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Ceratitis capitata Bacterial Symbionts: Implications in Insect Control †

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Abstract: The Mediterranean fly (Medfly), *Ceratitis capitata* causes important economic and agricultural losses due to its peculiar ability to invade and adapt to different climates throughout tropical and subtropical regions. Traditional control methods should be implemente by innovative tools among which those based on nsect symbiont seem very promising. Our study aimed to characterize the microbial communities of different anatomical districts (gut and reproductive organs) of three different strains of *C. capitata*, to determine whether selected symbionts could be translated into potential tools for the symbiotic control of medfly. While confirming the presence of *Asaia* in both organs, we revealed for the first time the presence of *Propionibacterium* and *Chroococcidiopsis* in the reproductive organs of Medfly. These findings paves the way for the development of control methods based on the use of symbiotic bacteria.

Keywords: *Ceratitis capitata*; *Asaia*; symbiotic control

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1. Introduction

Ceratitis capitata is one of the principal destructive pest of fruit production worldwide [1], because of its significant physical damage on fruits and vegetables and its economic impact on agriculture and forestry. Due to its ability to tolerate and adapt to a wide range of climates and its capability to attack very large hosts, *C. capitata* has a wide distribution in particularly in Mediterranean countries. Several strategies have been proposed to control the Medfly distribution. Among them, methods such as the insecticide bait spray and the sterile insect technique have been demonstrated to be effective methods for Medfly control [2, 3]. Although the chemical method works well in medfly control, it has some disadvantages related to its toxicity for the human being and rend the fruits or plants polluted by leaving residues on them. The sterile insect technique (SIT) has been successful in several countries releasing X-rays sterile medflies aimed to reduce the wild insect population. The study of microbiota could open new perspectives in Medfly control. Up to now, studies on microbiota composition of *C. capitata* are already limited [4-8]. Our study aimed to characterize the microbial communities of different anatomical districts (gut and reproductive organs) of three different strains of *C. capitata*, to determine whether selected symbionts could be translated into potential tools for the symbiotic control of medfly.

2. Materials and Methods

C. capitata rearing

The strains of *C. capitata* used in this work were: i) Guatemala strain, established in 1989 from wild pupae collected in Antigua (Guatemala); ii) La Réunion strain was established in 1994 from wild pupae collected near St. Denis (La Reunion, France); iii) ISPRA strain, established in 1968 at the European Community Joint Research Centre (Ispra, Italy) with wild flies from Sicily and Greece, and maintained in Pavia since 1979. These strains originated from the Department of Biology & Biotechnology, University of Pavia, where they are maintained under standard rearing conditions [9] and since 2018, they have been maintained in the insectary at the School of Biosciences & Veterinary Medicine, University of Camerino.

16. S rRNA gene sequencing

The 16S Miseq analysis was conducted on male and female organs (gut and reproductive organs) of three different *C. capitata* populations.

A single pool of 20 organs for each group was obtained from cohorts of 10-day-old insects dissecting in sterile conditions. Samples were homogenized with sterile 0.5-mm wide glass beads (Bertin) for 30s at 6800 rpm by automatic tissue homogenizer (Precellys 24, Bertin). Genomic DNA was extracted using a JetFlex Genomic DNA Purification kit (Invitrogen, Thermo Fisher Scientific) according to the manufacturer's instructions. A negative control consisting of a blank sample was included for each batch of extraction to control for contamination of bacteria possibly introduced during the DNA extraction. They were not further processed since no quantifiable extract was produced from each negative control.

16S sequencing analysis was conducted by LGC Genomics (Berlin, Germany). Libraries preparation was performed by amplifying the hypervariable region V3–V4 of 16S ribosomal RNA using 341F and 785R oligonucleotides [10]. Data were pre-processed using the Illumina bcl2fastq 2.17.1.14 software and reads sorted by amplicon inline barcodes. Sequencing adapter remnants were clipped from all reads. 16S pre-processing and OTU picking from amplicons were analyzed using Mothur 1.35.1 [11]. The sequence alignments were performed against the 16S Mothur-Silva SEED r119 reference alignment. OTU diversity was analysed with QIIME 1.9.0 [12] and annotations of putative species level of OTUs were obtained with NCBI BLAST+ 2.2.29 [13]. The raw data were submitted as BioProject accession number PRJNA682004 to NCBI database.

