

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Bacterial diversity shift determined by different diets in the gut of the spotted wing fly *Drosophila suzukii* is primarily reflected on acetic acid bacteria.**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1634704> since 2017-11-23T16:16:59Z

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

**This is the author's final version of the contribution published as:**

VACCHINI V., GONELLA E., CROTTI E., PROSDOCIMI E., MAZZETTO F., CHOUAIA B., CALLEGARI M., MAPELLI F., MANDRIOLI M., ALMA A., DAFFONCHIO D. – Bacterial diversity shift determined by different diets in the gut of the spotted wing fly *Drosophila suzukii* is primarily reflected on acetic acid bacteria. *Environmental Microbiology Reports* 9(2), 2017, 91–103.

**The publisher's version is available at:**

<http://onlinelibrary.wiley.com/doi/10.1111/1758-2229.12505/full>

**When citing, please refer to the published version.**



**Bacterial diversity shift determined by different diets in the gut of the spotted wing fly *Drosophila suzukii* is primarily reflected on acetic acid bacteria**

Journal:	<i>Environmental Microbiology and Environmental Microbiology Reports</i>
Manuscript ID	Draft
Journal:	Environmental Microbiology Reports
Manuscript Type:	EMIR - Brief report
Date Submitted by the Author:	n/a
Complete List of Authors:	Daffonchio, Daniele; King Abdullah University of Science and Technology, Biological and Environmental Sciences & Engineering Crotti, Elena; University of Milan, DEFENS
Keywords:	cultivation-dependent approach, fluorescent in situ hybridization (FISH), symbionts, green fluorescent protein, 16S rRNA gene pyrosequencing

SCHOLARONE™  
Manuscripts

1 **Bacterial diversity shift determined by different diets in the gut of the spotted wing fly *Drosophila***  
2 ***suzukii* is primarily reflected on acetic acid bacteria**

3

4 Violetta Vacchini<sup>1#</sup>, Elena Gonella<sup>2#</sup>, Elena Crotti<sup>1#</sup>, Erica M. Prosdocimi<sup>1°</sup>, Fabio Mazzetto<sup>2</sup>, Bessem  
5 Chouaia<sup>1§</sup>, Matteo Callegari<sup>1</sup>, Francesca Mapelli<sup>1</sup>, Mauro Mandrioli<sup>3</sup>, Alberto Alma<sup>2</sup> and Daniele  
6 Daffonchio<sup>1,4\*</sup>

7

8 <sup>1</sup>Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Università degli Studi  
9 di Milano, Milano, Italy

10 <sup>2</sup>Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), Università degli Studi di Torino,  
11 Grugliasco, Italy

12 <sup>3</sup>Dipartimento di Scienze della Vita (DSV), Università degli Studi di Modena e Reggio Emilia, Modena,  
13 Italy

14 <sup>4</sup>Biological and Environmental Sciences and Engineering Division (BESE), King Abdullah University  
15 of Science and Technology (KAUST), Thuwal 23955-6900, Kingdom of Saudi Arabia.

16 <sup>#</sup>These authors have equally contributed to the work

17 <sup>°</sup>Current address: Blizard Institute, Queen Mary University of London, United Kingdom

18 <sup>§</sup>Current address: Department of Entomology, 5142 Comstock Hall, Cornell University, Ithaca, NY,  
19 14853, United States of America

20 <sup>\*</sup>Corresponding Author: Daniele Daffonchio, BESE, Biological and Environmental Sciences and  
21 Engineering Division, King Abdullah University of Science and Technology (KAUST), Building 2,  
22 Level 3, Room 3236, Thuwal 23955-6900, Kingdom of Saudi Arabia; Tel: +966 (2) 8082884; Email:  
23 daniele.daffonchio@kaust.edu.sa.

24

25 **Running title:** Acetic Acid Bacteria of *Drosophila suzukii*

26

## 27 Abstract

28 The pivotal role of diet in shaping gut microbiota has been evaluated in different animal models,  
29 including insects. *Drosophila* flies harbour an inconstant microbiota among which acetic acid bacteria  
30 (AAB) are important components. Here, we investigated the bacterial and AAB components of the  
31 invasive pest *Drosophila suzukii* microbiota, by studying the same insect population separately grown  
32 on fruit-based or non-fruit artificial diet. AAB were highly prevalent in the gut under both diets (90 and  
33 92% infection rates with fruits and artificial diet, respectively). Fluorescent *in situ* hybridization and  
34 recolonization experiments with green fluorescent protein (Gfp)-labelled strains showed AAB capability  
35 to massively colonize insect gut. High-throughput sequencing on 16S rRNA gene indicated that the  
36 bacterial microbiota of guts fed with the two diets clustered separately. By excluding AAB-related  
37 OTUs from the analysis, insect bacterial communities did not cluster separately according to the diet,  
38 suggesting that diet-based diversification of the community is primarily reflected on the AAB  
39 component of the community. Diet influenced also AAB alpha-diversity, with separate OTU  
40 distributions based on diets. High prevalence, localization and massive recolonization, together with  
41 AAB clustering behaviour in relation to diet, suggest an AAB role in the *D. suzukii* gut response to diet  
42 modification.

43

## 44 Keywords

45 16S rRNA gene pyrosequencing, cultivation-dependent approach, fluorescent *in situ* hybridization  
46 (FISH), symbionts, green fluorescent protein

47

48 **INTRODUCTION**

49 The insect gut microbiota plays very critical and essential roles for the host biology, physiology and  
50 immunity (Hamdi *et al.*, 2011). Diet, together with other factors, such as environmental habitat, host  
51 developmental stage and phylogeny, profoundly affect its diversity and structure, consequently  
52 influencing insect functionality (Colman *et al.*, 2012; Yun *et al.*, 2014).

53 In last years, increased attention has been focused on the study of the bacterial microbiota associated to  
54 different species of drosophilid flies. *Drosophila* represents a powerful insect model for a vast array of  
55 studies, including the defence mechanism-based investigations and the exploration of host-commensal  
56 interactions (Erkosar *et al.*, 2013; Lee and Lee, 2014). With the aim to unravel host-microbiome  
57 interactions beyond laboratory boundaries, researchers have been prompted to investigate the gut  
58 microbiota diversity of different natural species of drosophilid flies (Chandler *et al.*, 2011; Wong *et al.*,  
59 2013; Cox and Gilmore, 2007). By using molecular techniques four bacterial families have been found  
60 to be commonly associated to field-captured or laboratory-reared flies, namely Enterobacteriaceae,  
61 Acetobacteraceae, Lactobacillaceae and Enterococcaceae (Brummel *et al.*, 2004, Chandler *et al.*, 2011,  
62 Corby-Harris *et al.*, 2007, Cox and Gilmore, 2007, Ren *et al.*, 2007, Ridley *et al.*, 2012, Ryu *et al.*, 2008,  
63 Sharon *et al.*, 2010, Storelli *et al.*, 2011, Wong *et al.*, 2011; Wong *et al.*, 2013). In particular,  
64 Acetobacteraceae (acetic acid bacteria, AAB) are among the dominant taxa in laboratory-reared *D.*  
65 *melanogaster* (Ryu *et al.*, 2008; Wong *et al.*, 2011). Conversely, field-captured *Drosophila* flies show  
66 an inconstant bacterial community, where AAB are, however, frequently associated (Wong *et al.*, 2013).  
67 AAB are a bacterial group widespread in sugar- and ethanol-rich matrices, such as flowers' nectar,  
68 fruits, vegetables and fermented matrices, all niches shared by drosophilid flies and from which they can  
69 pass to the *Drosophila* gut, a sugar- and ethanol-rich environment (Blum *et al.*, 2013; Cox and Gilmore,  
70 2007 Crotti *et al.*, 2010). AAB establish a delicate interaction with the insect innate immune system,  
71 being involved in the suppression of the growth of pathogenic bacteria in healthy individuals (Ryu *et al.*,  
72 2008), but also the modulation of the insulin pathway and the enhancement of the larval developmental

73 rate, body size, intestinal stem cells activity and energy metabolism (Shin *et al.*, 2011). A beneficial role  
74 of AAB has been also demonstrated for mosquito larval development (Chouaia *et al.*, 2012; Mitraka *et*  
75 *al.*, 2013).

76 The spotted wing fly *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), an endemic pest in  
77 South-East Asia, has been accidentally introduced in USA, Canada and Europe (Cini *et al.*, 2012;  
78 Hauser, 2011; Lee *et al.*, 2011). Unlike its relatives that attack rotten fruits, *D. suzukii* lays eggs on  
79 healthy soft summer fruits where the larvae grow (Walsh *et al.*, 2011; Mitsui *et al.*, 2006). So far, little  
80 information is available on the bacterial community associated to *D. suzukii* specimens collected in USA  
81 (Chandler *et al.*, 2014), while just few other publications studied *Wolbachia* infection (Mazzetto *et al.*,  
82 2015; Cattel *et al.*, 2016; Siozios *et al.*, 2013).

