

**TITLE:** DEVELOPMENT OF A NEW *Bacillus thuringiensis* SUBSP. *israelensis* ISOLATION METHODOLOGY FROM SOIL SAMPLES

**AUTHORS:** PEREIRA, A.L.S.; BRANDÃO, S.S.F.; OLIVEIRA, E.J.A.

**INSTITUTION:** INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA DE PERNAMBUCO - IFPE CAMPUS RECIFE (AVENIDA PROFESSOR LUIZ FREIRE, 500, CEP 50740-545, RECIFE-PE, BRAZIL)

**ABSTRACT:**

*Bacillus thuringiensis* subsp. *israelensis* (Bti) is the most widely used biopesticide in mosquitoes control, offering a target-specific action with no toxicity to humans and environmental-friendly. In addition, Bti strains are generally found to be resistant to different antibiotics, showing a particular resistance against penicillin. Several techniques have been used to isolate the entomopathogenic bacteria from the environment, especially from soil samples. Penicillin is also commonly used in selective media for Bti isolation, with a previous inoculation into an enrichment medium. Therefore, the goal of this study was to develop a Bti isolation technique with a higher selection from the soil by inoculating the pasteurized bacterial suspension directly on the medium with penicillin G, being tested against the standard method using an enrichment before the selective medium step. A sporulated suspension of Bti IPS-82 strain was pasteurized at 80°C for 12 min and tested in two situations: inoculation into nutrient agar for enrichment at 35°C for 24h followed by selective medium cultivation with penicillin G in concentrations of 0 to 100 mg/L and selective medium inoculation directly from the pasteurized suspension in penicillin G concentrations of 0 to 20 mg/L. The Bti growth was observed in all of the tested penicillin media concentrations of 0 to 100 mg/L (0, 10, 20, 40 and 100 mg/L) inoculated after enrichment phase, presenting a lower selective pressure than the direct inoculation method into selective medium, which exhibited growth only in lower penicillin concentration of 2,5 mg/L, with a reduced growth in 5 mg/L and no growth in 10 mg/L and 20 mg/L concentrations. The data were analyzed by t-tests and the 2,5 mg/L penicillin concentration was chosen for the use in selective media for the developed methodology. The results show a higher selective pressure when using pasteurization followed by selective medium cultivation, which might be advantageous for the Bti isolation from contaminated natural soil samples. The outsider species inhibition is being verified in the current test phase with several isolation tests with soil samples.

**Keywords:** bacterial isolation, biopesticides, entomopathogenic bacteria, penicillin G

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