'*Candidatus Erwinia dacicola*', a coevolved symbiotic bacterium of the olive fly *Bactrocera oleae* (Gmelin)

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The taxonomic identity of the hereditary prokaryotic symbiont of the olive fly *Bactrocera oleae* (Diptera: Tephritidae) was investigated. In order to avoid superficial microbial contaminants and loosely associated saprophytic biota, flies were surface-sterilized at the larval stage and reared under aseptic conditions until adult emergence. *B. oleae* flies originating from different geographical locations and collected at different times of the year were tested. Bacterial isolation was undertaken from the cephalic oesophageal bulb, which is known to be a specific site of accumulation for the hosted microsymbionts in the adult insect. Despite evidence of multiplication cycles taking place within the insect, attempts at cultivation of the isolated bacteria *ex situ* were not productive at any stage, leading to the choice of unculturable status definition. PCR amplification and nucleotide sequencing of the entire 16S rRNA gene consistently yielded a single sequence that displayed marked similarity with enterobacterial lineages, with closest matches (97 %) to *Erwinia persicina* and *Erwinia rhapontici*. The novel taxon differs from common intestinal bacterial species of fruit flies and from instances of culturable bacteria previously described in *B. oleae* raised without sterility precautions, which we also observed as minority occupants or occasional contaminants. The symbiont's identity is also distinct from *Pseudomonas savastanoi*. In all observations, the numerically dominant inhabitant of the olive fly oesophageal organ was the same unculturable organism, whose presence at later stages was also regularly observed in the midgut. A novel species is proposed, by virtue of its unique properties, under the designation '*Candidatus Erwinia dacicola*'.

INTRODUCTION

Symbiotic associations of micro-organisms with insects are widespread (Buchner, 1965; Moran & Baumann, 2000; von Dohlen *et al.*, 2001). Symbionts can be prokaryotes or eukaryotes and often can not be cultivated on common laboratory media (Smith & Douglas, 1987). In some associations, the host harbours the symbionts inside cells, such as the symbiosis between aphids and members of the genus *Buchnera* (Baumann *et al.*, 1995). The micro-organisms can provide a source of essential amino acids that

are lacking in the diet of their hosts. Each aphid acquires *Buchnera* from its mother (Douglas, 1998). Other symbionts are extracellular but live in close proximity to their hosts, such as the gut-inhabiting micro-organisms of termites (Breznak & Brune, 1994). Symbiosis can also be parasitic, such as that between *Wolbachia pipientis* and a variety of arthropods (O'Neill *et al.*, 1997; Werren *et al.*, 1995).

At the beginning of the last century, Petri (1909) described an example of hereditary symbiosis in the olive fly *Bactrocera* (= *Dacus*) *oleae* (Gmelin) (Diptera: Tephritidae) based on microscopic observations. Adults of *B. oleae*, which is the most important pest of olives across the entire range of the olive tree (Commonwealth Institute of Entomology, 1996), harbour micro-organisms inside a cephalic organ (oesophageal bulb or pharyngeal bulb), connected to the pharynx, in which the symbionts multiply rapidly, forming masses that reach the midgut (Fig. 1). The mother, endowed with

Published online ahead of print on 1 April 2005 as DOI 10.1099/ijs.0.63653-0.

Abbreviation: ARDRA, amplified 16S rDNA restriction analysis.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of '*Candidatus Erwinia dacicola*' isolate Patavina is AJ586620.

Fig. 1. Outline of the bacterial location within adult *Bactrocera oleae*. (a) Female fly laying its egg in an olive; the inserted folded ovipositor is visible. Bruises on the fruit surface are traces of previously laid eggs. (b) Anatomical depiction of the fly head in longitudinal section, with indication of the pharyngeal bulb (broken lines) leading to the visualization of its content, shown in the following panel [redrawn from Girolami (1973); original realized by hand from a real fly specimen under a camera lucida-equipped microscope]. (c) Scanning electron micrograph of the bacterial content present in the pharyngeal bulb. (d) Detail of (b); lumpy masses of bacteria (M) in transit towards the oesophagus and midgut are shown. (e) Transmission electron micrograph showing an insect cell (left) from the pharyngeal bulb epithelium and the bacteria stored within (right). (f) Transmission electron micrograph of the bacteria in longitudinal and cross section. Images (c), (e) and (f) are used courtesy of M. Mazzini. Bars: (a) 1 mm; (b) 100 μ m; (c, e) 1 μ m; (d) 200 μ m; (f) 0.1 μ m.

