Combination of Genomic and Proteomic Approaches to Characterize the Symbiotic Population of the Banana Aphid (Hemiptera: Aphididae)

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ABSTRACT  Aphids are known to live in symbiosis with specific bacteria called endosymbionts that have positive or negative impacts on their hosts. In this study, six banana aphid (Pentalonia nigronervosa Coquerel) strains from various geographical origins (Gabon, Madagascar, and Burundi) were screened to determine their symbiotic content, using complementary genomic (16S rDNA sequencing and specific polymerase chain reaction) and proteomic (two-dimensional difference gel electrophoresis coupled with protein identification by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry) approaches. Despite the geographical heterogeneity, the combined methods allowed us to identify the same two symbionts in the six aphids strains tested: Buchnera aphidicola and Wolbachia. Although B. aphidicola is found in almost all aphid species, the systematic presence of Wolbachia in banana aphids is particularly interesting, as this bacterium usually has a low prevalence in aphid species. Phylogenetic analyses showed that the Wolbachia sp. strain found in P. nigronervosa was very similar to the strain present in aphids of the genus Cinara, known to have developed a strong and long-term symbiotic association with Wolbachia. The high level of asexual reproduction in P. nigronervosa could be linked to the presence of Wolbachia, but its prevalence also suggests that this symbiotic bacterium could play a more essential role in its aphid host.

KEY WORDS  banana aphid, symbiosis, Buchnera aphidicola, Wolbachia

Many insects are known to live symbiotically with intracellular microorganisms that influence their development (Braendle et al. 2003), nutrition (Douglas 1998), reproduction and speciation (Thompson 1987, Simon et al. 2011), and protection and immunity (Teixeira et al. 2008). Aphids (Hemiptera: Aphidoidea), in particular, harbor several bacterial endosymbionts that are usually classified as obligate (primary) or facultative (secondary) symbionts. Primary symbionts are very similar, whereas secondary symbionts can vary in shape and structure (Abbot and Moran 2002).

Buchnera aphidicola is an obligate symbiont present in almost all aphid species (Buchner 1965, Baumann et al. 1995). Exceptions are species from the tribe Ceratiphidini that harbor extracellular yeast-like symbionts of the phylum Ascomycotina instead of B. aphidicola. This fact is sometimes interpreted as evidence of symbiont replacement (Fukatsu and Ishikawa 1996).

Buchnera provides aphids with essential amino acids that are not available in the plant phloem. It is therefore essential for the insect to survive (Baumann et al. 1995, Douglas 1998). Reciprocally, the bacterium cannot survive more than a few hours outside its host (Baumann and Moran 1997). This co-evolution between aphids and Buchnera has apparently led to the deletion of several genes that were not essential for a symbiotic lifestyle (Gil et al. 2002).

These symbiotic bacteria are located in specialized cells called bacterioocytes or mycetocytes that are aggregated in organs called bacteriomes or mycetomes. They are maternally transmitted by colonization of the developing eggs and embryos at an early stage, which could explain this symbiont distribution (Gómez-Valero et al. 2004).

In addition to Buchnera, aphids can harbor secondary symbionts (Tsuchida et al. 2005). These bacteria are not required for survival but they have various effects, either positive or negative, on their insect hosts: protection against high temperatures and natural enemies (Oliver et al. 2003), loss of fecundity and cytoplasmic incompatibility (Clancy and Hoffmann 1996, Fukatsu et al. 2001), fitness effects (Chen et al. 2000), host plant utilization (Tsuchida et al. 2004), and body color changes (Tsuchida et al. 2010). Secondary symbionts can differ among host species, and also

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among individual hosts within species (Oliver et al. 2010).

