The aim of the project is to verify the hypothesis that some species of gut-associated bacteria of larvae of insects from the family *Scarabaeidae* can exert an antagonistic action against bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. transmitted by entomopathogenic nematodes, which may constitute an important mechanism of defence against infection caused by these pathogens.

The bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. are obligate symbionts of entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis.* One of the stages of the development of these nematodes are free living invasive larvae that penetrate the layer of soil, typically to a depth of approx. 15 cm, in order to find the insects that inhabit it. They have the ability to actively seek out the host and then penetrate into its body through orifices of the respiratory and digestive systems, where they release mutualistic bacteria. Nematodes protect the mutualistic bacteria against adverse environmental factors. *Photorhabdus* spp. and *Xenorhabdus* spp. cause the death of the insect within approx. 24–48 hours and prepare an optimal environment for the growth and development of the nematode by decomposing the tissues of the host using lytic enzymes. These bacteria also have the ability to produce different types of compounds which inhibit the growth of other microorganisms present in the environment. So far there have been no reports on antagonistic mechanisms that would work in the opposite direction, i.e. production of substances that would inhibit the growth of the bacteria *Xenorhabdus* spp. and *Photorhabdus* spp by bacteria of the insect gut microflora. However, this type of interaction can be expected, since the optimal habitat of the insect gut microbiota is the digestive canal of a living host, and the bacteria released by entomopathogenic nematodes cause a rapid death of the insect. The bacteria that make up the intestinal microflora of insect larvae should have developed their own defense mechanisms that would inhibit the growth of pathogenic bacteria. It is therefore possible that some of the gut associated bacteria are capable of producing substances that have an antagonistic action against *Xenorhabdus* spp. and *Photorhabdus spp.*, and can protect both the gut associated microorganisms and the entire body of the insect. The planned research will enable the verification of this hypothesis.

It can be assumed that some of the bacteria present in the gut of *Scarabaeidae* can synthesize bacteriocins, as representatives of the genera *Pseudomonas, Serratia, Enterococcus* i *Lactococcus*, which are known for the production of bacteriocins, have been found among them. It is, therefore, not excluded that the gut of *Scarabaeidae* may also contain bacteria that produce compounds which exert an antagonistic action on the bacteria *Xenorhabdus* and *Photorhabdus* . It is also possible that some gut associated bacteria of *Scarabaeidae* may synthesize other kinds of substances with antibiotic activity. So far, there have been no large-scale studies on isolating antibiotics from gut associated bacteria of insects; however, this source of antibacterial substances is certainly worth more attention.

In the project presented in this proposal, experiments will be conducted using gut associated bacteria of *Scarabaeidae* larvae.The family *Scarabaeidae* consists of over 30 thousand beetle species worldwide. In Poland, they are represented, among others, by *Melolontha* spp and *Amphimallon* spp. The population of some representatives of this genus, particularly *Melolontha melolontha* and *Amphimallon solstitiale*, has significantly increased in recent years, placing intense pressure on the environment.

Entomopathogenic nematodes play an important role in regulating the number of harmful insects. Laboratory experiments have shown, however, that larvae of *M. melolontha* and *A. solstitiale* have a relatively low susceptibility to infection by entomopathogenic nematodes. Therefore, larvae of this species of beetle could be a promising source of gut bacteria capable of inhibiting the growth of the bacteria carried by *Steinernema* spp. and *Heterorhabditis* spp.

In the initial stage of the research, 1800 bacterial strains will be isolated from the midgut of of *M. melolontha* and *A. solstitiale*. Then isolates will be studied for their ability to inhibit the growth of five EPB species, using several types of growth inhibition plate assays. Isolates proved to be capable of inhibiting the growth of EPB will be identified by molecular methods. The final step of the study will involve a metagenomic analysis of the gut microflora of a larger number of *M. melolontha* and *A. solstitiale* larvae coming from different backgrounds. The experiments will allow to determine the spread of the previously identified isolates in populations of *M. melolontha* and *A. solstitiale*, which will provide an answer to the question of whether these bacteria are commonly found, or whether they are only present in the gut of few individuals. The study will also demonstrate whether the increased resistance to infection by EPN is correlated with the presence and/or the number of bacteria exhibiting antagonistic properties against EPB.

The results of the study will have a great cognitive importance. They will help explain the mechanisms of bacterial interspecies competition and the resulting role of the nematode-bacterium-insect complex in ecosystems. They will also play an important role in understanding the mechanisms of insect defence against infection by entomopathogenic nematodes and developing methods for overcoming these mechanisms. They will contribute to the development of biological methods of reducing the numbers of harmful insects and to limiting the use of chemicals in the environment.