SCIENTIFIC COMMUNICATION

Methodology for bioassay entomopathogenic *Bacillus* against sand fly (Diptera, Psychodidae, Phlebotominae) larvae

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ABSTRACT. The objective of this research is to present a method to bioassay entomopathogenic *Bacillus* species against sand fly (Diptera, Psychodidae, Phlebotominae) larvae. The vector *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera, Psychodidae, Phlebotominae) and some *Bacillus* strains were used in laboratory to develop this method. Some characteristics of this method are commented and the importance to develop a standard method to test pathogenicity of *Bacillus* species against phlebotomine larvae is also discussed.
KEY WORDS. Phlebotominae, *Bacillus*, bioassay, method

Due to the importance of phlebotomine species (Diptera, Psychodidae, Phlebotominae) as vectors of leishmaniasis, it is necessary to investigate strategies to be used in their control, especially against their immature stages. Bacterial species of the genus *Bacillus* have been used as an alternative control method for some Diptera of medical importance and the possibilities of using these enthomopathogenic agents against phlebotomine vectors were discussed (WARBURG 1991; WARBURG *et al.* 1991; OLIVEIRA FILHO & MELO 1994; PENER & WILAMOWSKI 1996; ROBERT *et al.* 1997). However, few studies have been done aiming to observe the pathogenic activity of *Bacillus* species to phlebotomine larvae (BARJAC *et al.* 1981; WERMELINGER *et al.* 1995a; PENER & WILAMOWSKI 1996; WERMELINGER *et al.* in press). Since it is difficult to compare results of bioassays which used different methodologies, it is important to develop and present techniques that could allow possible comparisons between such tests. Considering that no bioassay method with *Bacillus* for phlebotomine larvae has been described with details, the aim of this research is to present a methodology to bioassay entomopathogenic *Bacillus* against larvae of these insects.

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The technique was developed with *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera, Psychodidae, Phlebotominae), the vector of the American visceral leishmaniasis, obtained from a phlebotomine insectary of the laboratory in the Department of Entomology of the Oswaldo Cruz Institute (IOC) and with bacterial strains of *Bacillus thuringiensis* serovar *israelensis* (B.t.i.), *Bacillus sphaericus*, and *Bacillus thuringiensis* serovar *morrisoni* from the Department of Bacteriology of the same Institute, in Rio de Janeiro, State of Rio de Janeiro, Brazil. The results of these tests are presented in WERMELINGER *et al.* (1995a in press). *L. longipalpis* larvae was fed with industrialized food for aquarium fish (Vitormonio ®) which has been used in insectary to rear *L. longipalpis* and *Lutzomyia intermedia* (Lutz & Neiva, 1912) (Psychodidae, Phlebotominae) (RANGEL *et al.* 1985). This technique consists of groups of 20 young third instar larvae of *L. longipalpis*, selected by size (around 1 mm) and placed into Petri dish plates (150 mm by 20 mm), previously sterilized, each one representing one experimental unit. Each plate contained in its bottom a layer of Paris plaster approximately 5 to 10 mm thick, humidified with distilled water aiming to maintain the humidity above 90% within them. These Petri dishes were individualized in aseptic plastic pots, with 170 mm diameter and 70 mm height, lined with sterile paper filter, also humidified.

Second instar larvae of *L. longipalpis* were previously maintained in Petri dishes in similar conditions as the bioassay for adaptation before being used. At the beginning of the third stadium, larvae of *L. longipalpis* were placed in sterile Petri dishes with each one of them as one experimental unit. Depending on the test each one of them can have different number of replications and concentrations of the products. For example, WERMELINGER *et al.* (1995a) used three replications and eight concentrations and WERMELINGER *et al.* (in press) used at least four repetitions and two concentrations.

The inoculum must be quantified, mixed and homogenized with larval food before being used for larvae in treated groups. The concentrations of such inoculum are formed using biomass (mg of dry weight) bearing delta-endotoxin crystals and spores of *Bacillus*, obtained by bacterial growth in a New Brunswick 14 liter capacity fermentator loaded with soya flour, divalent metals and yeast extract liquid culture medium, during 22 hours at 33°C, and harvested with continuous centrifugation (3500 rpm). The biomass material is weighed within an Eppendorf and suspended in 0.5 ml distilled water, homogenized in vortex by 20 to 30 seconds and mixed with larval food (1 g), previously autoclaved at 120°C during 20 minutes and offered to sand fly larvae. After 48 hours all food not offered to larvae should be discarded. Larval food in the control lots is humidified with 0.5 ml distilled water aiming to standardize its texture. The food with inoculum and that humidified of the control lots are offered to larvae during seven days and changed every 48 hours. After this period, a normal food (without inoculum) is offered to the groups of larvae of *L. longipalpis* until they reach the pupal stage. The percentage of mortality is calculated according to the number of pupae obtained. This is important because the duration of the larval cycle of *L. longipalpis* increases when larvae of this species are treated with B.t.i., according to the results of BARJAC *et al.* (1981) and WERMELINGER *et al.* (1995b). It seems that BARJAC *et al.* (1981) and PENER & WILAMOSKI (1996) maintained larvae of *L.*

longipalpis during its whole larval cycle feeding on food containing the inoculum. In this present method, the period of seven days of inoculum ingestion by *L. longipalpis* larvae proved to be enough to study susceptibility patterns of this vector to entomopathogenic *Bacillus* (WERMELINGER *et al.* 1995a,b, in press). For this reason the period of seven days of feeding in contaminated food should be used in testing enthomatogenic *Bacillus* against Diptera vectors.

ACKNOWLEDGEMENTS. To the staff of the Laboratory of Bacterial Physiology of the Department of Bacteriology and to the Sandfly Insectary, of the Leishmaniasis and Onchocerciasis Vector Laboratory, of the Department of Entomology of Oswaldo Cruz Institute for technical support. To CNPq and FAPEMIG for support.

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Recebido em 19.VIII.1999; aceito em 25.VIII.2000.