Until recently, eight secondary symbionts have been described in aphids: *Serratia symbiotica*, also referred to as *PASS* for pea aphid secondary symbiont, *R*-type symbiont or *S*-symbiont (Koga et al. 2003); *Hamiltonella defensa*, also named *PABS* for pea aphid Bemisia-type symbiont (Darby et al. 2001); *Rickettsia* or *PAR* for pea aphid *Rickettsia* (Chen et al. 1996); *Spiroplasma* sp (Fukatsu et al. 2001); *Wolbachia* (Gómez-Valero et al. 2004); *Arsenophonus*; *Regiella insecticola* (Chen and Purcell 1997); and, more recently, a facultative symbiont of the genus *Rickettsiella* (Tsuchida et al. 2010).

Among these, *Wolbachia* is particularly interesting, as it can distort the reproducing capabilities of its host. *Wolbachia* has rarely been found in aphids (Werren et al. 1995), even if it is commonly found in insects (at least in 60% of arthropod species). This alphaproteobacterium is mainly maternally transmitted, but cases of horizontal transfer have been reported (O’Neill et al. 1992).

The banana aphid *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae) is the main vector of the most serious viral banana disease worldwide (Thomas and Iskra-Caruana 2000). Despite the negative impact of *P. nigronervosa* on banana production, its symbiotic population has never been investigated. The objective of this study is to gain robust information about the symbiotic populations in several *P. nigronervosa* strains from various geographical origins by combining three independent protocols. This work could provide some interesting prospects about aphid—plant—virus interactions, especially as some symbiont proteins are believed to interact with virus particles in the case of persistent viruses transmitted by aphids. These data could prove useful for the development of an efficient control method against BBTV.

### Materials and Methods

**Insects.** Aphids were maintained at 25°C in a long-day photoperiod (16-h light periods) on tissue-cultured banana plantlets of the Williams variety. Plants were replaced when they outgrew the boxes or when they were covered with honeydew. All aphid strains were reared in isolated growth chambers. Table 1 shows the different *P. nigronervosa* strains used in this study.

#### Table 1. *P. nigronervosa* strains used in this study

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Country of origin</th>
<th>City of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Burundi</td>
<td>Bujumbura (Center)</td>
</tr>
<tr>
<td>2</td>
<td>Burundi</td>
<td>Bujumbura (Isale)</td>
</tr>
<tr>
<td>3</td>
<td>Burundi</td>
<td>Bujumbura (Gbitoke)</td>
</tr>
<tr>
<td>4</td>
<td>Gabon</td>
<td>Libreville</td>
</tr>
<tr>
<td>5</td>
<td>Madagascar</td>
<td>Arivoninamano</td>
</tr>
<tr>
<td>6</td>
<td>Madagascar</td>
<td>Ambodiafantsy</td>
</tr>
</tbody>
</table>

**Genomic Approach.** DNA Extraction. Adult aphids were soaked in 100% ethanol for 1 min to eliminate confounding bacteria present on their surface before total DNA extraction using the Qiagen DNeasy blood and tissue kit (QUIGEN, Chatsworth, CA). DNA quality was evaluated using a NanoDrop spectrophotometer (NanoDrop ND-1000 Spectrophotometer, NanoDrop Technologies, Wilmington, DE). Two ratios of absorbance were measured: 260/280 nm and 260/230 nm. DNA was considered to be of good quality and purity when those ratios were around 1.8 for 260/280 and in the range of 2.0–2.2 for 260/230.

**16S rDNA Amplification and Sequencing.** This experiment was first conducted with *P. nigronervosa* strain 2 (Table 1), from Bujumbura (Isale), to screen symbiotic populations without any preconceived idea or bias linked to the use of specific primers. After DNA extraction from a single aphid, almost the entire length of bacterial 16S rDNA (≈1.5 kb) was amplified by polymerase chain reaction (PCR) using primers 16SA1 (5’-AGAGTTTGATCMTGGCTCAG-3’) and 16SB1 (5’-TACCGYTACCCGTGACTTC-3’) following the temperature program described by Fukatsu and Nikoh (1999). PCR amplifications were performed in 50 μl reactions containing 2 μl of DNA, 5 μl of 10X reaction buffer (Bioline), 5 μl deoxy nucleotide triphosphate mixture (25 mM each), 1.5 μl of MgCl2 (50 mM) 1 μl of each primer (25 μM), 0.2 μl of Taq polymerase (Hybripol, 1 U/μl), and 34.3 μl of water.