83 Considering AAB abundance and importance in drosophilid flies, we aimed to assess the effect of two  
84 different diets (i.e. based or not on fruit) on the diversity of bacterial and AAB microbiota of *D. suzukii*.  
85 Specifically, we evaluated the possibility that AAB are involved in the gut microbiota diversification  
86 when insects are exposed to two different alimentary regimes. For studying the effect of diets on the  
87 bacterial microbiota diversity, we first confirmed the significance of AAB in the *D. suzukii* gut. We  
88 determined their prevalence, the gut localization through fluorescent *in situ* hybridization (FISH) and  
89 the ability to recolonize the insect gut by using green fluorescent protein (Gfp)-tagged derivatives of a  
90 series of strains from a *D. suzukii* isolate collection. As a second step of the study we assessed the  
91 changes of the bacterial microbiota structure and diversity by means of cultivation-independent  
92 techniques.

93

## 94 RESULTS

95 **Prevalence of *Wolbachia* and AAB.** Since *Wolbachia* is a frequent symbiont of drosophilid flies, the  
96 prevalence of this bacterium has been evaluated on adults obtained both from fruit and artificial diet  
97 rearings. In flies reared on fruit *Wolbachia* showed an infection rate of 66% (33 out of 50 positive

98 specimens). *Wolbachia* prevalence was significantly lower (GLM,  $p < 0.05$ ) in individuals maintained  
99 on the artificial diet (infection rate of 28%, 14/50 positives). Conversely, AAB occurred in almost all of  
100 the analysed individuals reared on both food sources, with 90 and 92% infection rates in flies  
101 maintained on fruits and artificial diet, respectively (45 and 46 out of 50 individuals) with no significant  
102 difference in infection incidence (GLM,  $p=0.727$ ).

103

104 **AAB isolation.** Since the condition of fruit-based rearing is the closest to the diet of *D. suzukii* in field  
105 conditions, we concentrated our efforts on individuals reared on this diet; however, specimens reared on  
106 artificial diet have been also included in the analysis. The final collection included 234 isolates that were  
107 de-replicated according to the ITS fingerprinting profiles. 16S rRNA gene sequencing of representatives  
108 of each ITS profile identified the isolates as belonging to *Komagataeibacter*, *Gluconacetobacter*,  
109 *Acetobacter* and *Gluconobacter* genera (Yamada *et al.*, 2012a; 2012b), while only 16.3% of the isolates  
110 did not belong to Acetobacteraceae family (Tab. 1). Twenty-eight isolates have been affiliated to the  
111 *Acetobacter* genus, including the species *A. cibinongensis*, *A. indonesiensis*, *A. orientalis*, *A.*  
112 *orleanensis*, *A. peroxydans*, *A. persici* and *A. tropicalis*. *A. persici* and *A. indonesiensis* were the most  
113 represented species. Eighteen *Gluconobacter* isolates have been affiliated to three species, *G.*  
114 *kanchanaburiensis*, *G. kondonii* and *G. oxydans*. The unique isolate of *G. kondonii* in the collection has  
115 been collected from an adult fly fed on fruits, while *G. kanchanaburiensis* isolates have been obtained  
116 from specimens reared on artificial diet. Twelve isolates collected from adults fed on fruit showed high  
117 sequence similarity with *G. oxydans*. One hundred and twenty-three isolates have been assigned to  
118 *Gluconacetobacter* and *Komagataeibacter* genera. In particular, 118 *Komagataeibacter* isolates have  
119 been obtained from fruit-fed *Drosophila*. Due to the phylogenetic proximity of the species of this genus,  
120 discrimination at the species level was not possible with the actual 16S rRNA sequencing. *Ga.*  
121 *liquefaciens* isolates (no. 4) have been obtained from three pupae and one larva using the TA1 medium.

122 Finally, the attribution to either *Gluconacetobacter* or *Komagataeibacter* genera could not be  
123 discriminated according to the actual 16S rRNA sequence (Tab. 1).

124

125 **Localization of AAB in the *D. suzukii* gut and colonization by Gfp-labelled strains.** Fluorescent *in situ*  
126 hybridization (FISH) on the insect dissected organs using the AAB-specific probe AAB455, gave  
127 positive signals in the proventriculus and the gut (Fig. 1), whereas no fluorescence was detected in the  
128 absence of probe. The proventriculus epithelium gave a strong signal, observable by merging the  
129 interferential contrast (Fig. 1c) with the fluorescent (Fig. 1b) images. Magnification in fig. 1d allowed  
130 the visualisation of fluorescent AAB microcolonies adhering to the peritrophic matrix.

131 *Gluconobacter* cells have been observed in the midgut (Fig. 1g) suggesting the distribution of this genus  
132 in the inner side of the intestinal lumen. Fig. 1e-h show *Gluconobacter* distribution (Fig. 1g) in relation  
133 to the dispersal of *Eubacteria* (Fig. 1f), indicating that it is surrounded by other bacteria, presumably  
134 AAB (Fig. 1d). However, we could not ascertain such hypothesis because all the attempts to design  
135 specific probes effective for *Acetobacter*, *Gluconacetobacter* and *Komagataeibacter* genera, failed.

136 Strains *G. oxydans* DSF1C.9A, *A. tropicalis* BYea.1.23 and *A. indonesiensis* BTa1.1.44 have been  
137 successfully transformed with a plasmid carrying the Gfp cassette. Plasmid stability experiments  
138 showed that *G. oxydans* DSF1C.9A retained the plasmid with a relatively high percentage (73.1%),  
139 while this was not the case for strains BYea.1.23 and BTa1.1.44. Thus, colonization experiments of  
140 adult flies have been performed under antibiotic (kanamycin) administration in the insect food. The Gfp-  
141 labelled strains massively recolonized the fly foregut and midgut (Fig. 2); no auto-fluorescence has been  
142 observed in control flies. *G. oxydans* DSF1C.9A successfully colonized the crop, the proventriculus and  
143 the first part of the midgut (see the magnifications in Fig. 2b and 2c). The Gfp-labelled cells are clearly  
144 restricted to the epithelium side of the proventriculus, embedded in the peritrophic matrix (Fig. 2c).  
145 Likely, the midgut showed the same massive colonization pattern as the foregut (Fig. 2d-e). In this tract,  
146 small hernias are also visible by interferential contrast (indicated by black arrowheads in Fig. 2e),

147 probably due to microscopic damages produced during the dissection. These hernias appeared full of a  
148 gelatinous matrix that resulted Gfp-positive by CLSM, showing that Gfp-labelled cells are completely  
149 sunk in the gel and suggesting that the bacterial cells are actually contained by the peritrophic matrix.  
150 The black filaments around the organ are the Malpighian tubules, more evident in the CLSM picture  
151 (Fig. 2d). Also *A. tropicalis* BYea.1.23(Gfp), and *A. indonesiensis* BTa1.1.44(Gfp) strains successfully  
152 colonized the foregut and midgut (Fig. S1): since they showed an identical colonization pattern, only  
153 strain BYea.1.23(Gfp) images are shown. The labelled bacteria were present in the whole tract and they  
154 have been especially located close to the gut walls and within the peritrophic matrix (Fig. S1).

155

156 **Characterization of *D. suzukii* bacterial diversity by DNA-based analysis.** At first, to have a general  
157 view of the bacterial community associated to *D. suzukii*, DNA extracted from 32 specimens has been  
158 used, as template, in PCR-DGGE assays (targeting a fragment of the 16S rRNA gene, Tab. S1). In  
159 particular, five larvae (n. 1-5), one pupa (n. 6) and ten adults (n. 7-16; Fig. S2a-b) reared on fruits have  
160 been analysed, as well as four larvae (n. 29-32), four pupae (n. 25-28) and eight adults (n. 17-24) reared  
161 on the artificial diet (Fig. S2c). Consistent with previous data reported for other drosophilid flies  
162 (Chandler *et al.*, 2011; Wong *et al.*, 2013), *D. suzukii* specimens showed relatively simple bacterial  
163 communities with the presence of few prevalent bacterial taxa. The lowest variability in the community  
164 profiles has been observed among larvae reared on fruits and on the artificial diet: many PCR-DGGE  
165 bands were conserved among the samples belonging to the same diet. Conversely, only few conserved  
166 bands were detected among adults reared on fruits, which showed more complex profiles than larval  
167 ones either reared on fruits or on the artificial diet (Fig. S2a-c). PCR-DGGE profiles allowed observing  
168 the influence of diet on the insect bacterial community structure and composition (Fig. S2): the bacterial  
169 community of adults reared on fruit diet was clearly more complex than the one of adults reared on  
170 artificial diet. Moreover, PCR-DGGE sequencing results revealed high prevalence of AAB in insects  
171 reared on both diet substrates (Tab. S2).

