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Efficient Recovery of Plasmid DNA from *Erwinia herbicola* with High Nuclease Activity

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An efficient method is described for the isolation and purification of plasmid DNA from *Erwinia herbicola* (a bacterium with high nuclease activity), for which none of the standard methods yielded plasmid DNA in desired amounts. The procedure adopted here is based on elimination of the endogenous nuclease activity using guanidinium thiocyanate. The DNA preparation does not need a separate purification step. The method precludes the use of RNase, protase and phenol. The plasmid DNA preparation obtained by this method is of high purity suitable for restriction digestion, cloning, hybridization, preparation of DNA probes and sequencing.

*E. herbicola* (Catalog No. 21998; ATCC, Rockville, MD, USA) is an industrially important organism because of its ability to produce keto-sugar acids as intermediates of ascorbic acid. As a result, the organism is a good candidate for genetic manipulations. We studied the organism for the presence of plasmid DNA using several standard methods from Sambrook et al. (11) such as the alkaline lysis method (2), the sodium dodecyl sulfate (SDS) lysis method (6), the boiling method (7) etc. The gel picture revealed that the bacteria harbored the plasmid DNA, but the yields were invariably very low, and the isolated DNA was susceptible to degradation within a day, even when preserved at -20°C. Apparently, it shows that the organism possesses high endogenous nuclease activity.

Various other methods described for the isolation of plasmid DNA from prokaryotic organisms were also tried (1,3-5,9). However, plasmid DNA could not be recovered easily and efficiently in reasonably large quantities from this bacteria. Several modifications in the known standard procedures were also unable to increase plasmid DNA yield or inhibit its degradation.

Guanidinium thiocyanate, a strong protein denaturant, has been success-

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