3. Results

The microbiome sequencing of male and female organs of three different populations of *C. capitata* generated a total of 4.2 M reads, varying among samples (minimum = 64.760, maximum = 701.290), with an average of 352.944 reads. Analysis of the rarefaction curves indicated an adequate sampling quality, suggesting a coherent amount of sequence reads per sample. The Principal Coordinates Analysis (PCoA) plots show the high similarity of microbial composition among all guts analyzed, while a more specific microbial community in ISPRA reproductive organs was represented. No substantial difference in microbial composition is observed between males and females in any strain. (Fig. 1).

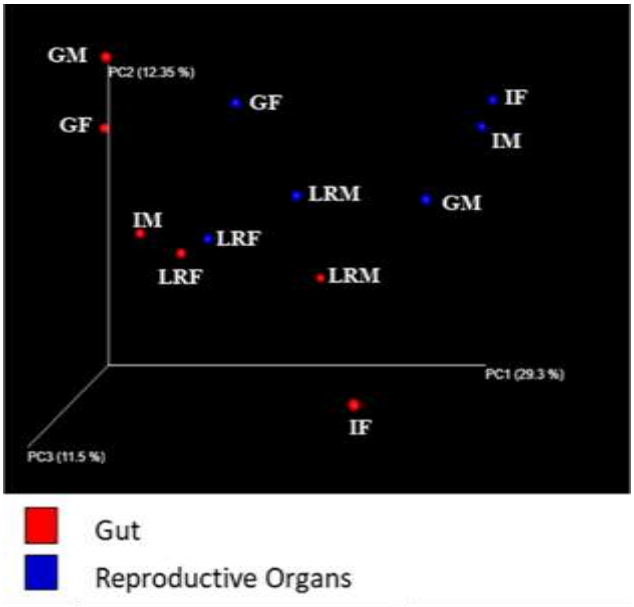

Figure 1. Principal Coordinates Analysis (PCoA) plots of samples colored according to different organs (gut = red; reproductive organs = blue). GM: Guatemala male; GF: Guatemala female; LRM: La Réunion male; LRF: La Réunion female; IM: ISPRA male; IF: ISPRA female. GutM: male gut; GutF: female gut; ROM: male reproductive organ; ROF: female reproductive organ.

At phylum level, Proteobacteria results the most prevalent in all groups, in particular in guts (males and females 99%). The phyla Actinobacteria and Firmicutes were detected in reproductive organs. Bacteroides phylum was revealed in reproductive organs of La Réunion (males: 2.7%) and ISPRA strains (male: 6% and females: 2.3%). Additionally, Cyanobacteria phylum was present in ISPRA strain reproductive organs (male: 15% and females: 7.8%) (Fig. 2; Table S1).

