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*Bacillus* species have been used to produce a number of enzymes on an industrial scale. Some of these are encoded in harbouring plasmids. We have previously reported the isolation of plasmid from 2 species of *Bacillus*. The objective of this study was the characterization of plasmid(s) found in *Bacillus licheniformis* strain L4 by determining the number of plasmids in this strain and their size, by restriction enzyme digestion. Plasmid DNA was extracted from a large scale preparation by alkaline lysis procedure, and purified in a CsCl/ethidium bromide density gradient. Purified plasmid, DNA when subjected to agarose gel electrophoresis, revealed 3 bands of approximate size 5, 11 - 13, and > 23 kb indicating either the presence of more than one conformation of a plasmid or the presence of more than one plasmid in the extract. The digestion of each (electroeluted) DNA band with KpnI, size separation by agarose gel electrophoresis, and Southern hybridization with total plasmid DNA oligolabelled with <sup>32</sup>P as a probe revealed an identical DNA banding pattern with all 3 plasmid DNA bands indicating the presence of only one plasmid in this strain. The digestion of total plasmid DNA with *EcoRI*, *Hind III*, *KpnI* and *PvuII* revealed the presence of 2 sites each of *EcoRI*, *KpnI* and *PvuII*, and 4 sites of *Hind III* in this plasmid.

Based on the results of the restriction enzyme digestion of this plasmid, the size of the plasmid was estimated to be 12 - 13 Kb. The complete restriction map of this plasmid has to be determined by DNA sequencing as the resolution power of agarose gel electrophoresis is not adequate to detect small fragments generated by restriction enzyme digestion.

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