172 Thus, to sturdily investigate the diet influence on the insect bacterial community, 16S rRNA gene  
173 pyrosequencing was performed on 14 specimens, including eight individuals reared on fruits and six on  
174 the artificial diet and considering different developmental stages (five larvae, two pupae and seven  
175 adults). Variability among the samples has been reported (Tab. S3; Fig. 3a). Using the Shannon Index to  
176 measure  $\alpha$ -diversity in each sample and plotting it on a rarefaction curve, we confirmed the saturation of  
177 the bacterial diversity associated to the samples (Fig. S3). We obtained in total 178,856 reads after  
178 quality evaluation and chimera removal. The different ecological estimators showed that, on average,  
179 the bacterial communities associated with the specimens reared on fruits exhibited a greater diversity  
180 than those from individuals reared on artificial diet ( $118 \pm 42$  and  $78 \pm 24$  OTUs, respectively; Tab. S3).  
181 As a matter of fact, the microbiota of *D. sukuzii* specimens reared on fruit showed on average a greater  
182 richness ( $\text{Chao1} = 137.4 \pm 48.3$ ), a higher diversity ( $H' = 2.5 \pm 0.75$ ) and a higher evenness ( $J = 0.52 \pm$   
183  $0.13$ ), when compared to the microbiota of flies reared on artificial diet ( $\text{Chao1} = 91.4 \pm 31.1$ ;  $H' = 1.75$   
184  $\pm 0.67$ ;  $J = 0.4 \pm 0.13$ ).

185  $\beta$ -diversity has been evaluated through principal coordinates analysis (PCoA) on the similarity matrix  
186 obtained by UniFrac. The two principal components explain 49.67% of the variation (Fig. 3b). PCoA  
187 showed three clusters of samples ( $p < 0.05$ ): the first one encompasses the two larvae and the sole pupa  
188 reared on the artificial diet; the second one includes all the adults reared on the artificial diet, while the  
189 third is constituted by all the specimens reared on fruits (Fig. 3b). Interestingly, the exclusion of AAB  
190 OTUs from the analysis showed a loss of the clustering pattern observed before (Fig. 3c). Specifically,  
191 the three abovementioned clusters were not significantly different one to each other ( $p > 0.05$ ),  
192 highlighting that AAB could be more responsive than other bacterial groups following diet  
193 modification. Thus, we evaluated the distribution of AAB at OTU level among the specimens exploring  
194 the 16S rRNA gene pyrosequencing dataset: a clustering tendency of the samples in relation to the  
195 different diets has been further observed (Fig. 3d).

196 Looking to the bacterial community's composition, the results showed that the average percentage of  
197 reads belonging to Acetobacteraceae family was 24.8% per specimen (18% in case of fruit-reared  
198 insects and 33.9% for specimens fed with artificial diet; Fig. 3a). At genus level, 16S rRNA gene  
199 pyrosequencing revealed that in *D. suzukii* specimens, reared on fruit and on the artificial diet,  
200 Acetobacteraceae family was composed mainly by the genera *Acetobacter* and *Gluconobacter* (average  
201 20% of 3.9% out of the total reads respectively, Fig. S4; Tab. S4).  
202 Interestingly, reads affiliated to Rickettsiales, to which *Wolbachia* genus belongs, have been detected  
203 only in flies reared on fruits, with an average of 27.5%, confirming results obtained by PCR-DGGE  
204 (Fig. 3a; Fig. S2). *Wolbachia* was the only representative of Rickettsiales order in the dataset. Reads  
205 clustering within Rhodospirillales order (the order to which Acetobacteraceae belongs) were present in  
206 all the specimens with different abundance; in some cases it reached percentages of 85.2 and 85.4 out of  
207 the total number of sequences per sample (MF1 and PP2, respectively). Members of other orders such as  
208 Enterobacteriales, Xanthomonadales, Lactobacillales, Rhizobiales, Burkholderiales and  
209 Sphingobacteriales constituted relevant fractions of the remaining bacterial communities (Fig. 3a).

210

## 211 DISCUSSION

212 Prevalence, FISH and 16S rRNA gene PCR-DGGE and pyrosequencing analyses confirmed that AAB  
213 are invariably present in *D. suzukii* gut in our experimental conditions. In *D. melanogaster* and other  
214 insects, AAB have been demonstrated as prevalent symbionts with important biological roles (Shin *et*  
215 *al.*, 2011; Chouaia *et al.*, 2012; Mitraka *et al.*, 2013). For instance, *Acetobacter tropicalis*, a species that  
216 we found in *D. suzukii*, was previously described in association with the olive fruit fly *Bactrocera oleae*  
217 (Kounatidis *et al.*, 2009).

218 Localization and intimate association of AAB with *D. suzukii*, revealed by FISH (Fig. 1), support the  
219 hypothesis that these bacteria may indeed influence the gut functionality. In the midgut, AAB  
220 localization along with the peritrophic matrix suggests a bacterial interaction with the host gut

221 epithelium. Moreover, recolonization experiments with Gfp-labelled strains (i.e. *G. oxydans* DSF1C.9A,  
222 *A. tropicalis* BYea.1.23 and *A. indonesiensis* BTa1.1.44) strongly supported the capability of AAB to  
223 colonize the gut (Fig. 2 and Fig. S1). As indicated elsewhere (Favia *et al.*, 2007), recolonization  
224 experiments have been performed under the antibiotic pressure of kanamycin, a required procedure  
225 when Gfp cassette is encoded on a plasmid to avoid the loss of the plasmid itself. Certainly, the use of  
226 antibiotic could have a negative side effect on the insect host and other gut symbionts. Further  
227 investigations could help in verifying if the used concentration of antibiotic might have detrimental  
228 effects for the host and/or the gut microbiota. However, such investigation was beyond the purpose of  
229 the experiments that were designed to assess which gut portions were recolonized by the strains. For *A.*  
230 *tropicalis* a very similar gut localization pattern to that of *D. suzukii* has been already observed in the  
231 olive fruit fly *B. oleae* (Kounatidis *et al.*, 2009), where the bacterium was observed in contact with the  
232 gut epithelium of the insect, entrapped in a polysaccharidic matrix. Similarly, in other insects, such as  
233 the leafhopper *Scaphoideus titanus*, and *Anopheles* and *Aedes* mosquitoes, other AAB of the genus  
234 *Asaia* massively colonize the epithelia of the gut and the reproductive organs (Crotti *et al.*, 2009;  
235 Damiani *et al.*, 2010; Favia *et al.*, 2007; Gonella *et al.*, 2012). The AAB localization observed in the gut  
236 of *D. suzukii* confirmed that guts of sugar-feeding insects are primary habitat for AAB, in which they  
237 establish strict topological and presumably functional connections with the epithelial cells (Crotti *et al.*,  
238 2010; Chouaia *et al.*, 2014).

239 *D. suzukii* microbiota diversity has been investigated at little extent and just one paper has been  
240 published describing the insect bacterial community (Chandler *et al.*, 2014). By the use of a next  
241 generation sequencing (NGS) technique, authors analyzed pools of specimens collected from cherries  
242 sampled at different developmental stages, showing an high frequency of the gamma-Proteobacterium  
243 *Tatumella*, while the two AAB *Gluconobacter* and *Acetobacter* genera were found at lower abundance  
244 (Chandler *et al.*, 2014). Conversely, in our study, sequences related to *Tatumella* genus have not been  
245 retrieved in any of the analysed samples, but a high prevalence of AAB have been found (average of

246 24.8%). Insects in Chandler and colleagues' work (2014) have been collected in USA, while our  
247 populations derive from Italian field-collected individuals. Moreover, different variable regions on 16S  
248 rRNA gene have been amplified in the two studies. Such environmental and methodological differences  
249 may explain the differences between our and the Chandler *et al.* work (2014). However, further  
250 investigations are needed to determine *Tatumella* prevalence in different *D. suzukii* populations,  
251 considering with special attention insects collected in different locations, as already mentioned by  
252 Chandler *et al.* (2014).