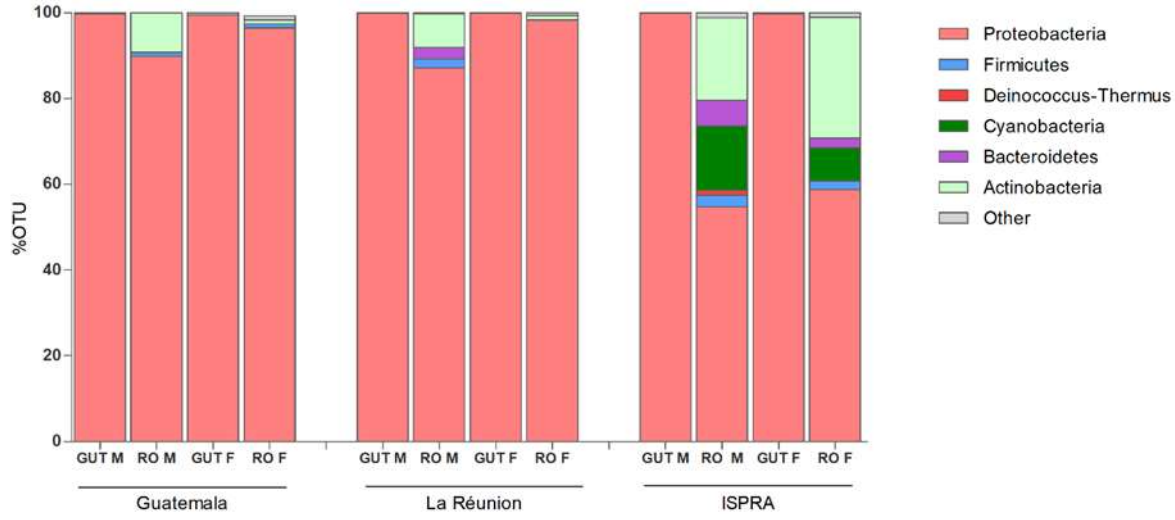

Figure 2. Phylum level composition (% of OTUs) in different organs of three different population of *C. capitata*. Only OTUs representing > 1% of the total reads are represented. RO: reproductive organs; F: females; M: males.

At genus level, among the Proteobacteria phylum, *Klebsiella*, belonging to the class of Gammaproteobacteria, resulted the most abundant bacteria with a range around 85-98% in the guts and 80-33% in male and female reproductive organs of all three population, except in the La Rèunion female where the massive *Providencia* is highly represented

(95,7%) while *Klebsiella* to 1.8%. *Providencia* was detected with a different percentage range (6-95%) in all samples although in male gut of La Réunion strain and female gut of ISPRA strain the percentage was lower that 1% (0.3% and 0.5% respectively) (Fig. 3; S2).

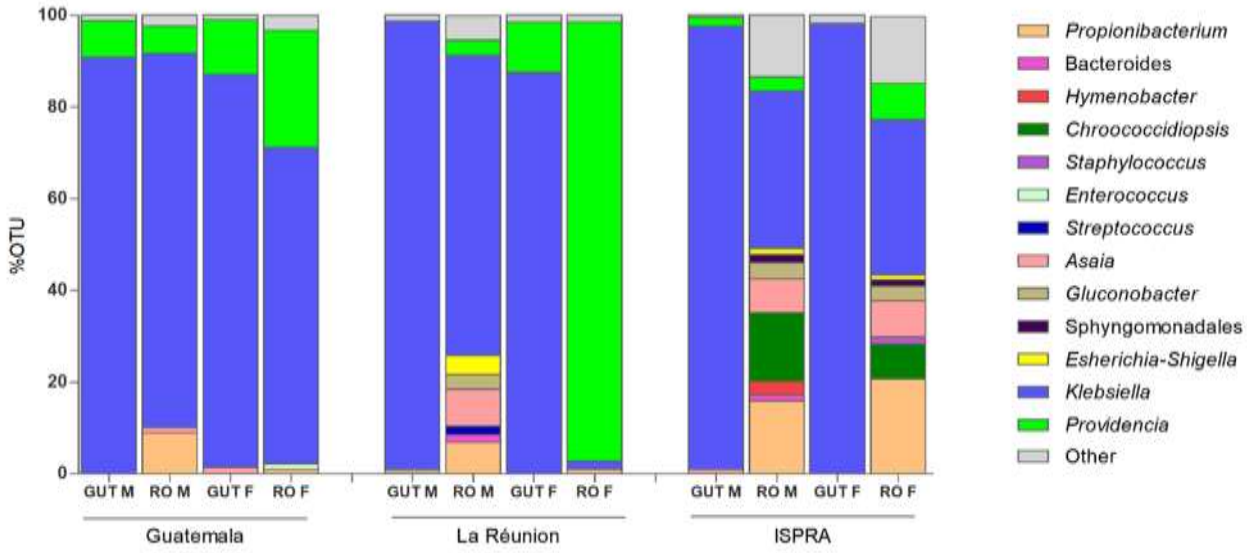

Figure 3. Genus level composition (% of OTUs) in different organs of *C. capitata*. Only OTUs representing > 1% of the total reads are represented. RO: reproductive organs; M: male; F: female.