To determine if several sequences from different microorganisms could be in the amplified DNA, PCR products with the correct size were cloned with the TOPO TA-cloning kit and the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA), using TOP10 competent cells and following the manufacturer’s instructions. The length of the inserted fragment was checked, and colonies of interest were cultured in 1.5 ml of Luria-Bertani medium supplemented with ampicillin and subjected to a classical plasmid extraction protocol (Sambrook and Russel 2006).

Products were then digested with the four-base recognizing restriction endonuclease *HaeIII* (New England BioLabs, Ipswich, MA) and electrophoresed in agarose gels to type the cloned 16S rDNA.

A dye terminator-labeled cycle sequencing reaction was performed on the purified plasmids with sequencing primers 16SA1 and EUB1405R (5’-AGAGTTTGATCMTGGCTCAG-3’) following the temperature program described by Katwa et al. (2010). The reaction products were analyzed with a 3130 Genetic Analyzer (Applied Biosystems, Carlsbad, CA).

The resulting sequences were cleaned from plasmid and primer sequences, Contigs were made using ATGC sequence assembly software (GENETYX Corporation, Tokyo, Japan) and were further compared with databases using blastn. The complete 16S rDNA sequences generated in this study were recorded in GenBank under accession numbers KC522606 for *Wolbachia* and KC638397 for *B. aphidicola*.

The experiment was completed by the analysis of the V3 region of the 16S rDNA for all the aphid strains described in Table 1, using universal primers F 357...
The amplified PCR products were cloned using the Fermentas CloneJET PCR cloning kit (St. Leon-Rot, Germany). Plasmid DNA was then purified using the Fermentas GeneJET Plasmid Miniprep kit and sent to Macrogen (Seoul, Korea) for sequencing.

To confirm the results obtained from 16S rDNA characterization, a targeted detection of symbionts was made for all the aphid strains listed in Table 1, using the specific primers listed in Table 2 and the thermal cycle described by Tsuchida et al. (2002). Positive controls in all PCR reactions. A negative control was also made, using water instead of DNA.

Symbiont Detection by Targeted PCR. To confirm the results obtained from 16S rDNA characterization, a targeted detection of symbionts was made for all the aphid strains listed in Table 1, using the specific primers listed in Table 2 and the thermal cycle described by Tsuchida et al. (2002).

To avoid false-positive and false-negative results, genomic DNA samples from several *Acyrthosiphon pisum* (Hemiptera: Aphididae) strains known to harbor specific symbionts (obtained from T. Tsuchida, Toyama University, Japan) were used as positive controls in all PCR reactions. A negative control was also made, using water instead of DNA. Diagnostics PCR were made on 20 individuals for each aphid strain.

Phylogenetic Analyses. Phylogenetic trees were constructed using MEGA 5 software (Tamura et al. 2011), and all the alignments were done using Muscle (Edgar 2004). Neighbor-joining trees were created using MEGA 5 software (Tamura et al. 2011), and all the alignments were done using Muscle (Edgar 2004).

Results

Genomic Approach. Almost the entire eubacterial 16S rDNA from the symbionts of one Burundian strain of *P. nigronervosa* (strain 2, from Bujumbura [Isale]) was amplified, cloned, and sequenced. Analysis of the cloned fragments revealed nine sequence types that could be associated to two different bacterial species.

The generated contig sequence associated to the first organism was 1,449-bp-long and showed 96% sequence similarity with the 16S rDNA sequences of the same strain, using a Percoll gradient according to the protocol of Charles and Ishikawa (1999).

