

ISOLATION AND CHARACTERIZATION OF *Bacillus thuringiensis* STRAINS FROM ANGOLA

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Bacillus thuringiensis is a sporigen bacterium widely distributed in soil. During sporulation

B. thuringiensis is characterized by production of parasporal crystals composed of protein molecules of different weights (27-140 KDa) known as delta-endotoxin or insecticidal crystal proteins that are toxic to various insects. In insects, after ingestion, delta-endotoxins are converted in smaller polipeptides by proteases. The interaction between active toxin and the midgut epithelium causes pores in the cellular membranes of the epithelium and successively osmotic shock.

Thirty-nine strains were isolated in soil samples collected from different regions of Popular Republic of Angola. *B. thuringiensis* strains were analyzed by morphology, production of spores and crystals and Polymerase chain reaction (PCR) technique.

A rapid analysis of *B. thuringiensis* strains predictive of insecticidal activity was established by using PCR.

Primers specific to regions of high homology within genes encoding two major classes of *B. thuringiensis* crystal proteins were used to generate a PCR product profile characteristic of each insecticidal class. Included in the screen were PCR primers specific for *cryI* (LEP) and *cryIV* (DIP) which are insecticidal for lepidopteran and dipteran respectively. Sequences of the primers are following:

DIPIA 5' CAAGCCGCAAATCTTGTGGA
DIPIB 5' ATGGCTTGTTTCGCTACATC
LEPIA 5' CCGGTGCTGGATTTGTGTTA
LEPIB 5' AATCCCGTATTGTACCAGCG

Results

Most of identified strains reacted with *cryI* gene primers (LEP) while only three showed to harbor *cryIV* gene (DIP). Results are summarized in table 1.

Table 1. PCR amplification of LEP and DIP genes.

isolates	LEP	DIP	is.	LEP	DIP	is.	LEP	DIP
AF1	-	-	AF21	-	-	AF41	-	-
AF2	-	-	AF22	-	-	AF42	+	-
AF5	-	-	AF23	-	-	AF44	+	-
AF6	-	-	AF24	-	-	AF45	+	-
AF9	+	-	AF25	+	-	AF46	+	-
AF10	+	-	AF27	-	-	AF47	+	-
AF11	-	-	AF30	-	-	AF48	-	-
AF12	-	-	AF31	-	-	AF49	+	-
AF13	-	-	AF32	-	-	AF50	-	+
AF16	-	-	AF33	+	-	AF51	-	+
AF17	-	-	AF37	+	-	AF53	-	+
AF18	-	-	AF38	-	-	AF54	+	-
AF19	-	-	AF40	-	-	AF55	-	-

Eighteen *B. thuringiensis* isolates were selected for a primary toxicity test against *Ceratitis capitata* (Dip. Tephritidae): 3 out of 18 (AF42, AF33 and AF37) caused a mortality (86,3 %, 62,1 % and 25,7 % respectively) statistically different from the control on larvae. We did not find any activity on adults with the exception of AF18 that showed a slight mortality of 24,3 % in 7 days. The potential activity of *B. thuringiensis* against Medfly and mosquitos could be used in future as pests biocontrol and thus replaces chemical insecticides.