253 It is widely recognized the importance of diet in shaping the insect bacterial community (Montagna *et*  
254 *al.*, 2015; Colman *et al.*, 2012; Yun *et al.*, 2014). Particularly, in *D. melanogaster* the establishment and  
255 maintenance of the microbiota are determined by bacterial intake from external sources (Blum *et al.*,  
256 2013). Differences in the diversity and dominance of bacterial species associated to several *Drosophila*  
257 species are thus related to food source (Wong *et al.*, 2011). This has been substantiated by Chandler and  
258 coworkers (2011) who observed that individuals of different *Drosophila* species reared on different food  
259 sources enriched a similar microbiota when moved to the same medium. With the present study, we  
260 confirmed that also in case of *D. suzukii* there are differences in the bacterial communities between  
261 animals reared on fruits and on artificial diet (Fig. 3). Specifically, the fruit-based diet determined a  
262 higher diversity in the bacterial community rather than the artificial diet, confirming what already  
263 reported in literature about the reduction of the insect microbial community complexity in case of  
264 artificial diet-fed animals in comparison to natural diet-fed ones (Lehman *et al.*, 2009). In our study, the  
265 fruit-based diet can be considered similar to the natural one *D. suzukii* is exposed to in orchards. The  
266 diet appeared as a more important factor than the life stage in discriminating the insect associated  
267 microbiota, since discrimination at the life stage was possible only between juvenile stages and adults  
268 reared on the artificial diet ( $p < 0.05$ ; Fig. 3b). Chandler *et al.* (2011), analyzing clone libraries of the  
269 bacterial community associated to different species of *Drosophila* flies, field-collected or reared in the  
270 laboratory, found AAB in both types of individuals: sequences related to *Commensalibacter* and

271 *Acetobacter* have been retrieved, while the authors reported the nearly complete lack of *Gluconobacter*  
272 sequences and the complete lack of *Gluconacetobacter* ones within their samples. In our 16S rRNA  
273 gene-based survey of the *D. suzukii* microbiota, *Acetobacter* and *Gluconobacter* have been detected  
274 while *Gluconacetobacter* and *Komagataeibacter* have not, although isolates of these two genera have  
275 been obtained. The 16S rRNA sequence phylogenetic proximity of AAB genera and the small region,  
276 targeting the bacterial 16S rRNA gene used in our PCR amplifications (about 500 bp), could have  
277 masked the discrimination of *Gluconacetobacter* and *Komagataeibacter* sequences (Fig. S4). In this  
278 perspective, the use of multiple primer pairs and the choice of longer regions (however taking into  
279 account limitations of the current NGS techniques) could lead to a more representative view of the  
280 structure of the host bacterial community. Another factor that might have introduced biases in the  
281 microbiota analysis is the DNA extraction method. Even though in our work, DNA has been extracted  
282 through one of the most widely used, cost-effective and efficient methods available for DNA extraction,  
283 i.e. the using sodium dodecyl sulfate-proteinase K-CTAB treatment, the parallel use of alternative  
284 methods on the same set of samples might help to better evaluate the reliability of the obtained data.  
285 Our results indicated that AAB may play a role in structuring the gut community. In the AAB OTUs  
286 distribution in relation to the specimens, a clustering pattern based on the food source was recognized  
287 (Fig. 3d), further strengthening the results of the clustering already observed in fig. 3b. Such findings  
288 indicate that AAB are primarily involved in the response to the diet, and suggest that they may be  
289 directly or indirectly involved in the bacterial community shift following a different diet exposition. We  
290 have evaluated the impact of the diet on the bacterial community, without considering the AAB  
291 contribution: by excluding AAB OTUs from the analyzed dataset, we found the loss of the previously  
292 observed clustering pattern ( $p > 0.05$ ; compare Figs. 3b and 3c). Taken together, these data highlight not  
293 only the differentiation of the AAB community in response to the diet type, but also indicate that AAB  
294 are crucial in determining samples' grouping along with diet variation. It is also noteworthy that the  
295 insects reared on the artificial diet originated from the same field population of the fruit-fed insects.

296 Another variable that could be associated with the distinction of the samples between fruit-fed and  
297 artificial diet-fed animals is the presence of *Wolbachia*, but we concluded that it cannot be considered as  
298 a driver of the bacterial community modification in this case. Although *Wolbachia* was detected by  
299 PCR-DGGE and 16S rRNA barcoding just in fruit-fed samples, the complementary PCR analysis  
300 performed for determining *Wolbachia* in the two diet groups, demonstrated its presence in the artificial  
301 diet-fed animals. *Wolbachia* is generally considered as intracellular reproductive manipulator, described  
302 in many insect species, including different *Drosophila* spp. (Werren *et al.*, 2008; McGraw and O'Neill,  
303 2004). The different incidence in samples reared on fruits respect to the artificial diet could be explained  
304 by the presence of inhibitory compounds in the artificial diet, hindering or somehow temporarily  
305 influencing *Wolbachia* growth. Lack of *Wolbachia* by high throughput sequencing in flies reared on  
306 artificial diet could be the result of the number of analyzed insects (n. = 6), since the *Wolbachia*  
307 prevalence rate in our *D. suzukii* population has been verified to be 28%. On the other hand, the  
308 *Wolbachia* strain associated to *D. suzukii* has been reported to be imperfectly maternally transmitted,  
309 showing polymorphic infection (Hamm *et al.*, 2014). Moreover, the results could indicate a  
310 diversification of infection rates linked to the diet source; indeed, prevalence analysis pointed out a  
311 lower infection rate than previously reported in a similar population (Mazzetto *et al.*, 2015).

312 A competition phenomenon between *Asaia* and *Wolbachia* has been described to occur at the level of  
313 mosquito gonads (Rossi *et al.*, 2015) and *Asaia* has been indicated as responsible for inhibiting  
314 *Wolbachia* transmission in mosquitoes (Hughes *et al.*, 2014). In this study, we could not observe  
315 competition phenomena between AAB and *Wolbachia*. However, no specific investigations have been  
316 performed at gonad level. It should be underlined that so far competition has been described only for  
317 *Asaia*, a symbiont that has never been described in *D. suzukii* or other *Drosophila* flies.

318 In conclusion, AAB's high prevalence in individuals fed on both diet types, their localization and ability  
319 to massively recolonize the insect gut indicate that AAB are major components of the *D. suzukii*  
320 microbiota and, similarly to *D. melanogaster*, they might play important roles in the physiology and

321 behaviour of the host. The AAB diversity shifts and their weight in determining the clustering behaviour  
322 of the bacterial microbiota in relation to diet might indicate their crucial role in determining the  
323 microbiota response to diet in *D. suzukii* gut.

324

## 325 EXPERIMENTAL PROCEDURES

326 **Insects.** Field-captured larvae of *D. suzukii* emerging from blueberries, raspberries and blackberries in  
327 orchards of the Cuneo province, (Piedmont, North-West Italy) in summer 2013 have been reared for at  
328 least eight generations in laboratory condition both on fruits (strawberries, blueberries, grapes and kiwi  
329 fruits) and on a sugar-based artificial diet (composed with 71 g of corn flour, 10 g of soy flour, 5.6 g of  
330 agar, 15 g of sucrose, 17 g of brewer's yeast, 4.7 ml of propionic acid, 2.5 g of vitamins mix for each Kg  
331 of the preparation) at the Dipartimento di Scienze Agrarie, Forestali e Alimentari (DISAFA), University  
332 of Torino. Insects have been kept in plastic cages (24 × 16 × 12 cm) in a growth chamber at 25 ± 1 °C,  
333 65 ± 5% RH and 16L:8D photoperiod, until collected for analyses (Tab. S1). Bacterial community  
334 evaluation was carried out on 2<sup>nd</sup>-3<sup>rd</sup> instar larvae, pupae, and 7-20 day-old adults.

335

336 **Prevalence of AAB and Wolbachia and AAB isolation.** Prevalences of *Wolbachia* and AAB have been  
337 evaluated as described in Method S1. The strategy of isolation was to collect as many AAB isolated  
338 colonies as possible according to diversity of colony morphology obtained from different sources (the  
339 insect specimens) and different media. A bacterial collection has been obtained and identified as  
340 indicated in Method S2. 16S rRNA sequences of representative isolates have been deposited in the ENA  
341 database under the accession numbers LN884027-LN884133.