Analytical Two-Dimensional Gel Electrophoresis. We used the protocol described by Francis et al. (2010), with slight modifications. Protein quantification was carried out using the RCDC quantification kit from Bio-Rad (Hercules, CA). The protein extracts were then labeled with three different CyDyes (GE Healthcare Europe GmbH, Freiburg, Germany): Cy2 (blue, 488 nm), Cy3 (green, 532 nm), and Cy5 (red, 633 nm). The labeling was done by mixing 12.5 μg of each protein sample with the corresponding CyDye for 30 min. The labeling was then stopped by adding 10 mM lysine.

The samples corresponding to the different treatment groups were: a total protein extraction from *P. nigronervosa*, a symbiont protein extraction of *P. nigronervosa*, and a total protein extraction of an A. pisum clone with a unique endosymbiont (*B. aphidicola*). The three samples were analyzed per pair and all combinations were tested. For each gel, samples were labeled either with Cy3 or Cy5 and mixed with an internal reference standard protein mixture (pooled from equal aliquots from all the experimental samples) labeled with Cy2. Protein two-dimensional electrophoresis and picking and identification by mass spectrometry were performed as described by Francis et al. (2010).

Table 2. Specific primers used in this study

<table>
<thead>
<tr>
<th>Target symbiont</th>
<th>Target gene</th>
<th>Primer name</th>
<th>Primer sequence (5’-3’)</th>
<th>Product size (kb)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buchnera</td>
<td>16SrDNA</td>
<td>16SA1</td>
<td>AGACTTTGATCMTGCGCTCAG</td>
<td>0.321</td>
<td>Fukatsu and Nikoh 1998</td>
</tr>
<tr>
<td>PASS</td>
<td>16SrDNA</td>
<td>16SA1</td>
<td>AGACTTTGATCMTGCGCTCAG</td>
<td>0.48</td>
<td>Fukatsu and Nikoh 1998</td>
</tr>
<tr>
<td>PAUS</td>
<td>16SrDNA</td>
<td>16SA1</td>
<td>AGACTTTGATCMTGCGCTCAG</td>
<td>0.2</td>
<td>Sandström et al. 2001</td>
</tr>
<tr>
<td>PABS</td>
<td>16SrDNA</td>
<td>PABS</td>
<td>TACCGGATCCTGCGCTCAG</td>
<td>1.66</td>
<td>Darby et al. 2001</td>
</tr>
<tr>
<td>Rickettsia</td>
<td>16SrDNA</td>
<td>16SA1</td>
<td>AGACTTTGATCMTGCGCTCAG</td>
<td>0.2</td>
<td>Fukatsu and Nikoh 1998</td>
</tr>
<tr>
<td>Rickettsiella</td>
<td>16SrDNA</td>
<td>16SA1</td>
<td>AGACTTTGATCMTGCGCTCAG</td>
<td>0.26</td>
<td>Tsuchida et al. 2010</td>
</tr>
<tr>
<td>Spiroplasma</td>
<td>16SrDNA</td>
<td>16SA1</td>
<td>AGACTTTGATCMTGCGCTCAG</td>
<td>0.51</td>
<td>Fukatsu and Nikoh 1998</td>
</tr>
<tr>
<td>Wobacula</td>
<td>16SrDNA</td>
<td>16SA1</td>
<td>AGACTTTGATCMTGCGCTCAG</td>
<td>0.45</td>
<td>Fukatsu and Nikoh 1998</td>
</tr>
<tr>
<td>Arsenophonus sp</td>
<td>16SrDNA</td>
<td>16SA1</td>
<td>AGACTTTGATCMTGCGCTCAG</td>
<td>0.96</td>
<td>Tsuchida et al. 2002</td>
</tr>
</tbody>
</table>

(5’-CCTACGCGACGCGACCAGCAGCAG-3’) and R 538 (5’-ATTACCCGGGCCTGCTGCTG-3’) (Muyzer et al. 1993).
B. aphidicola of the aphids Uroleucon ambrosiae Thomas (Hemiptera: Aphididae), *A. pisum*, and *Uroleucon sonchi* L. (Hemiptera: Aphididae).

