

Identification of a gene (*mob14-3*) encoding a mobilization protein from the *Bacillus thuringiensis subsp. israelensis* plasmid pTX14-3

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Submitted April 2, 1991 EMBL accession no. X56204

Bacillus thuringiensis is a gram-positive bacterium well known for its insecticidal activity towards larvae of insects. *B. thuringiensis subsp. israelensis* (Bti) is one of the few subspecies which are toxic to larvae of dipteran aquatic insects such as mosquitos and black flies. Due to this insecticidal property, Bti has a high commercial importance for control of several human disease vectors (for a review see (1)).

Most insecticidal crystal protein genes are located on large plasmids in *B. thuringiensis* (2) and it has been shown that these plasmids are able to promote their own inter-cellular transfer by a 'conjugation-like' process (3). *B. thuringiensis subsp. israelensis* harbors several small 'cryptic' plasmids, two of which have been cloned in *Bacillus subtilis* and *Escherichia coli* (4). These two plasmids have, in our laboratory, shown to be mobilizable in broth mating experiments between different Bti strains (manuscript in preparation).

A region of one of the cloned Bti plasmids (pTX14-3) (4, 5), containing an open reading frame (ORF) on 1463 nucleotides (res. # 100–1563), has been sequenced. The deduced amino acid sequence has a very strong homology to a gene (*mob2*) from a *B. thuringiensis subsp. thuringiensis* plasmid pGI2 (6). Thus, 60% of the last third of the translated sequence (from amino acid # 323 to 488) are identical to those found in Mob2 protein (97/165). Furthermore, the *mob2* gene has been shown to code for a mobilization protein with considerable homology to other gram positive mobilization proteins (7).

Inter-cellular transfer of the cloned Bti plasmid pTX14-3 may be due to a mobilization protein. The following arguments support this possibility: 1) The strong sequence homology between *mob2* and the ORF from pTX14-3; 2) *mob2* codes for a mobilization protein; 3) upstream the ORF is a sequence similar to the reported mobilization protein binding sequences (RSA) (figure) (7), and 4) the plasmid pTX14-3 is mobilizable in Bti strains. Thus, our present findings suggest a mobilization gene, which we designate the sequence ORF from pTXI4-3 *mob14-3*.

A number of *B. thuringiensis* replicons has recently been cloned for the design of cloning vectors (8). The features of mobilizable vectors may be of great importance in the development of recombinant *B. thuringiensis* strains and in future genetic studies. Yet, awareness on *B. thuringiensis* plasmids ability to exhibit horizontal transfer should be emphasized in the context of the development of commercial strains.

ACKNOWLEDGEMENT

We thank Jesper Damgaard for excellent technical assistance.

REFERENCES

1. Andrews,R.E.,Jr., Faust,R.M., Wabiko,H. and Raymond,K.C. (1987) *Crit. Rev. Biotechnol.* **6**, 163–232.
2. Kronstad,J.W., Schnepf,H.E. and Whiteley,H.R. (1983) *J. Bacteriol.* **154**, 419–428.
3. Gonzalez,J.M.,Jr., Brown,B.J. and Carlton,B.C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6951–6955.
4. Boe,L., Nielsen,T.T., Madsen,S.M., Andrup,L. and Bolander,G. (1991) *Plasmid* (in press).
5. Clark,B.D., Boyle,T.M., Chu,C.-Y. and Dean,D.H. (1985) *Gene* **36**, 169–171.
6. Mahillon,J., Hespel,F., Pierssens,A.M. and Delcour,J. (1988) *Plasmid* **19**, 169–173.
7. Josson,K., Soetaert,P., Michiels,F., Joos,H. and Mahillon,J. (1990) *J. Bacteriol.* **172**, 3089–3099.
8. Baum,J.A., Coyle,D.M., Gilbert,M.P., Jany,C.S., and Gawron-Burke,C. (1990) *Appl. Environ. Microbiol.* **56**, 3420–3428.

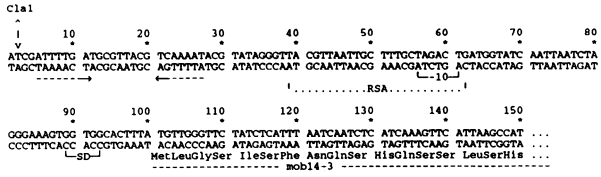

Figure 1. Upstream region of the *mob14–3* gene. The Pribnow box (–10), a possible stem loop (symbolized by arrows) and a potential Shine-Dalgarno (SD) sequence are indicated.