342

343 **Localization of *D. suzukii* AAB by fluorescent in situ hybridization (FISH) and colonization**  
344 **experiments with Gfp labelled strains.** FISH has been carried out on tissues and organs dissected from  
345 mass-reared *D. suzukii* adults in a sterile saline solution. The dissected organs have been fixed for two

346 minutes at 4°C in 4% paraformaldehyde and washed in Phosphate-Buffered Saline (PBS). All  
347 hybridization experiment steps have been performed as previously described (Crotti *et al.*, 2009;  
348 Gonella *et al.*, 2012), using fluorescent probes, specifically designed for the acetic acid bacterial group  
349 (AAB455, sequence GCGGGTACCGTCATCATCGTCCCCGCT) and for *Gluconobacter* (Go15,  
350 sequence AATGCGTCTCAAATGCAGTT and Go18, sequence GTCACGTATCAAATGCAGTTCCC).  
351 The universal eubacterial probe, Eub338 (sequence GCTGCCTCCCGTAGGAGT), has been used to  
352 detect the localization of the overall bacterial abundance and presence in the organs analysed (Gonella *et*  
353 *al.*, 2012). Probes for AAB and Eubacteria have been labelled at the 5' end with the fluorochrome Texas  
354 Red (TR; absorption and emission at 595 nm and 620 nm, respectively), whereas probes Go15 and  
355 Go18 have been labelled with indodicarbocyanine (Cy5; absorption and emission at 650 nm and 670  
356 nm, respectively). After hybridization, the samples have been mounted in anti-fading medium and then  
357 observed in a laser scanning confocal microscope SP2- AOBS (Leica). Hybridization experiments in the  
358 absence of probes have been performed as negative controls.

359 *G. oxydans* strain DSF1C.9A, *A. tropicalis* BYea.1.23 and *A. indonesiensis* BTa1.1.44 have been  
360 transformed through electroporation introducing the plasmid pHM2-Gfp (Favia *et al.*, 2007) as  
361 described in **Method S3**. Plasmid stability has been verified for the transformants as reported in **Method**  
362 **S4**. Recolonization experiments using *G. oxydans* DSF1C.9A(Gfp), *A. tropicalis* BYea.1.23(Gfp) and *A.*  
363 *indonesiensis* BTa1.1.44(Gfp) have been performed as indicated in **Method S5**.

364

#### 365 ***Characterization of the D. suzukii bacterial community through molecular ecology approaches.***

366 Immediately after collection larval, pupal and adult individuals of *D. suzukii* have been washed once  
367 with ethanol 70% and twice with saline and immediately stored at -20°C in ethanol until molecular  
368 analyses. Total DNA has been individually extracted from larvae, pupae and adults by sodium dodecyl  
369 sulfate-proteinase K-cethyltrimethyl ammonium bromide (CTAB) treatment, as described in Raddadi *et*  
370 *al.* (2011).

371 PCR-DGGE has been performed as described in Method S6. The obtained sequences have been  
372 deposited in the EMBL database under the accession numbers LN884134-LN884176.  
373 Genomic DNA previously extracted from designated individuals (codes: LF1, LF2, LF3, PF1, MF1,  
374 FF2, FF3, MF4, LP1, LP3, PP2, FP1, FP3, and MP3, Tab. S1, Tab. S3) were used in 16S rRNA gene  
375 pyrosequencing as described in Method S7. 16S rRNA gene sequences obtained from 16S rRNA gene  
376 pyrosequencing analysis have been deposited in European Nucleotide Archive with accession numbers  
377 PRJEB10109. The OTU table obtained from 16S rRNA gene pyrosequencing analysis has been filtered  
378 and only OTU sequences of AAB have been kept. Statistical significance ( $p < 0.05$ ) of sample  
379 distribution in different clusters along Axis 1 of PCoA analysis has been examined by t-test using the  
380 software GraphPad Prism version 5.03. Heatmap based on the distribution of AAB OTUs has been  
381 prepared as described in Method S8.

382

### 383 FUNDING INFORMATION

384 King Abdullah University of Science and Technology supported the study through the baseline research  
385 funds to D.D. This work was partially funded by Consorzio di Ricerca Sperimentazione e Divulgazione  
386 per l'Ortofrutticoltura Piemontese, within the project "Programma di ricerca, sperimentazione e  
387 dimostrazione agricola in frutticoltura e orticoltura – 2014 – Indagini sul nuovo dittero esotico  
388 *Drosophila suzukii* responsabile di gravi danni alle drupacee". E.C. acknowledges personal support from  
389 "Piano Sviluppo di Ateneo: Linea B-Dotazione annuale per attività istituzionale" in the project "Acetic  
390 acid bacteria cell factories".

391

### 392 REFERENCES

393 Bextine, B., Lauzon, C., Potter, S., Lampe, D., and Miller, T.A. (2004) Establishment of a genetically  
394 marked insect-derived symbiont in multiple host plants. *Curr Microbiol* 48: 327-331.

- 395 Blum, J.E., Fischer, C.N., Miles, J., and Handelsman, J. (2013) Frequent replenishment sustains the  
396 beneficial microbiome of *Drosophila melanogaster*. mBio 4. e00860-13.
- 397 Brummel, T., Ching, A., Seroude, L., Simon, A.F., and Benzer, S. (2004) *Drosophila* lifespan  
398 enhancement by exogenous bacteria. Proc Natl Acad Sci U S A 101:12974-9.
- 399 Cattel, J., Kaur, R., Gibert, P., Martinez, J., Fraimout, A., Jiggins, F., Andrieux, T., Siozios, S., Anfora,  
400 G., Miller, W., Rota-Stabelli, O., Mouton, L. (2016) *Wolbachia* in European populations of the  
401 invasive pest *Drosophila suzukii*: regional variation in infection frequencies. PLoS ONE 11:  
402 e0147766.
- 403 Chandler, J.A., James, P.M., Jospin, G., and Lang, J.M. (2014) The bacterial communities of  
404 *Drosophila suzukii* collected from undamaged cherries. PeerJ 2: e474.
- 405 Chandler, J.A., Lang, J.M., Bhatnagar, S., Eisen, J.A., and Kopp, A. (2011) Bacterial communities of  
406 diverse *Drosophila* species: Ecological context of a host-microbe model system. PLoS Genet 7(9):  
407 e1002272.
- 408 Chouaia, B., Gaiarsa, S., Crotti, E., Comandatore, F., Degli Esposti, M., Ricci, I., *et al.* (2014) Acetic  
409 acid bacteria genomes reveal functional traits for adaptation to life in insect guts. Genome Biol Evol  
410 6(4): 912–920.
- 411 Chouaia, B., Rossi, P., Epis, S., Mosca, M., Ricci, I., Damiani C., *et al.* (2012). Delayed larval  
412 development in *Anopheles mosquitoes* deprived of *Asaia* bacterial symbionts. BMC Microbiol 12:  
413 S2.
- 414 Cini, A., Ioriatti C., and Anfora G. (2012) A review of the investigation of *Drosophila suzukii* in Europe  
415 and draft research agenda for integrated pest management. Bull Insectol 65: 149-160.
- 416 Colman, D.R., Toolson, E.C., Takacs-Vesbach, C.D. (2012) Do diet and taxonomy influence insect gut  
417 bacterial communities? Mol Ecol 21:5124-37.

- 418 Corby-Harris, V., Habel, K.E., Ali, F.G., and Promislow, D.E.L. (2007) Alternative measures of  
419 response to *Pseudomonas aeruginosa* infection in *Drosophila melanogaster*. J Evol Biol 20(2):526-  
420 33.
- 421 Cox, C., and Gilmore, M. (2007) Native microbial colonization of *Drosophila melanogaster* and its use  
422 as a model of *Enterococcus faecalis* pathogenesis. Infect Immun 75:1565-1576.
- 423 Crotti, E., Damiani, C., Pajoro, M., Gonella, E., Rizzi, A., Ricci, I., *et al.* (2009) *Asaia*, a versatile acetic  
424 acid bacterial symbiont, capable of cross-colonizing insects of phylogenetically-distant genera and  
425 orders. Environ Microbiol 11: 3252-3264.
- 426 Crotti, E., Rizzi, A., Chouaia, B., Ricci, I., Favia, G., Alma, A., *et al.* (2010) Acetic acid bacteria, newly  
427 emerging symbionts of insects. Appl Environ Microbiol 76: 6963-6970.
- 428 Damiani, C., Ricci, I., Crotti, E., Rossi, P., Rizzi, A., Scuppa, P., *et al.* (2010) Mosquito-bacteria  
429 symbiosis: the case of *Anopheles gambiae* and *Asaia*. Microb Ecol 60 (3): 644-654.
- 430 Eriksson, A., Anfora, G., Lucchi, A., Lanzo, F., Virant-Doberlet, M., and Mazzoni, V. (2012)  
431 Exploitation of insect vibrational signals reveals a new method of pest management. PLoS One 7:  
432 e32954.
- 433 Erkosar, B., Storelli, G., Defaye, A., and Leulier F. (2013) Host-intestinal microbiota mutualism:  
434 "learning on the fly". Cell Host Microbe 13: 8-14.
- 435 Favia, G., Ricci, I., Damiani, C., Raddadi, N., Crotti, E., Marzorati, M., *et al.* (2007) Bacteria of the  
436 genus *Asaia* stably associate with *Anopheles stephensi*, an Asian malarial mosquito vector. Proc Natl  
437 Acad Sci USA 104: 9047–9051.
- 438 Gonella, E., Crotti, E., Rizzi, A., Mandrioli, M., Favia, G., Daffonchio, D., and Alma, A. (2012)  
439 Horizontal transmission of the symbiotic bacterium *Asaia* sp. in the leafhopper *Scaphoideus titanus*  
440 Ball (Hemiptera: Cicadellidae). BMC Microbiol 12(Suppl 1): S4.