The sequence associated to the second organism (1,425 bp) was 97% similar to the 16S rDNA sequences of Wolbachia symbionts isolated from various insect species, including three aphid species. Phylogenetic analyses further confirmed these results and the identity of the detected symbiont (Fig. 1). The constructed tree showed that the 16S rDNA sequence corresponding to Wolbachia from *P. nigronervosa* clustered with Wolbachia sequences from other aphid species, including Cinara cedri Mineur (Hemiptera: Aphididae), Cinara p inne (Hemiptera: Aphididae), Cinara p inne (Hemiptera: Aphididae), and Aphis nerii Boyer de Fonscolombe (Hemiptera: Aphididae).

In addition, the V3 region of the bacterial 16S rDNA was amplified for five other *P. nigronervosa* strains. Amplification repeatedly yielded a heterogeneous amplification product of 200 bp. Ninety-six colonies were randomly selected for each aphid strain after cloning. Sequencing of the purified products revealed the presence of *B. aphidicola* and Wolbachia in all aphid strains.

Specific PCR tests made with the primer pairs 16SA1/ApiP2 and 16SA1/W2 yielded amplification products of 321 and 450 bp, respectively, confirming the presence of *B. aphidicola* and Wolbachia in all the collected *P. nigronervosa* strains.

None of the other primers listed in Table 2 produced PCR amplification products, suggesting the absence of other symbionts.
Proteomic Approach. We used the two-dimensional difference gel electrophoresis method, which only requires a single gel to reproducibly detect differences between fluorescently tagged protein samples (Ullu et al. 1997). This technique allowed us to identify six proteins belonging to *B. aphidicola* and two proteins belonging to *Wolbachia*, confirming their presence in the banana aphids we sampled (Table 3).

In particular, we detected a GroEL protein and an OmpF-like protein, both known to be abundant in *B. aphidicola*, and an ankyrin repeat domain protein that is specific to *Wolbachia*.

### Discussion

Symbiotic populations hosted by aphids can be very diverse and complex. New facultative symbionts are regularly described (Guay et al. 2009, Tsuchida et al. 2010) and novel effects of endosymbionts on host physiology are still unraveled (Hosokawa et al. 2010). Additional undiscovered associations between aphids and bacterial symbionts are therefore quite likely to exist (Russell and Moran 2006). The necessity to couple several methods for studying symbiosis and its relevance has already been highlighted (Fukatsu and Nikoh 1998). The endosymbiotic microbiota is indeed complex, with multiple symbionts coexisting inside one insect body, and this can sometimes lead to artifacts when using genomics-based methods only. It is therefore useful to analyze the microbial DNA sequences obtained in connection with other methods. That is why we chose to combine genomic and proteomic approaches in this study, to get a full view of the symbiotic population in the banana aphid.

Through the combination of those two methods, two bacterial symbionts were identified of the six banana aphid strains we screened: *B. aphidicola* and *Wolbachia*.

Surprisingly, and despite geographical heterogeneity, there was no difference in symbiont composition between strains. It is indeed usual to detect differences in secondary symbiont occurrences between aphids of the same species, especially when host plants and geographical origins vary (Oliver et al. 2010). However, examples of stable associations exist but remain rare (Gómez-Valero et al. 2004).

The coexistence of two symbionts in the banana aphid is not remarkable by itself, as multiple endosymbioses is known to occur in aphids (Tsuchida et al. 2002, Moran and Dunbar 2006): up to half of aphid species may contain a secondary symbiont (Chen et al. 1996). However, co-infections are often unstable (Moran and Dunbar 2006), because the coexistence of multiple symbionts can be costly for the host (lower growth and fecundity rates).