contractile perianal glands that become filled with bacteria, transmits symbionts to the eggs during oviposition. A bacterial cap-like mass is typically found around the egg's micropile. The bacteria multiply inside intestinal caeca of all larval stages, while their exact location inside pupae is not known. Adults harbour large amounts of bacteria in the oesophageal organ before emerging from their puparium (Petri, 1909). The same author suggested that the symbiont might be '*Bacterium*' (*Pseudomonas*) *savastanoi*, the causal agent of the olive knot disease, as it could be rescued from larvae; however, he pointed out that, if this were the case, the cultured cells would represent just a minimal fraction of the whole bacterial mass multiplying in the larval blind sacs and in the adult's oesophageal bulb. By comparing the exiguous

c.f.u. counts on nutrient plates with the microscopically visible bacterial volume filling the appropriate organs, he postulated that the bacteria hosted by the olive fly would exist in a state of prevailing non-culturability. Petri also warned that the ascription of the symbiont to '*B. savastanoi*' was to be considered a provisional assessment to be either confirmed or disproved once novel methods of cultivation or taxonomic identification were available to resolve the doubt. He admitted that, if the true symbiont were actually a different species, the cultured '*B. savastanoi*' would have had no longer to be seen as a fraction of cells having regained *ex situ* culturability, but rather as one of the opportunistic contaminants invading accessible cavities which he encountered on different occasions. It is worth remarking that, as

early as in 1909, a naturalist correctly took into account the possibility of a viable but not culturable (VBNC) condition and envisaged the need for direct identification tools to address the issue unambiguously. In his 132-page publication, Petri (1909) exhaustively reports analyses on over 1000 flies at all stages from egg, larva, pupal through to imaginal, along with efficient ways of sterile fly rearing coupled to different bacterial cultivation strategies. In later analyses, neither Buchner (1965) nor Hagen (1966) disputed the *P. savastanoi* designation. In our own initial studies, started over three decades ago (Girolami & Cavalloro, 1972), consistent with Petri's result, we could not observe any multiplication of oesophageal bulb bacteria, either under aerobic or anaerobic conditions, on different growth media. At the same time, we could verify, upon rearing insects on artificial media, the effects of a progressive loss of the bacteria, seen as lower vitality and fertility of the flies, accompanied by a shrinking of the oesophageal bulb to about one-third of its normal volume. As a consequence, moreover, this cavity, lacking its typical resident microflora, becomes much more prone to be invaded by other microbial species of various kinds, which can often result in deleterious infections for the insect (Girolami & Cavalloro, 1972). Other studies have shown that larvae developing from eggs laid from adults deprived of their bacteria are unable to develop in the olives (Hagen, 1966; Hagen & Tassan, 1972). The basis of the symbiotic advantage for the flies has been postulated to be a nutritional effect, both in terms of enhanced dietary protein hydrolysis and the synthesis of required amino acids lacking in the olive pulp (Tsiropoulos, 1980). The evidence agrees on a symbiotic interaction between *B. oleae* and its hosted microbiota, while issues that remain are the taxonomic nature of the symbiont, as well as its culturability *ex insecto*, on which, as described above, and reviewed by Manousis & Ellar (1988), different hypotheses had been put forward.

Today's availability of DNA-based methodologies, combined with the fast development of DNA sequence databases, enables the taxonomist to approach the description of non-culturable entities objectively, including insect symbionts (Baumann & Moran, 1997), whose identity and phylogeny can now be assessed. To this end, the present work focuses on the unculturable bacteria found inside the oesophageal bulb and the midgut of the olive fly *B. oleae*, in order to clarify the systematic placement of these microorganisms and trace their phylogenetic relationships.

METHODS

Insect origin. In November 2001, two groups of flies in the larval stage were collected in the field from unripe olives in Bari (southern Italy) and in the Lake Garda area (north-eastern Italy). Another two groups of flies were collected from overripe olives in the Liguria area (north-western Italy) in May 2002 and on green olives in September 2004.

