. leopardina* during the present study (*Sphingobacterium*, *Bacillus* and *Asaia*) is known as an insect symbiont and none showed a stable infection. These species may thus represent environmental bacteria, probably originating from the wasp's gut. Because *M. leopardina* is a polyphagous hyperparasitoid that attacks both *Aphytis* species and *P. diaspidis*, the presence of *Wolbachia*, *Cardinium* and perhaps the other bacteria may reflect the particular bacteria found in the host attacked by the hyperparasitoid.

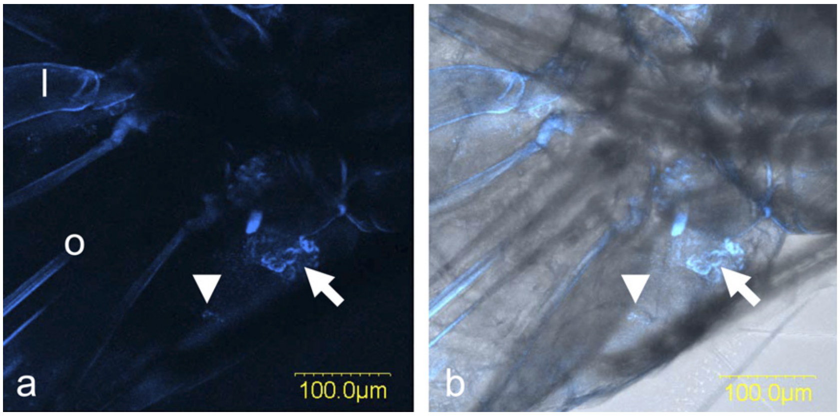

Fig. 1. FISH of *P. diaspidis* female. A specific signal for *Cardinium* is found in the germarium area (arrow), and the mature oocyte (arrow head). (a) fluorescence signal; (b) combined bright field and fluorescence. Autofluorescence of chitin is notable in the legs (l) and ovipositor (o).

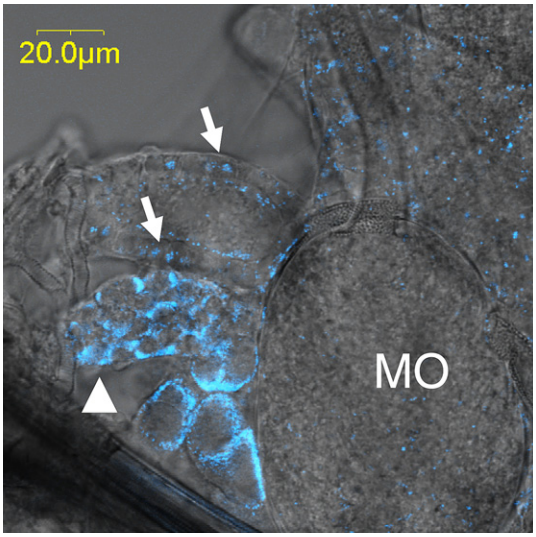

Fig. 2. FISH of *P. diaspidis* ovary. An optical section (1 μm) representing *Cardinium* distribution in the ovary. Note the concentration of *Cardinium* in the follicle cells (arrow), the germarium (arrow head), and the mature oocyte (MO).

The possibility that *Cardinium* is involved in the parthenogenetic reproduction of *P. diaspidis* (Zchori-Fein and Perlman, 2004), is supported by the current data, which demonstrate that *P. diaspidis* does not host any major symbiotic bacterium other than *Cardinium*. Our results place the arthropod genus *Plagiomerus* on the list of *Cardinium* hosts for which the symbiont is implicated in parthenogenesis, which has up to now included only the genera *Encarsia* and *Aspediotus*. Screening of different species of the genus, as well as other genera in the family Encyrtidae, is required in order to establish the prevalence of this association.

Fluorescence *in situ* hybridization reveals the presence of *Cardinium* specifically in the ovaries of *P. diaspidis*, where the bacteria are aggregated mainly inside the follicle cells (Figs. 1 and 2). This observation is in agreement with previous findings, in which parthenogenesis-inducing *Cardinium* was detected mainly in the follicle and nurse cells, and to a lesser extent in the oocytes of two *Encarsia* species (*E. pergandiella* and *E. hispida*) observed under an electron microscope (Zchori-Fein et al., 2001; Zchori-Fein et al., 2004). Similar studies on two close relatives of *Cardinium* revealed a distribution pattern that is not restricted to the reproductive tissues. In the first, Marzorati et al. (2005) showed that although *Cardinium* is present in high numbers in the gonads of its *Scaphoideus titanus* (Cicadellidae) host, it could also be detected in the fat body and salivary glands of both males and females. In the second study, the recently described *Paenicardinium endonii*, a symbiont of the nematode *Heterodera glycines*, was observed in tissues such as the pseudocoelom and intestine, in addition to its presence in the reproductive tissues (Noel and Atibalentja, 2006). The role *Cardinium* and *Paenicardinium* play in these host's biology is not yet clear, and further study is required in order to establish a possible connection between the symbiont's distribution pattern and its phenotype.

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