*B. aphidicola* has been detected in almost all the aphid species tested so far in the literature, and found to be essential for their normal development and reproduction. We confirmed its presence in the banana aphid by 16S rDNA sequencing, targeted PCR, and a proteomic approach. Interestingly, a GroEL protein from *B. aphidicola* was identified. This molecular chaperone is considered as the most abundant protein from *B. aphidicola* and an ankyrin repeat domain protein that is specific to *Wolbachia*.

**Table 3. Symbiont proteins identified in this study**

<table>
<thead>
<tr>
<th>MW</th>
<th>pI</th>
<th>Score</th>
<th>Coverage</th>
<th>Peptide no.</th>
<th>Protein identification</th>
<th>Accession</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>42,729</td>
<td>5.55</td>
<td>206</td>
<td>56%</td>
<td>16</td>
<td>OmpF-like porin</td>
<td>NP_240177</td>
<td>Buchnera aphidicola</td>
</tr>
<tr>
<td>57,988</td>
<td>5.14</td>
<td>225</td>
<td>59%</td>
<td>35</td>
<td>Chaperonin GroEL</td>
<td>YP_002468346</td>
<td>Buchnera aphidicola</td>
</tr>
<tr>
<td>48,470</td>
<td>6.23</td>
<td>50</td>
<td>15%</td>
<td>6</td>
<td>MesJ protein</td>
<td>YP_005619666</td>
<td>Buchnera aphidicola</td>
</tr>
<tr>
<td>59,662</td>
<td>9</td>
<td>58</td>
<td>20%</td>
<td>13</td>
<td>Anthranilate synthetase</td>
<td>AAR99727.1</td>
<td>Buchnera aphidicola</td>
</tr>
<tr>
<td>129,552</td>
<td>5.88</td>
<td>64</td>
<td>8%</td>
<td>9</td>
<td>Rec-C</td>
<td>YP_0025361.1</td>
<td>Buchnera aphidicola</td>
</tr>
<tr>
<td>65,260</td>
<td>9.14</td>
<td>56</td>
<td>25%</td>
<td>14</td>
<td>Sulfitreductase subunit beta</td>
<td>YP_005619933.1</td>
<td>Buchnera aphidicola</td>
</tr>
<tr>
<td>78,763</td>
<td>8.81</td>
<td>72</td>
<td>15%</td>
<td>12</td>
<td>Ankyrin repeat domain</td>
<td>WP_010405977.1</td>
<td>Wolbachia</td>
</tr>
<tr>
<td>45,578</td>
<td>8.21</td>
<td>84</td>
<td>27%</td>
<td>10</td>
<td>Fic family protein</td>
<td>NP_367022</td>
<td>Wolbachia</td>
</tr>
</tbody>
</table>

MW, molecular weight; pI, isoelectric point; Score, Mowse score according to Mascot research (Pappin et al. 1993); Coverage, percentage of the protein sequence identified; Peptide no., number of peptide hits for each protein; Accession, accession number on NCBI. Organism: related organism for protein identification.
fact that a nearly systematic occurrence of *Wolbachia* was recorded in another aphid species of the genus *Pentalonia* (Pentalonia caladii [Hemiptera: Aphididae]) in Hawaii (Jones et al. 2011).

The presence of *Wolbachia* in the banana aphid was confirmed by the detection of an ankyrin repeat domain protein. While these domains are relatively rare in bacteria, the *Wolbachia* genome is however characterized by an unusually large number of genes encoding for proteins containing ankyrin repeat domains (Iturbe-Ormaetxe et al. 2005) that are proposed to play a functional role in symbiont biology and in host–symbiont interactions (Wu et al. 2004, Iturbe-Ormaetxe et al. 2005). In particular, genes encoding proteins with ankyrin repeat domains are believed to be linked to the cytoplasmic incompatibility phenotype (Tram et al. 2003, Iturbe-Ormaetxe et al. 2005).