Insect surface sterilization and rearing under microbiologically controlled conditions. The olives were kept in the laboratory until the release of mature larvae. All subsequent operations

were carried out under a laminar flow hood. The larvae were put in a 1% sodium hypochlorite solution for 2–3 min and then left to pupate in sterilized sand moistened with a 0.5% sodium hypochlorite solution. After 2–3 days, the pupae could be handled without being damaged and were removed from the sand, immersed again in 1% sodium hypochlorite solution for 5 min and rinsed with sterilized distilled water. The pupae were inserted separately into small, sterilized, open vials. The vials were enclosed in larger sterile vials, closed with cotton, containing LBA medium (1⁻¹: 10 g Bacto-tryptone, 5 g Bacto-yeast extract, 10 g NaCl, 16 g agar) on the bottom. A drop of sterile 50% glucose solution was applied to a small metal mesh located on the top of the larger vial to feed the newly emerged adults.

Insect dissection. Oesophageal bulbs and midguts containing bacteria were extracted under a stereoscope 2–3 days after the emergence of adults, by dissecting the flies using sterile forceps under a laminar flow. Ovipositors were removed from the abdomens of female adults under the same conditions. For bacterial analyses, the dissected oesophageal organs and midguts were placed in 2 µl distilled water on a sterile microscope slide and gently squeezed with a sterile pipette tip. The content was aspirated and transferred to sterile Eppendorf tubes. The ovipositors were placed directly in sterile microtubes. For inoculation on agar media, oesophageal bulbs and midguts were dissected, collected with sterile forceps and streaked directly onto the medium. For inoculation in liquid media, insects were dissected inside a drop of medium, on a sterile glass slide, the organs were squeezed onto a drop of the same medium and transferred into tubes containing the medium by using a sterile pipette.

Attempts at cultivation of bacteria on different media. The contents of oesophageal bulbs and guts, obtained as described above, were transferred directly onto agar media or suspended in liquid media. The following seven substrates were tested: brain heart infusion, malt agar, MacConkey agar, trypticase soy broth, MRS agar (De Man, Rogosa, Sharpe), LB medium and tryptone/yeast extract (all from Merck). These media had been successfully used for *in vitro* cultivation of a 'near-*Erwinia*' species of gut bacteria associated with the insect thrip *Frankliniella occidentalis* (de Vries *et al.*, 2001). All the media were inoculated with the content of oesophageal bulbs and guts. As control, two plates for each medium were streaked with the content of oesophageal bulbs and guts of some non-surface-sterilized flies. Plates were incubated at 23 °C and first inspected after 72 h.

Morphological characterization of bacteria. Specimens for microscopic observations were obtained by air-drying microbial suspensions resulting from squashing an excised oesophageal bulb in a water drop on a glass slide. Bacteria were heat-fixed and Gram-stained to be observed under bright-field microscopy. Total cell counts were carried out by resuspending the whole oesophageal bulb content in 1 ml water in an Eppendorf tube and staining the suspension by using the BacLight viability kit (Molecular Probes). Cells were subsequently observed and counted in epifluorescence microscopy. Transmission and scanning electron micrographs of different organs of *B. oleae*, obtained as described by Mazzini & Vita (1981), were kindly made available by Professor Massimo Mazzini (Università della Tuscia, Viterbo, Italy).

DNA extraction and 16S rRNA gene amplification. DNA was extracted from the content of oesophageal bulbs, midguts or ovipositors as described by Palmano *et al.* (2000). PCRs were carried out in 25 µl containing 1 µl from the nucleic acid extraction, 200 µM dNTPs, 0.8 µM of each primer, 0.625 U *Taq* DNA polymerase (Amersham Biosciences) and 2.5 µl 10× PCR buffer (500 mM KCl, 100 mM Tris/HCl, pH 9, 15 mM MgCl₂). Thermal processing, conducted in an INC PTC-100 programmable thermal

controller (MJ Research), consisted of 95 °C for 2 min followed by 35 cycles of 96 °C for 30 s, 50 °C for 30 s, 72 °C for 90 s and a final extension at 72 °C for 10 min.

The universal bacterial 16S rRNA primers used were fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rP1 (5'-ACGGTTACCTTGTTACGACTT-3') (Weisburg *et al.*, 1991), yielding an amplicon of approximately 1500 bp. PCR products were separated by electrophoresis on a 1% agarose gel and visualized under UV following staining with ethidium bromide. Amplified rDNA restriction analysis (ARDRA) using the endonuclease *HhaI* was carried out on the amplicons to compare the profile with those obtained from colonies of culturable bacteria occasionally arising on plates.

DNA sequencing. The amplification products from 11 samples, eight from Bari, one from Lake Garda and two from Liguria, all from single oesophageal bulbs, were analysed by dideoxynucleotide sequencing. In addition, a sample from the content of a midgut and one from an ovipositor (both from Liguria) were included.

