Susceptibility of wireworm larvae (Agriotes sordidus) (Coleoptera: Elateridae) to isolates of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) and their symbiotic bacteria (Morganellaceae: Photorhabdus and Xenorhabdus) from France under laboratory conditions

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Susceptibility of wireworm larvae (*Agriotes sordidus)* (Coleoptera: Elateridae) to isolates of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae) and their symbiotic bacteria (Morganellaceae: *Photorhabdus* and *Xenorhabdus*) from France under laboratory conditions

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Abstract: Wireworms, the larvae of click beetles (Coleoptera: Elateridae), are considered economically important pests of a wide variety of crops in many parts of the world. In France, the main agricultural pests are *Agriotes sordidus*, *A. lineatus* and *A. sputator*. The use of chemical pesticides is more and more controversial due to their detrimental effect to both environment and human health. The development of effective biological control agents is therefore particularly desirable to control this insect pest. Our study aims to evaluate the virulence of entomopathogenic nematodes (EPNs) strains belonging to five different species (*Steinernema feltiae, S. carpocapsae, S. affine, S. boemarei* and *Heterorhabditis bacteriophora*) and their symbiotic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.) against *A. sordidus* larvae in laboratory conditions. The infestations with EPN strains were performed at two different temperatures, 18 °C and 23 °C, and the mortality rates of wireworm larvae were measured within 72 h after exposure. The wireworm larvae susceptibility to EPNs is mainly low and strain or species specific. These results are in agreement with previous studies which state that wireworms are generally considered to be resistant to EPN infection. We then measured the entomopathogenicity of about twenty EPN-symbiotic bacteria by direct injection of the bacterial culture (10^4 ufc per larvae) in the haemolymph of *A. sordidus* larvae. One strain of the panel, *Xenorhabdus kozodoii* FR48, exhibited a high level of entomopathogenicity by killing almost 100 % of the wireworm larvae within 48 hours post injection. We showed that *X. kozodoii* FR48 was able to multiply in the haemolymph suggesting that septicaemia was the cause of the insect death. In order to better understand the genetic mechanisms involved in the virulence of *Xenorhabdus* towards wireworm larvae, the genomic sequences of the entomopathogenic strain *X. kozodoii* FR48 and of two other non-pathogenic strains of *X. kozodoii* (FR71 and FR74) were compared. We used comparative genomics tools (https://mage.genoscope.cns.fr/microscope) to identify in the genome of *X. kozodoii* FR48 some specific genes and genomic regions putatively involved in the virulence. Among the candidate genes, we identify a large number of genes encoding for proteins of unknown function and a large genomic region which may be involved in the modification of LPS. This genetic locus is closely related to a well-described locus in the bacterium *Vibrio. vulnificus*, both in terms of the organisation of the genes and their sequence similarity. This locus is involved in the synthesis of a modified sialic acid which is a major virulence factor of *V. vulnificus*. In the case of *X. kozodoii* FR48 strain, we showed that this locus was highly expressed in insect haemolymph (*G. mellonella* and *S. littoralis*), which suggests a potential role in the entomopathogenicity of this bacterium. Our study describes for the first time that a

bacterium of the *Xenorhabdus* genus is entomopathogenic towards wireworm larvae, thus opening up new promising perspectives for the biocontrol of this pest. Further investigations are needed to better understand the interactions between *X. kozodoii* FR48 and wireworm larvae.