The phylogenetic tree based on the 16S rDNA (Fig. 1) shows banana aphid *Wolbachia* among other *Wolbachia* strains hosted in various insect and nematode species. When constructing this tree, we were confronted with nodes with low bootstrap values. Classifying *Wolbachia* strains in invertebrates was already considered as a hard task by several authors (Fenn et al. 2006, Augustinos et al. 2011). Indeed, horizontal transfer events of *Wolbachia* genome fragments are known to occur and to make phylogenetic analysis more complicated, as nuclear gene copies would evolve in a different way than cytoplasmic copies of *Wolbachia* genes.

Regarding our tree, we can nevertheless see that the *Wolbachia* strain recorded in *P. nigronervosa* is very similar to the strains in aphid species of the genus Cinara. This is interesting because several studies have confirmed the prevalence of *Wolbachia* in *Cinara* species of different origins, also suggesting a strong and long-term symbiotic association with it (Gómez-Valero et al. 2004, Wang et al. 2009).

The *Wolbachia* sp. found in the banana aphid is also close to the one found in phytophagous spider mites of the genus Bryobia (Acari: Tetranychidae). Both these mites and aphids of the genus Cinara are characterized by a high level of asexual reproduction, and this phenomenon is believed to be directly linked to the presence of *Wolbachia* (Gómez-Valero et al. 2004, Ros et al. 2008). The capacity of *Wolbachia* to induce parthenogenesis, cytoplasmic incompatibility, and feminization has indeed been well studied in various insect species (Rousset et al. 1992, Stouthamer et al. 1993, Clancy and Hoffmann 1996) and is believed to promote bacterial spread within a species.

Similarly to Bryobia and Cinara, *P. nigronervosa* is characterized by parthenogenetic reproduction; hardly any sexual morphs are observed (Footitt et al. 2010). This could be the consequence of a stable infection by *Wolbachia*. Nevertheless, complete asexuality is rare in the animal kingdom (Burt 2000) and asexuals are considered to have limited adaptive potential (Ros et al. 2008). Bacterial infection could however play a role in the adaptive success of asexual species. An important presence and a stable infection of the banana aphid by *Wolbachia* could thus be explained if the bacterium has positive effects on the banana aphid. This hypothesis is strengthened by the fact that superinfections in insects are likely to be maintained when hosts receive more benefits than costs from symbiosis (Oliver et al. 2006).

Potential beneficial roles of *Wolbachia* have been broadly investigated these last years. Studies have been made on mosquitoes (*Diptera: Culicidae*) and Drosophila (*Diptera: Drosophilidae*) evidencing that *Wolbachia* could manipulate the host antioxidant system in a manner that is beneficial to host survival (Brennan et al. 2008, Brownlie et al. 2009). The bacterium was also shown to improve its host fitness in certain conditions (Dobson et al. 2002, Weeks et al. 2007).

*Wolbachia* was shown to play a nutritional role in Drosophila, but also in the bedbug *Cimex lectularius* L. (Hemiptera: Cimicidae), in which it was identified to be associated to bacteriocytes (Brownlie et al. 2009, Hosokawa et al. 2010). Regarding its prevalence, a possible role of *Wolbachia* in the banana aphid could be, similarly to *Buchnera*, to supply essential nutrients and vitamins to its host or to protect it from plant-produced defense molecules. *P. nigronervosa* effectively feed on banana phloem, which is rich in phenolic compounds (Pothavorn et al. 2010) known to have negative effects on aphids in various host plants (Goławska 2010). Arthropod *Wolbachia* are mostly known to be parasites, but the possibility of a more mutualistic role in insects could not be excluded (Fukatsu et al. 2001, Weeks et al. 2007).

This study identified *B. aphidicola* and *Wolbachia* in six strains of banana aphids from various origins. Combining proteomic and genomics methods allowed us to get a thorough view of the symbiont population of *P. nigronervosa* for the first time. Our results suggest that a strong link exists between *P. nigronervosa* and *Wolbachia*, and this long-term relationship could explain the anholocyclic status of the aphid species. A possible role of *Wolbachia* in banana aphid nutrient supply cannot be excluded and will deserve further investigation.

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