Additionally, to *Klebsiella* and *Providencia*, some bacteria belonging to the phylum Proteobacteria (class Alphaproteobacteria) such as *Asaia* and *Gluconobacter* were detected, which were present in all samples albeit with variable percentages (see Supplementary Information Table S2). Considering the 1% cut-off for sample analysis, *Asaia* was present in male reproductive organs (1.1%) and female gut (1.4%) in Guatemala strain, male reproductive organs (8%) of La Réunion strain and male gut (1%), male (7.4%) and female (8%) reproductive organs of ISPRA strain. *Gluconobacter* was mostly detected in males (guts 1% and reproductive organs 3.2%) of La Réunion strain and, in male (3.6%) and female (3.2%) reproductive organs of ISPRA strain. Just in the ISPRA strain, the bacterium *Chroococcidiopsis*, belonging to the phylum Cyanobacteria, was detected in male (14,9%) and female (7.6%) reproductive organs. In all strain of *C. capitata*, *Propionibacterium* was detected only in male and female reproductive organs with a range of 1-20%

As already reported in several studies, no *Wolbachia* was detected in any samples [14].

4. Discussion

In our study we described the microbial composition of three different strains of *C. capitata*. Although the three different populations were reared under the same conditions, which is reflected in a homogeneous microbial community in the intestines dominated by *Klebsiella* and *Providencia*, as described in previously studies [15-18], highly different microbial communities were detected in the reproductive organs. As previously described in Comandatore et al. 2021 [19], *Asaia* was isolated from Ceratitis capitata adult of all the three population. Our results demonstrated the presence of *Asaia* in reproductive organs, together with *Propionibacterium* and *Chroococcidiopsis* thar resulted specifically associated in these districts of medfly. Particularly, *Chroococcidiopsis* was found in *C. capitata* ISPRA strain only. Our results open the way to future studies to verify the complexity of symbionts relationship and their role in the insect fitness. This study improves the knowledge of microbiota associated with *C. capitata* and offers new element to implement the pest management programs. Moreover, since we observed the presence of *Asaia* and the contextual absence of *Wolbachia* in the reproductive organs and considering the competition

phenomena that occurred between these two symbionts in some mosquitoes, in-deep investigations could be further performed to better evaluate the impact of microbial competition in the applications of *Wolbachia*-male sterile technique approach in Medfly.

Supplementary Materials: Table S1: % OTU Phyla in *C. capitata* strains; Table S2: % OTU Genera in *C. capitata* strains.

Author Contributions: Conceptualization, GF and CD.; methodology, AC and CD.; software, AC.; validation, AC, GF. and CD.; formal analysis, AC, IR, GF andCD.; investigation, AC, AS, IR, GG, GF and CD.; resources, GG.; data curation, AC, AS, GG, CD.; writing—original draft preparation, AC; writing—review and editing, AC, GF and CD.; visualization, AC, GG, AS, IR, GF and CD; supervision, GF and CD.; project administration, CD.; funding acquisition, GF. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: BioProject accession number PRJNA682004 to NCBI database.

Conflicts of Interest: The authors declare no conflict of interest.

Table S1. % OTU Phyla in *C. capitata* strains.