PCR products were purified with a QIAquick PCR purification kit (Qiagen) and used as template for sequencing with an ABI PRISM automatic DNA sequencer. Additional primers were devised from the sequences obtained in order to walk through the entire 16S rRNA gene sequence: fR2 (5'-CGTGCTCAGTTCAGTG-3'), fL2 (5'-GGAAGTGCATTCGAACTG-3'), rR2 (5'-CTCGTGTGTGAAATGTTGG-3') and rL2 (5'-AAGGCACTAAGGCATCTCTG-3').

Phylogenetic analysis. A BLASTN GenBank analysis of the sequences obtained was run through the NCBI website. The sequences were subsequently aligned with their closest relatives as downloaded from the RDP database (Cole *et al.*, 2003) using the BioEdit sequence alignment editor (Hall, 1999). Distance matrices were calculated using the substitution model of Jukes & Cantor (1969). Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values were calculated with 100 replicates as a measure of reliability (Felsenstein, 1985).

RESULTS AND DISCUSSION

Insect rearing under microbiologically controlled conditions

As one of the critical aspects of this research was ascertaining whether or not the bacteria found within the insect are the result of a specific interaction involving hereditary transmission of an unculturable bacterium to the offspring, particular precautions were undertaken to minimize exposure to the potential intake of microbes from the environment. Such necessity led us to devise an original method which successfully ensured a minimized level of contamination. Upon isolation of *B. oleae* from olives, an immersion in a sodium hypochlorite solution was carried out both at larval and pupal stage and sterile conditions were observed throughout the remaining parts of the life cycle. Possible external microbial contamination was tested on newly hatched adults by leaving them in contact with agarized media suitable for bacterial growth on which they were allowed to land and walk within the sterile recipient vial where they emerged from the pupal stage. Routinely, no colonies could be detected after up to several days of incubation inside these vials upon testing over 100 adult flies. Only on three occasions, during 2 years of work, two,

supposedly contaminant, bacterial colonies and one fungal colony arose in the tubes. In contrast, many bacterial and fungal colonies regularly developed on the medium in control vials containing adults that emerged from untreated pupae. The newly emerged flies were kept alive in the vials before dissection for 2 or 3 additional days and fed with sterile glucose solution. No bacterial colonies developed, even from adults faeces on the bottom of the tubes, confirming the efficiency of the aseptic treatment. Nevertheless, the oesophageal bulbs and the midguts of the same adults contained profuse amounts of bacteria, as judged by microscopy. Since in this study, as in previous ones (Petri, 1909; Girolami & Cavalloro, 1972), masses of bacteria have often observed to be continuously conveyed to the alimentary tract during adult life, for a period that can extend up to several months, we are led to conclude that bacteria continue to multiply within the oesophageal bulb. If this were not the case, considering the observed frequent rate of emission of bacterial masses down the midgut, the oesophageal bulb would be empty within a few days. On the contrary, we have observed that adults keep their full load of bacteria in the bulb for periods of months while continuing the periodic discharge and transit of bacterial cells through their alimentary tract. Consequently, the midgut of the adults, including those reared under aseptic conditions, contains abundant bacteria. These, like those isolated from the oesophageal bulb, are not able to grow on standard microbiological media. In flies obtained under these microbiologically controlled conditions, the bacteria are also found in the ovipositor and transmitted to the surface of the laid egg with no apparent difference from the situation of flies growing in the wild. Our analyses, as described below, verified the presence of unique, pure and identical sequences from bacteria isolated from the oesophageal bulbs, midgut or ovipositor, from flies originating independently from three Italian regions located as far as 800 km apart.

Bacterial cultivation attempts

Repeated attempts at bacterial isolation from the content of both oesophageal bulbs and midguts were pursued by streaking on several microbiological media. No colonies developed on the plates in the majority of cases. Only occasionally, sporadic colonies, in numbers which were orders of magnitude lower (1–30 c.f.u.) than those of bacterial cells actually streaked, appeared on plates. However, their amplified 16S rRNA gene sequence displayed an ARDRA profile completely different from the one consistently found upon direct PCR of the unculturable bulk bacteria residing in the oesophageal bulb. The same result was obtained for liquid media. Conversely, in all plates inoculated with bacterial masses from non-surface-sterilized insects, numerous bacterial and fungal colonies developed, in line with reports from other studies (Tsiropoulos, 1983; Belcari *et al.*, 2003), but, even in this case, c.f.u. counts were, by far, never large enough to account for the actual cell numbers actively maintained within the fly, suggesting that, irrespective of

the presence of other bacteria consequent to the lack of surface sterilization treatments, the main symbiont core was always represented by a non-culturable entity. These results essentially confirm the repeated observations reported by Petri (1909).