- 441 Goodhue, R.E., Bolda, M., Farnsworth, D., Williams, J.C., and Zalom F.G. (2011) Spotted wing  
442 drosophila infestation of California strawberries and raspberries: economic analysis of potential  
443 revenue losses and control costs. *Pest Manag Sci* 67: 1396-1402.
- 444 Hamdi, C., Balloi, A., Essanaa, J., Crotti, E., Gonella, E., Raddadi, N., Ricci, I., Boudabous, A., Borin,  
445 S., Manino, A., Bandi, C., Alma, A., Daffonchio, D., Cherif, A., 2011. Gut microbiome dysbiosis and  
446 honeybee health. *J Appl Entomol* 135: 524–533.
- 447 Hamm, C.A., Begun, D.J., Vo, A., Smith, C.C.R., Saelao, P., Shaver, A.O., Jaenike, J., and Turelli, M.  
448 (2014) *Wolbachia* do not live by reproductive manipulation alone: infection polymorphism in  
449 *Drosophila suzukii* and *D. subpulchrella*. *Mol Ecol* 23: 4871–4885.
- 450 Hauser, M. (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera:  
451 Drosophilidae) in the continental United States, with remarks on their identification. *Pest Manag Sci*.  
452 67: 1352–1357.
- 453 Hughes, G.L., Dodson, B.L., Johnson, R.M., Murdock, C.C., Tsujimoto, H., Suzuki, Y., Patt, A.A., Cui,  
454 L., Nossa, C.W., Barry, R.M., Sakamoto, J.M., Hornett, E.A., Rasgon, J.L. (2014) Native  
455 microbiome impedes vertical transmission of *Wolbachia* in *Anopheles* mosquitoes. *Proc Natl Acad*  
456 *Sci U S A* 111:12498-503.
- 457 Kounatidis, I., Crotti, E., Sapountzis, P., Sacchi, L., Rizzi, A., Chouaia, B., *et al.* (2009) *Acetobacter*  
458 *tropicalis* is a major symbiont of the olive fruit fly (*Bactrocera oleae*). *Appl Environ Microbiol* 75:  
459 3281-328.
- 460 Lee, J.C., Bruck, D.J., Deves, A.J., Ioriatti, C., Vogt, H., and Baufeld, P. (2011) In Focus: Spotted wing  
461 drosophila, *Drosophila suzukii*, across perspectives. *Pest Manag Sci* 67: 1349-1351.
- 462 Lee, K.-A., and Lee, W.-J. (2014) *Drosophila* as a model for intestinal dysbiosis and chronic  
463 inflammatory diseases. *Dev Comp Immunol* 42(1):102-10.

- 464 Lehman, R.M., Lundgren, J.G., and Petzke, L.M. (2009) Bacterial communities associated with the  
465 digestive tract of the predatory ground beetle, *Poecilus chalcites*, and their modification by laboratory  
466 rearing and antibiotic treatment. *Microb Ecol* 57:349-358.
- 467 Mazzetto, F., Gonella, E., and Alma, A. (2015) *Wolbachia* infection affects female fecundity in  
468 *Drosophila suzukii*. *Bull Insectol* 68: 153-157.
- 469 McGraw, E.A., O'Neill S. (2004) *Wolbachia pipientis*: intracellular infection and pathogenesis in  
470 *Drosophila*. *Curr Opin Microbiol* 7:67-70.
- 471 Mitraka, E., Stathopoulos, S., and Siden-Kiamos, I. (2013) *Asaia* accelerates larval development of  
472 *Anopheles gambiae*. *Pathog Glob Health* 107: 305-311.8
- 473 Mitsui, H., Takahashi, H.K., and Kimura, M.T. (2006) Spatial distributions and clutch sizes of  
474 *Drosophila* species ovipositioning on cherry fruits of different stages. *Popul Ecol* 48: 233-237.
- 475 Montagna, M., Chouaia, B., Mazza, G., Prosdocimi, E.M., Crotti, E., Mereghetti, V., *et al.* (2015)  
476 Effects of the diet on the microbiota of the red palm weevil (Coleoptera: Dryophthoridae). *PLoS One*  
477 10: e0117439.
- 478 Raddadi, N., Gonella, E., Camerota, C., Pizzinat, A., Tedeschi, R., Crotti, E., *et al.* (2011) “*Candidatus*  
479 *Liberibacter europaeus*” sp. nov. that is associated with and transmitted by the psyllid *Cacopsylla*  
480 *pyri* apparently behaves as an endophyte rather than a pathogen. *Environ Microbiol* 13: 414–426.
- 481 Ren, C., Webster, P., Finkel, S.E., and Tower, J. (2007) Increased internal and external bacterial load  
482 during *Drosophila* aging without life-span trade-off. *Cell Metab* 6:144–152.
- 483 Ridley, E.V., Wong, A.C.N., Westmiller, S., and Douglas, A.E. (2012) Impact of the resident microbiota  
484 on the nutritional phenotype of *Drosophila melanogaster*. *PLoS One* 7: e36765.
- 485 Rossi, P., Ricci, I., Cappelli, A., Damiani, C., Ulissi, U., Mancini, M.V., Valzano, M., Capone, A., Epis,  
486 S., Crotti, E., Chouaia, B., Scuppa, P., Joshi, D., Xi, Z., Mandrioli, M., Sacchi, L., O'Neill, S.L.,  
487 Favia, G. (2015) Mutual exclusion of *Asaia* and *Wolbachia* in the reproductive organs of mosquito  
488 vectors. *Parasit Vectors* 8:278.

- 489 Ryu, J.H., Kim, S.H., Lee, H.Y., Bai, J.Y., Nam, Y.D., Bae, J.W., *et al.* (2008) Innate immune  
490 homeostasis by the homeobox gene *Caudal* and commensal-gut mutualism in *Drosophila*. *Science*  
491 319: 777–782.
- 492 Siozios, S., Cestaro, A., Kaur, R., Pertot, I., Rota-Stabelli, O., and Anfora, G. (2013) Draft genome  
493 sequence of the *Wolbachia* endosymbiont of *Drosophila suzukii*. *Genome Announc.* 1(1), pii.00032-  
494 13.
- 495 Sharon, G., Segal, D., Ringo, J.M., Hefetz, A., Zilber-Rosenberg, I., and Rosenberg, E. (2010)  
496 Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. *Proc Natl Acad*  
497 *Sci USA* 107: 20051-20056.
- 498 Shin, S.C., Kim, S.H., You, H., Kim, B., Kim, A.C., Lee, K.A., *et al.* (2011) *Drosophila* microbiome  
499 modulates host developmental and metabolic homeostasis via insulin signaling. *Science* 334: 670-  
500 674.
- 501 Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., and Leulier, F. (2011) *Lactobacillus plantarum*  
502 promotes *Drosophila* systemic growth by modulating hormonal signals through TOR\_dependent  
503 nutrient sensing. *Cell Metab* 14: 403-414.
- 504 Walsh, D.B., Bolda, M.P., Goodhue, R.E., Dreves, A.J., Lee, J.C., Bruck, D.J., Walton, V.M., O'Neals,  
505 S.D., and Zalom, F.G. (2011) *Drosophila suzukii* (Diptera: Drosophilidae): Invasive pest of ripening  
506 soft fruit expanding its geographic range and damage potential. *Int J Pest Manage* 1: 1-7.
- 507 Werren, J.H., Baldo, L., Clark, M.E. (2008) *Wolbachia*: master manipulators of invertebrate biology.  
508 *Nat Rev Microbiol* 6:741-51.
- 509 Wong, A., Chaston, J., and Douglas, A. (2013) The inconstant gut microbiota of *Drosophila* species  
510 revealed by 16S rRNA gene analysis. *ISME J* 7: 1922-1932.
- 511 Wong, A., Ng, P., and Douglas, A. (2011) Low diversity bacterial community in the gut of the fruitfly  
512 *Drosophila melanogaster*. *Environ Microbiol* 13: 1889-1900.