	Guat Gut M	**Guat RO M**	**Guat Gut F**	**Guat RO F**	**La Re GUT M**	**La Re RO M**	**La Re GUT F**	**La Re OR F**	**ISPRA GUT M**	**ISPRA RO M**	**ISPRA GUT F**	**ISPRA RO F**
Taxonomy	%	%	%	%	%	%	%	%	%	%	%	%
Bacteria;Acidobacteria	0	0	0	0	0	0	0	0	0	0	0	0,2
Bacteria;Actinobacteria	0	9,2	0	1	0	7,9	0	1,1	0	19,3	0	28,2
Bacteria;Armatimonadetes	0	0	0	0	0	0	0	0	0	0,8	0	0
Bacteria;Bacteroidetes	0	0	0	0,7	0	2,7	0	0,5	0	6	0,1	2,3
Bacteria;Cyanobacteria	0	0	0	0	0	0	0	0	0	15	0	7,8
Bacteria;Deinococcus-Thermus	0	0	0	0,2	0	0	0	0	0	1,2	0	0,8
Bacteria;Firmicutes	0,2	1	0,4	1,7	0	2	0,1	0,1	0	2,6	0,1	1,9
Bacteria;Fusobacteria	0	0	0	0	0	0,2	0	0	0	0,2	0	0
Bacteria;Gemmatimonadetes	0	0	0	0	0	0	0	0	0	0,1	0	0
Bacteria;Proteobacteria	99,8	89,8	99,5	96,4	99,9	87,2	99,9	98,3	99,9	54,8	99,8	58,8
Other	0,2	0	0,5	0,9	0,1	0,2	0,1	0,6	0,1	1,1	0,2	1
Other	0,2	0	0,5	0,9	0,1	0,2	0,1	0,6	0,1	1,1	0,2	1

* Guat = Guatemala strain, La Re = La Réunion strain, ISPRA = ISPRA strain, RO = Reproductive organs, M = male, F = female.

Table S2. % OTU Genera in *C. capitata* strains.

	Guat Gut M	Guat RO M	Guat Gut F	Guat RO F	La Re GUT M	La Re RO M	La Re GUT F	La Re RO F	La Re GUT M	La Re RO M	ISPRA GUT M	ISPRA RO M	ISPRA GUT F	ISPRA RO F
Taxonomy														
Propionibacterium	0	9	0	1	0	6,9	0	1	0	6,9	0	15,9	0	20,7
Bacteroides	0	0	0	0,7	0	1,7	0	0,4	0	1,7	0	1,4	0	0
Hymenobacter	0	0	0	0	0	0	0	0	0	0	0	2,9	0	0,7
Chroococcidiopsis	0	0	0	0	0	0	0	0	0	0	0	14,9	0	7,6
Staphylococcus	0	0,3	0	0	0	0,2	0	0	0	0,2	0	0,4	0	1,5
Enterococcus	0,2	0,4	0,4	1,2	0	0	0,1	0	0	0	0	0	0	0
Streptococcus	0	0,2	0	0	0	1,8	0	0	0	1,8	0	0,5	0	0,4
Asaia	0,2	1,1	1,4	0,4	0,6	8	0,5	0,4	0,6	8	1	7,4	0,6	8
Gluconobacter	0,3	0,3	0,4	0,3	1	3,2	0,6	0,2	1	3,2	0,3	3,6	0,2	3,2
Sphingomonadales	0	0	0	0,3	0	0	0	0	0	0	0	1,7	0	1,3
Escherichia-Shigella	0	0,2	0	0	0	4,2	0	0	0	4,2	0	1,3	0	1
Klebsiella	90,8	81,6	85,7	69	97,7	65,4	87,5	1,8	97,7	65,4	96,6	34,4	98,2	33,9
Providencia	8	6	11,9	25,6	0,3	3,3	11	95,7	0,3	3,3	1,9	3,1	0,5	7,9
Other	1,2	2,3	1	3,2	1,3	5,5	1,5	1,5	1,3	5,5	0,5	13,4	1,8	14,9

* Guat = Guatemala strain, La Re = La Réunion strain, ISPRA = ISPRA strain, RO = Reproductive organs, M = male, F = female.

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