DNA amplification and nucleotide sequencing

The DNA extracted upon lysing the bacteria from oesophageal bulbs, guts or ovipositor, was used as a template for the PCR amplification of the 16S rRNA gene. A PCR product of the expected size of about 1500 bp was obtained in all cases with the primers fD1/rP1. The corresponding nucleotide sequences of the PCR-amplified 16S rRNA gene were obtained. In all, we compared the content of the oesophageal bulb, the midgut or the ovipositor of 13 flies developed from mature larvae grown inside unripe or overripe olives from different regions in Italy and collected at different periods of the year. The sequences were found to be all identical (GenBank accession no. AJ586620). This confirms that a single bacterial species represents the entire, or at least the largest fraction of the symbiotic microflora carried inside *B. oleae* adults raised through the above procedure. As the same 16S rRNA gene sequence was detected in all individuals analysed, regardless of place of collection, period of the year and ripeness of the olives, and as the same bacteria, also filling specialized anal glands present in *B. oleae*, were found in ovipositors, the data concur to rule out that these bacteria could originate from food or the environment and suggest, as Petri indicated in 1909, that, instead, they are maternally transmitted. In this respect, it is worth adding that, as shown by Petri (1909) and confirmed by our own observations, as soon as the *B. oleae* embryo completes its development in the egg, the unhatched larva already contains the bacterial symbiont within its blind sacs even before it has had a chance to start feeding on the olive pulp. This further underlines that its associated bacteria, which were shown to be deposited on the laid egg's micropile by the mother (Petri, 1909), are presumably acquired from that contingent rather than being recruited exogenously via nutrition.

Phylogenetic analysis

Similarity studies against the GenBank database indicated that the *B. oleae* symbiont belongs to the family *Enterobacteriaceae* within the class 'Gammaproteobacteria'. Its 16S rRNA gene sequence is 97% similar to those of *Erwinia persicina* (GenBank accession no. Z96086) and *Erwinia rhapontici* (AJ233417), two weak plant pathogens belonging to the subgroup of *Erwinia amylovora*. A somewhat lower similarity was found to an as-yet unidentified bacterium detected in studies on aphid symbionts (GenBank accession no. AB004763).

Fig. 2 reports a neighbour-joining tree based on 16S rRNA gene sequences, showing the taxonomic position of the symbiont of *B. oleae* with respect to its closest relatives. The *B. oleae* symbiont represents a distinct branch of the phylogenetic tree, well supported by a high bootstrap value.

The result of nucleotide sequencing therefore shows that the symbiont is not *P. savastanoi* (presently regarded as a subspecies of *Pseudomonas syringae*) as suggested theoretically by Petri (1909) and later by Hellmuth (1956), Buchner (1965) and Hagen (1966), but questioned by our previous work (Girolami & Cavalloro, 1972). In this respect, this work contributes to the closing of a century-old debate on the identity of the olive fly symbiont. As quoted, Petri (1909) had simply hypothesized that symbionts inside oesophageal bulbs could be *P. savastanoi*, the causal agent of olive knot disease, as he obtained small hyperplasiae on olive tree twigs inoculated with the content of oesophageal bulbs; but, at the same time, he left the possibility open that this species could just be a mere minority co-occupant of organs primarily invaded by a different, yet to be determined, unculturable species. As we observed in our previous work, *B. oleae* flies can often contain minor amounts of plate-culturable bacteria in their oesophageal organ, among which those crowding the *Olea europaea* carposphere and phyllosphere are the most prone to be recruited, thus giving a high chance of finding the olive knot causal agent. Other species that can be occasionally found include

Fig. 2. Neighbour-joining tree showing the phylogenetic position of the *B. oleae* symbiont based on 16S rRNA gene sequence analysis. Bootstrap values > 50% are reported on nodes. GenBank accession numbers are indicated.

Klebsiella pneumoniae, *Pantoea agglomerans* (Tsiropoulos, 1983) and, in some instances, the one that Hellmuth (1956) referred to as '*Agrobacterium luteum*' and that corresponds morphologically to the organism that Petri (1909) termed '*Ascobacterium luteum*'.