- 513 Yamada, Y., Pattaraporn, Y., Vu, H.T.L., Muramatsu, Y., Ochaikul, D., and Nakagawa, Y. (2012b)  
514 Subdivision of the genus *Gluconacetobacter* Yamada, Hoshino and Ishikawa 1998: the proposal of  
515 *Komagatabacter* gen. nov., for strains accommodated to the *Gluconacetobacter xylinus* group in the  
516  $\alpha$ -Proteobacteria. *Ann Microbiol* 62: 849–859.
- 517 Yamada, Y., Yukphan, P., Lan, Vu, H.T., Muramatsu, Y., Ochaikul, D., Tanasupawat, S., and  
518 Nakagawa, Y. (2012a) Description of *Komagataeibacter* gen. nov., with proposals of new  
519 combinations (Acetobacteraceae). *J Gen Appl Microbiol* 58: 397-404.
- 520 Yun, J.H., Roh, S.W., Whon, T.W., Jung, M.J., Kim, M.S., Park, D.S., Yoon, C., Nam, Y.D., Kim, Y.J.,  
521 Choi, J.H., Kim, J.Y., Shin, N.R., Kim, S.H., Lee, W.J., Bae, J.W. (2014) Insect gut bacterial  
522 diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host.  
523 *Appl Environ Microbiol* 80:5254-64.

524

525 **TABLE**

526 **Table 1.** Identification of cultivable bacteria associated to *D. suzukii*. All the isolates showed a  
 527 percentage of identity >97% in relation to the indicated species.  
 528

Isolates	No. isolates	LP	PP	AP fly	AF fly
<i>Acetobacter tropicalis</i>	1	0	0	0	1
<i>Acetobacter orleanensis/malorum/cerevisiae</i>	4	0	0	0	4
<i>Acetobacter peroxydans</i>	1	0	0	0	1
<i>Acetobacter indonesiensis</i>	10	0	1	1	8
<i>Acetobacter persici</i>	10	0	1	1	8
<i>Acetobacter orientalis</i>	1	0	0	0	1
<i>Acetobacter cibirongensis</i>	1	0	0	0	1
<i>Gluconacetobacter liquefaciens</i>	4	1	3	0	0
<i>Komagataeibacter</i> sp	118	0	0	0	118
<i>Gluconacetobacter/Komagataeibacter</i> sp.	1	0	0	0	1
<i>Gluconobacter kondonii</i>	1	0	0	0	1
<i>Gluconobacter oxydans</i>	12	0	0	0	12
<i>Gluconobacter kanchanaburiensis</i>	5	3	1	1	0
<i>Pseudomonas geniculata</i>	1	0	0	1	0
<i>Serratia</i> sp.	8	2	6	0	0
<i>Micrococcus</i> sp.	5	0	0	0	5
<i>Microbacterium foliorum</i>	2	0	0	0	2
<i>Streptococcus salivarius</i>	1	0	0	1	0
<i>Staphylococcus</i> sp.	12	0	0	0	12
<i>Paenibacillus</i> sp.	2	0	0	0	2
<i>Lactococcus lactis</i>	1	0	0	0	1
<i>Lactobacillus plantarum</i>	1	0	1	0	0
<b>Total</b>	<b>202</b>	<b>6</b>	<b>13</b>	<b>5</b>	<b>178</b>

529 LP: larvae fed with artificial diet; PP: pupae fed with artificial diet; AP: Adults fed with artificial diet; AF: Adults fed with  
 530 fruit diet  
 531

532

## 533 FIGURES

534 **Figure 1.** AAB localization in the gut of *D. suzukii*. (a-d) FISH of the insect gut after hybridization with  
 535 the Texas red-labelled probe AAB455, matching AAB. (a) Superposition of the interferential contrast  
 536 (c) and the FISH (b) pictures of the midgut close to the proventriculus that is indicated by white arrows  
 537 [for a scheme of the morphology of the initial part of the midgut and the upstream region refer to panel  
 538 (a) of Figure 3]. (d) Magnification of the image in (b). The massive presence of AAB adherent to the  
 539 peritrophic matrix (the black line below the first layer of cells indicated by black arrows) is observed.  
 540 (e-h) FISH of posterior midgut with the Texas red-labelled universal eubacterial probe Eub338 (f) and  
 541 the Cy5-labelled probe specific for *Gluconobacter*, Go615 and Go618 (g). (e) Intestine portion pictured  
 542 by interferential contrast. (h) Superposition of hybridization signals of Eubacteria (red) and  
 543 *Gluconobacter* (blue). Bars = 50  $\mu\text{m}$ .

544

545 **Figure 2.** Colonization of *D. suzukii* foregut and midgut by Gfp-labelled *G. oxydans* DSF1C.9A1  
 546 documented by confocal laser scanning microscopy. (a) The scheme represents the first tract of the  
 547 digestive system and shows the different gut portions highlighted in the next panels. (b-d) Digestive  
 548 tract portions including the crop, the proventriculus and the first part of the midgut. (c, d) Magnified  
 549 views of the crop (c) and the proventriculus (d) showed in (b). Masses of fluorescent cells are observed  
 550 in the crop (arrows). When the fluorescent strain cells reach the proventriculus (d), they colonize the gut  
 551 part close to peritrophic matrix. (e-f) Interferential contrast (f) and confocal laser scanning (e) pictures  
 552 of the posterior midgut of *D. suzukii* massively colonized by the *G. oxydans* strain labelled with Gfp.  
 553 Small hernias (arrowhead) are shown. In some cases, the gelatinous matrix in the hernias present  
 554 fluorescent cells. Bars = 50  $\mu\text{m}$ .

555

556 **Figure 3.** Bacterial diversity associated with *D. suzukii* by 16S rRNA gene pyrosequencing. (a) 16S  
 557 RNA gene pyrosequencing describing bacterial communities, at order level, associated with *D. suzukii*.

558 Names, under histograms, refer to fly specimens; in columns, the relative abundances in percentages of  
559 the identified orders are showed. Sequences that did not match with anything in the database are  
560 indicated as “Unclassified sequences”; bacterial sequences that have not been assigned to any  
561 taxonomical group are indicated as “Bacteria\_unclassified”; bacterial orders under 3% representation  
562 per sample have been grouped and indicated as “Class. Bac. Orders under 3%”. (b) Principal coordinate  
563 analysis (PCoA) on the phylogenetic  $\beta$ -diversity matrix on *D. suzukii* samples, considering all the  
564 bacterial OTUs. (c) Principal coordinate analysis (PCoA) on the phylogenetic  $\beta$ -diversity matrix on *D.*  
565 *suzukii* samples, considering all the bacterial OTUs, except for the ones belonging to AAB group. Red  
566 circle indicates fruit-fed individuals, while blue circles mark specimens fed on the artificial diet. (d)  
567 Distribution of AAB in *D. suzukii* hosts. The relative abundance of AAB OTUs, determined at 97%  
568 identity, is showed in the heatmap. Coloured scale represents OTUs abundance for each sample  
569 (indicated on the vertical axis). In bold are indicated samples from fruit-rearing; the remaining samples  
570 are related to artificial diet-fed animals. First letter of codes refers to the fly stage (M: male adult; F:  
571 female adult; L: larva; P: pupa); second letter of codes refers to feeding system (F: fruit-based diet; P:  
572 artificial diet); third letter of codes is related to subsequent number of samples.

