

A new endosymbiotic bacterium species associated with a nematode species of the genus *Xiphinema* (Nematoda, Longidoridae).

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Nematodes are the third largest group of metazoans; among them, the Family Longidoridae comprises two main genera of plant parasitic nematodes, *Xiphinema* and *Longidorus*, which contain several virus-vector species, e.g. the species *X. index*, the vector of grape fanleaf virus (GFLV), a serious pathogen of grapes. Bacterial endosymbionts of plant-parasitic nematodes represent a field of research that has become active in recent years. In this work we present a detailed characterization of the endosymbiont bacterium found in the nematode *X. pachtaicum* from the rhizosphere of sour orange trees (*Citrus x aurantium* L.) from Cordoba, Spain, and, based on morphological, phylogenetic and genomic characteristics propose a novel candidate genus and species for this uncultured bacterium (strain IAST). An intracellular bacterium, strain IAST, was observed to infect several species of the plant-parasitic nematode genus *Xiphinema* (*X. astaregiense*, *X. incertum*, *X. madeirense*, *X. pachtaicum*, *X. parapachydermum* and *X. vallense*). The bacterium could not be recovered on axenic medium. The localization of the bacterium (via light and fluorescence in situ hybridization microscopy) is in the *X. pachtaicum* females clustered around the developing oocytes, primarily found embedded inside the epithelial wall cells of the ovaries, from where they are dispersed in the intestine. Transmission electron microscopy (TEM) observations supported the presence of bacteria inside the nematode body, where they occupy ovaries and occur inside the intestinal

epithelium. Ultrastructural analysis of the bacterium showed cells that appear as mostly irregular, slightly curved rods with rounded ends, 0.8–1.2 μm wide and 2.5–6.0 μm long, possessing a typical Gram-negative cell wall. The peptidoglycan layer is, however, evident only occasionally and not detectable by TEM in most cells. Another irregularly occurring shell surrounding the endosymbiont cells or the cell clusters was also revealed, probably originating from the host cell membrane. Flagella or spore-like cells do not occur and the nucleoid is diffusely distributed throughout the cell. This endosymbiont is transmitted vertically through nematode generations. These results support the proposal of IAST as a new species, although its obligate intracellular and obligate endosymbiont nature prevented isolation of a definitive type strain. Strain IAST is therefore proposed as representing '*Candidatus Xiphinematocola pachtaicus*' gen. nov., sp. nov.