In terms of comparisons with other insect interactions, the *Erwinia* that we found is not related to *Buchnera aphidicola*, the primary symbiont of aphids. Its lineage is instead closer to the 'fruit-fly-associated bacteria' commonly present both on the infested fruits and in the oesophageal bulb or intestine of fruit flies, which are considered to be 'rapidly growing aerobic organisms with no special growth requirements' (Lloyd *et al.*, 1986). Among these, the most common species are *Klebsiella oxytoca* (Rossiter *et al.*, 1983), *Enterobacter cloacae* and *Pantoea agglomerans* (Lloyd *et al.*, 1986).

The relationship observed for the olive fly represents to our knowledge the first example of an obligate symbiotic relationship described among the Tephritidae; the insect also differs from all other known members of the family in the peculiar structure of its oesophageal bulb. Indeed, those of other fruit flies within the genera *Rhagoletis*, *Ceratitis*, *Anastrepha*, *Dacus* and *Bactrocera* (with the exclusion of *B. oleae*) are similar among themselves but very different from that of *B. oleae* and were described as '*Ceratitis*-type' oesophageal bulbs (Girolami, 1973). In that work, we demonstrated that such an organ was present in all Tephritidae and not only in the olive fly, as previously supposed (Buchner, 1965). We have also investigated, with the same procedure, other tephritids in which the oesophageal bulb is very different from the one of *B. oleae* and the symbionts, when present, belong to species which are different from '*Candidatus Erwinia dacicola*' (C. Capuzzo, L. Mazzon and V. Girolami, unpublished results).

It could be hypothesized that such marked morphological differences of the oesophageal bulb, an organ whose purpose is apparently to host bacteria, may reflect coevolution with a specific symbiotic bacterial species absent in other fruit flies.

Due to its phenotype and properties, namely its specific ecological niche, the unique mutual interaction with the animal host and distinct phylogenetic lineage, the *B. oleae* symbiont described herewith is proposed to represent a novel taxon within the family *Enterobacteriaceae*. Complying with the guidelines introduced by Murray & Stackebrandt (1995), given the as-yet *in vitro* unculturable status of the bacteria, a *Candidatus* designation is chosen.

Description of '*Candidatus Erwinia dacicola*'

Erwinia dacicola (da.ci'co.la. N.L. n. *Dacus* the generic name of the host fly; L. suff. -cola from L. masc. or fem. n. *incola* an inhabitant, dweller; N.L. masc. or fem. n. *dacicola* an inhabitant of *Dacus*).

[(*Enterobacteriaceae*) NC; G-; R; NAS (AJ586620); oligonucleotide sequences of unique regions of the 16S rRNA

gene are 5'-CCUUUUUGAUUGACGU-3' and 5'-CGAAGAGGUUAAUAAC-3'; S (*B. oleae*, oesophageal bulb); M]. Straight to moderately curved rod-shaped cells, 2–2.7 × 0.5–0.6 µm. Cell envelope structurally coherent with the Gram-negative model; presence of inner and outer membrane with relatively thinned periplasmic space visible in TEM preparations, abundant polyribosomal bodies, visible nucleoid network, presence of small polyphosphate-like electron-opaque granules and of sparse glycogen-like inclusions; absence of evident polyhydroxybutyrate bodies, absence of capsular envelope and other external layers. Negative to Gram staining. Unculturable on microbiological media. Symbiont of the olive fly *Bactrocera* (= *Dacus*) *oleae* Gmelin (Diptera, Tephritidae). Located exclusively in association with its host species within the following structures: larval blind sacs, imaginal oesophageal bulb, mid- and hindgut, anal glands, ovipositor and egg surface near the micropile. Basis of assignment: 16S rRNA gene sequence (GenBank accession no. AJ586620). G+C content of the 16S rRNA gene sequence is 55 mol%.

ACKNOWLEDGEMENTS

The authors are grateful to Sara Alberghini and Alessia Pisccedda, for skilful collaboration in the practical tasks of bacterial cultivation attempts and insect rearing. Valerio Giaccone and Riccardo Miotti Scapin are acknowledged for part of the chemical supplies. Massimo Mazzini is credited for the kind availability of transmission electron micrographs. We are particularly thankful to Stefano Saguato for providing the olives from the Liguria area. This work was made possible in part thanks to the following grants: Progetto di Ateneo dell'Università di Padova CPDA 028245 e PRIN n. 2002074345.

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