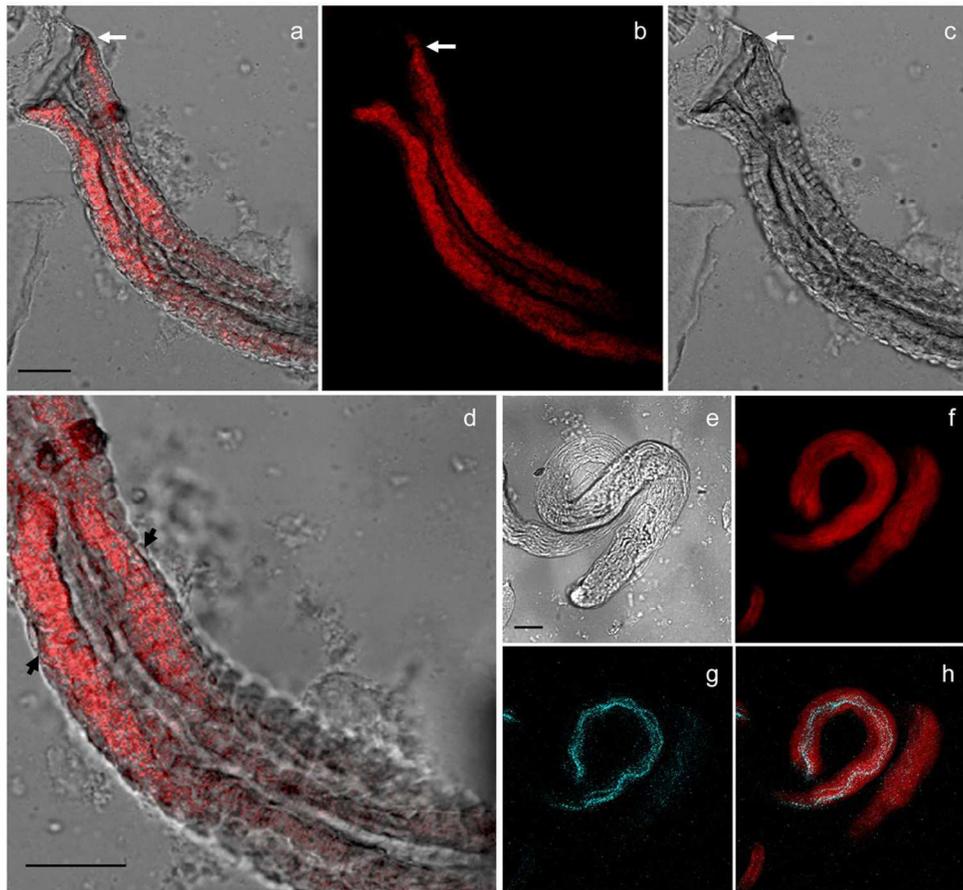


Figure 1. AAB localization in the gut of *D. sukii*. (a-d) FISH of the insect gut after hybridization with the Texas red-labelled probe AAB455, matching AAB. (a) Superposition of the interferential contrast (c) and the FISH (b) pictures of the midgut close to the proventriculus that is indicated by white arrows [for a scheme of the morphology of the initial part of the midgut and the upstream region refer to panel (a) of Figure 3]. (d) Magnification of the image in (b). The massive presence of AAB adherent to the peritrophic matrix (the black line below the first layer of cells indicated by black arrows) is observed. (e-h) FISH of posterior midgut with the Texas red-labelled universal eubacterial probe Eub338 (f) and the Cy5-labelled probe specific for *Gluconobacter*, Go615 and Go618 (g). (e) Intestine portion pictured by interferential contrast. (h) Superposition of hybridization signals of Eubacteria (red) and *Gluconobacter* (blue). Bars = 50  $\mu$ m.

205x189mm (300 x 300 DPI)

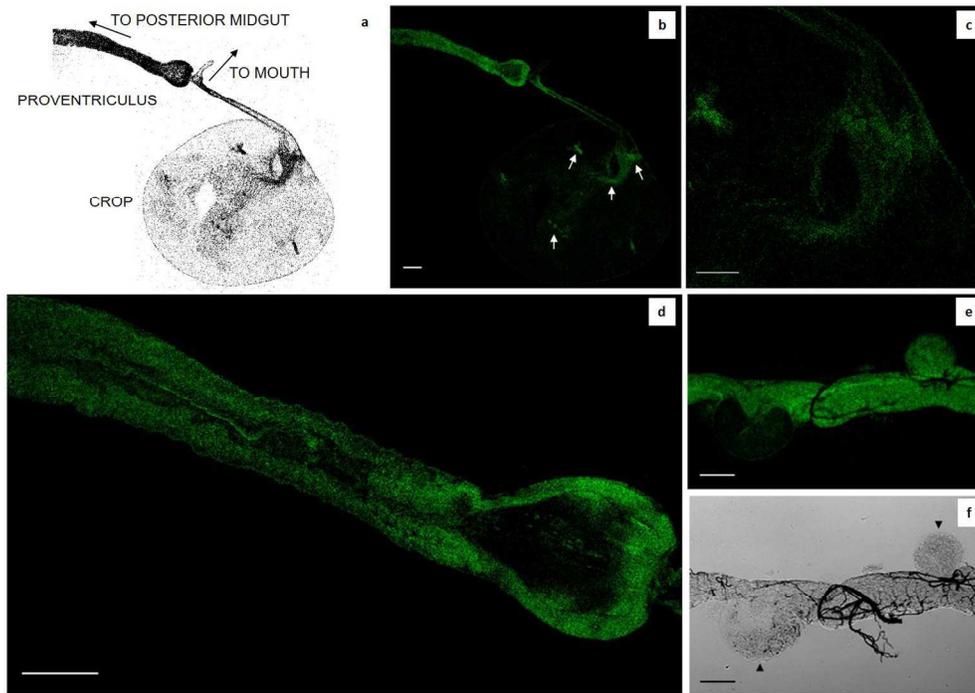


Figure 2. Colonization of *D. sukukii* foregut and midgut by Gfp-labelled *G. oxydans* DSF1C.9A1 documented by confocal laser scanning microscopy. (a) The scheme represents the first tract of the digestive system and shows the different gut portions highlighted in the next panels. (b-d) Digestive tract portions including the crop, the proventriculus and the first part of the midgut. (c, d) Magnified views of the crop (c) and the proventriculus (d) showed in (b). Masses of fluorescent cells are observed in the crop (arrows). When the fluorescent strain cells reach the proventriculus (d), they colonize the gut part close to peritrophic matrix. (e-f) Interferential contrast (f) and confocal laser scanning (e) pictures of the posterior midgut of *D. sukukii* massively colonized by the *G. oxydans* strain labelled with Gfp. Small hernias (arrowhead) are shown. In some cases, the gelatinous matrix in the hernias present fluorescent cells. Bars = 50  $\mu$ m.

277x194mm (300 x 300 DPI)

Only

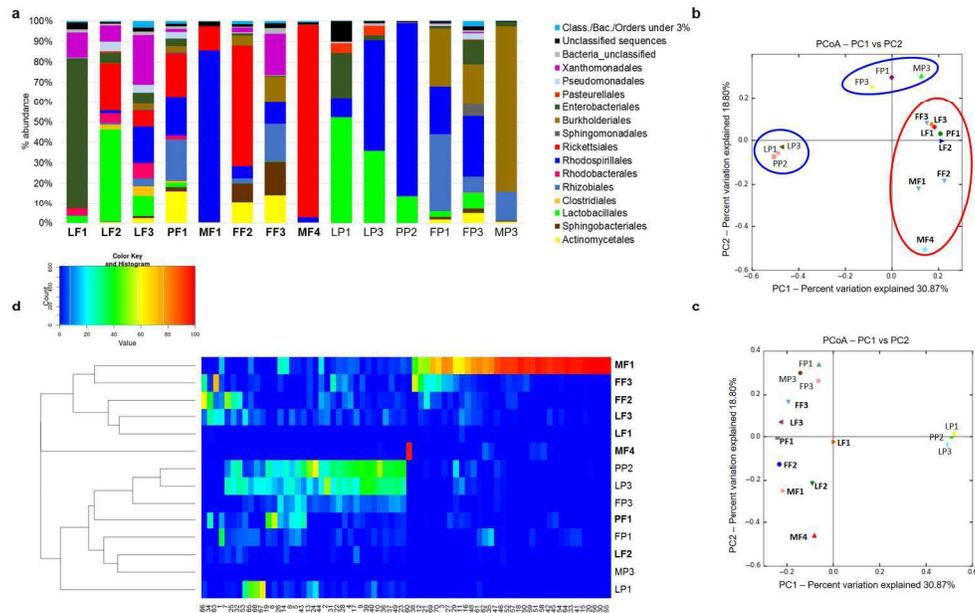


Figure 3. Bacterial diversity associated with *D. sukuzii* by 16S rRNA gene pyrosequencing. (a) 16S RNA gene pyrosequencing describing bacterial communities, at order level, associated with *D. sukuzii*. Names, under histograms, refer to fly specimens; in columns, the relative abundances in percentages of the identified orders are showed. Sequences that did not match with anything in the database are indicated as "Unclassified sequences"; bacterial sequences that have not been assigned to any taxonomical group are indicated as "Bacteria\_unclassified"; bacterial orders under 3% representation per sample have been grouped and indicated as "Class. Bac. Orders under 3%". (b) Principal coordinate analysis (PCoA) on the phylogenetic  $\beta$ -diversity matrix on *D. sukuzii* samples, considering all the bacterial OTUs. (c) Principal coordinate analysis (PCoA) on the phylogenetic  $\beta$ -diversity matrix on *D. sukuzii* samples, considering all the bacterial OTUs, except for the ones belonging to AAB group. Red circle indicates fruit-fed individuals, while blue circles mark specimens fed on the artificial diet. (d) Distribution of AAB in *D. sukuzii* hosts. The relative abundance of AAB OTUs, determined at 97% identity, is showed in the heatmap. Coloured scale represents OTUs abundance for each sample (indicated on the vertical axis). In bold are indicated samples from fruit-rearing; the remaining samples are related to artificial diet-fed animals. First letter of codes refers to the fly stage (M: male adult; F: female adult; L: larva; P: pupa); second letter of codes refers to feeding system (F: fruit-based diet; P: artificial diet); third letter of codes is related to subsequent number of samples.

372x231mm (150 x 150 DPI)

