RESEARCH NOTE

Bacillus sphaericus Entomocidal Potential Determined by Polymerase Chain Reaction

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The mosquitocidal activity of *Bacillus sphaericus* toxic strains is related to the capacity of some strains to produce a binary toxin composed of 51 and 42 kDa peptides which in combination form a crystal (P Baumann et al. 1987 *J Bacteriol 169:* 4061-4067). Some toxic strains also produce the 100 kDa toxin encoded by the *mtx* gene. This gene was first cloned from the weakly entomocidal strain SSII-1 (T Thanabalu et al. 1991 *J Bacteriol 173*: 2776-2785). This toxin shows regional homology to ADP-ribosyltransferase-type toxins and seems to be membrane located (T Thanabalu et al. 1993 *J Bacteriol 175*: 2314-2320). The well known, highly toxic strains harbour the 42, 51 and 100 kDa genes.

The detection of binary toxin is often done by phase contrast microscopy through the presence of crystalline inclusions in a culture grown between 24 to 48 hr. The presence of 100 kDa toxin can be determined by the cytotoxic effect (Thanabalu et al. 1993 *loc. cit.*) or by dot blot hybridization (L Liu et al. 1993 *Appl Environ Microbiol 59*: 3470-3473). In this paper, we present polymerase chain reaction (PCR) analysis to determine in a rapid and accurate way the presence of the genes related to the *B. sphaericus* entomocidal potential.

B. sphaericus 2362, 1593, 2297 and SSII-1 were from Culture Collection of Genus *Bacillus*-CCGB (TV Guaycurús & L Rabinovitch 1992 *Catalog of Culture Collection Genus Bacillus*, p. 89-92, Instituto Oswaldo Cruz). *B. sphaericus* isolated from different Brazilian soils were identified by morphological characteristics, i.e., rod-shaped cells with round, terminal spores.

The primers used in the PCR were derived from the sequence of the 51 and 42 kDa peptide genes (L Baumann et al. 1988 *J Bacteriol 170*: 2045-2050) and 100 kDa toxin *mtx* gene (Thanabalu et al. 1991 *loc. cit.*). For the binary toxin genes we designed two sets of primers used for the nested PCR reaction. Sequences and positions are listed in Table. A loopful of cells from a *B. sphaericus* colony on an overnight nutrient yeast salt mineral (NYSM) agar plate (AA Yousten & EW Davidson 1982 *App Env Microbiol 44*: 1449-1455) was transferred to a tube with 500 µl destilled water. It was boiled for 10 min, frozen and thawed twice. To a total volume of 50 µl containing 1.25 U of *Taq* DNA polymerase and 3mM $MgCl_2$, was added 5 µl of DNA suspension and 20 pmol of each primer. The mixtures were subjected to 30 temperature cycles (94°C 30 sec, 55°C 30 sec and 72°C 1 min) on a programmable heating block. After the first PCR with the BSN1/BSN2 and BSN3/BSN4 pairs of primers was acomplished, 1 µl from these reactions was used as template for the nested PCR stage under the same first round conditions.

Known highly toxic *B. sphaericus* strains 2362, 2297, 1593 and the low toxic SSII-1 strain were used for the PCR conditions. Using both sets of primers of binary toxin genes we got the specific size band, as expected, based on the sequence gene data. Primers BS1/BS2 gave a 523 bp fragment and BSN1/BSN2 a 1053 bp fragment, both corresponding to 51.4 kDa toxin gene. Primers BS3/BS4 and BSN3/BSN4 amplified respectively a 478 and 720 bp fragments both from 41.9 kDa toxin gene. The set of primers 100.1/100.2 are homologus to *mtx* gene with a 700 bp PCR product (Fig.). The PCR was positive for all pairs of primers using DNA from 2362, 2297 and 1593. In the SSII-1 strain only 100 kDa toxin gene was detected. Dot blots were carried out confirming the PCR results (data not shown). For improving the sensitivity and

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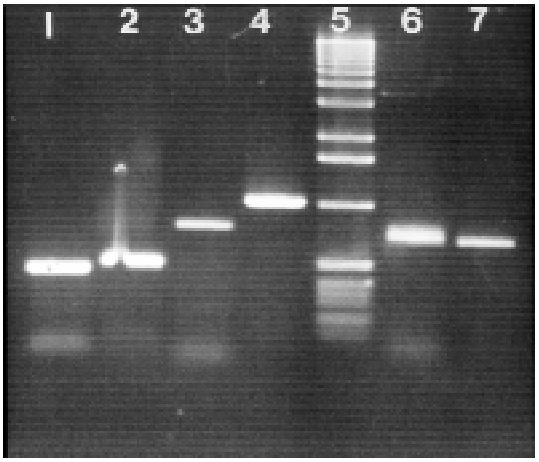
specificity of the binary gene detection, the nested PCR was applied. In some cases there were either no detectable amplified products or no specific products, after the first round. However, after the second round using inner primers, a clear DNA band with the expected size was then amplified. The nested PCR makes also the verification of amplified products by hybridization with inner probes or by restriction enzyme analysis unnecessary.

In some preliminary experiments we checked out this procedure with 12 newly isolated strains of *B. sphaericus* from Brazilian soil. All samples produced the specific PCR products to the three target genes. The PCR analysis was shown to be a reliable method for a prescreen of *B. sphaericus* isolates which should then be tested in a subsequent biological assay.



Agarose gel (1%) eletrophoresis of nucleic acid amplification products from *Bacillus sphaericus* 2362 DNA template. Lane 1, BS3/BS4; lane 2 BS1/BS2; lane 3, BSN3/BSN4, lane 4, BSN1/BSN2 and lane 6 100.1/100.2 primers. Lane 5, 1 Kb DNA ladder (BRL). Lane 7, 100.1/100.2 polymerase chain reaction product from SSII-1 strain.

TABLE

Primers used in the polymerase chain reaction (PCR) and nested PCR to detect the mosquitocidal genes in *Bacillus sphaericus*

Primer	Sequence 5'- 3'	Position	Target gene
BSN1	CACGGAATGGTTATGGTT	0640-0658	51.4 kDa
BSN2	AGGTGCATTAGGATACGA	1675-1693	51.4 kDa
BSN3	GTACATTCGCGTTATGG	2060-2077	41.9 kDa
BSN4	GTATCATAGGTGAACC	2846-2862	41.9 kDa
BS1	GTAGGGCGCTTGACAGTAGG	0977-0996	51.4 kDa
BS2	GGCCTATTTAGCCCCCTTG	1474-1493	51.4 kDa
BS3	GGCATAATGGGTCCGT	2221-2237	41.9 kDa
BS4	GAGCGCGGACCACATGC	2683-2700	41.9 kDa
100.1	CCAGGGGGAATTCGTC	1807-1822	100 kDa
100.2	GAGCTACTGTTCTCAC	2483-2498	100 kDa

The primers BSN1/BSN2 and BSN3/BSN4 were used in the first PCR round to the 51 and 42 kDa genes respectively, the BS1/BS2 and BS3/BS4 in the nested PCR round.