Bacillus sphaericus synthesizes a proteinaceous inclusion during sporulation which is toxic, upon ingestion, to certain species of mosquito larvae (Lacey and Undeen, 1986). Some other Bacilli also form larvicidal toxins but their targets differ. Their larvicides have been characterized biochemically and the genes have been cloned and sequenced in several instances. Since B. sphaericus was relatively poorly studied genetically, and because a previously cloned larvicide gene from this organism (Ganesan et al., 1983) showed signs of instability (Louis and Szulmajster, 1985), an E. coli library of B. sphaericus DNA was constructed and screened for clones showing larvicidal activity.

DNA from B. sphaericus was digested partially by EcoRI and ligated to pBR322 at its EcoRI site. Transformants were selected for resistance to ampicillin and tetracycline. Larvicidal clones were detected using Culex quinquefasciatus as the assay organism. Toxic clones were characterized by partial restriction mapping, subcloning and maxicell analysis. The larvicidal activity was also measured.
Two larvicidal clones were identified. Plasmids pAS233 and pAS377 were recovered from them and they contained inserts of 8.6 and 15.7 kb, respectively. Subcloning reduced the insert sizes to 3.6 kb for pAS233 and to 4.3 kb for pAS377 with retention of toxicity. The biological activity of the parents and subclones were similar with LC₅₀ values of approximately 10 μg cell protein per ml of assay volume. pAS233 also conferred larvicidal activity against Anopheles subpictus whereas clones containing pAS377 were innocuous to these larvae. Since sporulated B. sphaericus 1593 gave an LC₅₀ of 1-10 ng cell protein per ml, it was evident that the recombinant clones were significantly less active. A protease-deficient strain was then used as host for the plasmids but no increase in activity was observed indicating that proteolysis of the recombinant protein in E. coli was not a likely reason for the reduced activity.

Maxicell analysis indicated that subclones of pAS377 encoded a 29 kDa peptide whereas no product could be identified for pAS233 subclones. (Other workers have used antibodies (Baumann et al., 1987) and DNA sequencing (Hindley and Berry, 1987) to show that a 43 kDa protein was encoded by inserts similar to that of the pAS233 subclones.)
The subcloned insert of pAS233 contained its own promoter whereas the subcloned insert of pAS377 depended on a vector promoter. Since the insert of pAS233 was expressed from its own promoter, higher expression was expected if the insert could be cloned into *B. subtilis* which is closer physiologically to *B. sphaericus* than the latter is to *E. coli*. The insert of pAS233 was transferred to *B. subtilis* but no increase in toxicity was seen relative to the *E. coli* clones. Expression occurred during vegetative growth and did not increase in the stationary phase unlike fermentations of *B. sphaericus* in which larvicidal activity is seen mainly during sporulation (Lacey and Undeen, 1986).

**LIST OF REFERENCES**


