A simple and rapid method for the preparation of gram-negative bacterial genomic DNA

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Two major protocols for the preparation of bacterial genomic DNA have been commonly used (1, 2). These methods provided powerful means of preparing genomic DNA; however, both of them are rather complex and time-consuming. Presented here is a simple and rapid method for extraction of bacterial genomic DNA. This method is effective in producing digestible genomic DNA from a variety of gram-negative bacteria, including those of the genera *Xanthomonas, Pseudomonase, Agrobacterium*, and *Rhizobium*. All of them normally produce copious amounts of polysaccharides (2, 3), the most common problem affecting DNA purity, which can inhibit the activity of many molecular biological enzymes (2). The method also has several advantages of not requiring any enzymes for expensive cell lysis materials. It requires only one chloroform extraction, and the entire process can be accomplished within one hour.

DNA was extracted as follows: 1.5 ml of a saturated culture was harvested with centrifugation for 3 min at 12,000 rpm. The cell pellet was resuspended and lysed in 200 μl of lysis buffer (40 mM Tris−acetate pH 7.8, 20 mM sodium−acetate, 1 mM EDTA, 1% SDS) (4) by vigorous pipetting. To remove most proteins and cell debris, 66 μl of 5M NaCl solution was added and mixed well, and then the viscous mixture was centrifuged at 12,000 rpm for 10 min at 4°C. After transferring the clear supernatant into a new vial, an equal volume of chloroform was added, and the tube was gently inverted at least 50 times when a milky solution was completely formed. Following centrifugating at 12,000 rpm for 3 min, the extracted supernatant was transferred to another vial and the DNA was precipitated with 100% EtOH, washed twice with 70% EtOH, dried in speed-vac, and redissolved in 50 μl 1× TE buffer. If required, RNA could be removed by adding RNase in the lysis step for 30 min at 37°C.

In order to determine the efficiency of this technique, we compared it with published procedures (1, 2) using *Xanthomonas campestris* pv. *citri* as cell strain (Figure 1). For unknown reasons, the isolated genomic DNA with the CTAB method (2) always coextracted with a large amounts of RNA. This result was also found on the DNA extracted from other bacterial strains (data not shown). But with our method, a comparatively small amount of RNA was coextracted, as with the ordinary method (1). Furthermore, after restriction enzyme digestion, the area above 23 kb of the treated DNA with our method was clearer than with the other two methods (Figure 1, lane 6), showing that the method was more effective in producing digestible genomic DNA. To determine whether the method is widely applicable, we tested 7 more gram-negative bacterial strains (6 genera). A positive result was obtained. The method was quite good for the tested bacteria including those genera for which polysaccharide contamination normally impedes DNA purification (Figure 2).

Although vigorous pipetting was done in the lysis step of our method, no significant shearing of DNA was found (Figure 1, lane 3; Figure 2). The yield of DNA with our method was good (50−150 μg per 10^9 cells containing RNA) with a 260/280 nm ratio ≥2.0. The quality and quantity of DNA obtained by this method was better than those obtained by other common methods. Moreover, our method is simple and time-saving.

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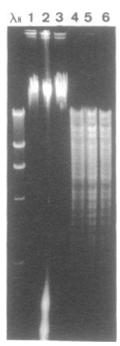

Figure 1. 1% agarose gel electrophoresis of *Xanthomonas campestris* pv. *citri* genomic DNA extracted as described in ref. 1 (lane 1), in ref. 2 (lane 2), and in this work (lane 3). From lane 4 to lane 6 showed EcoRI-digested DNAs in above order.

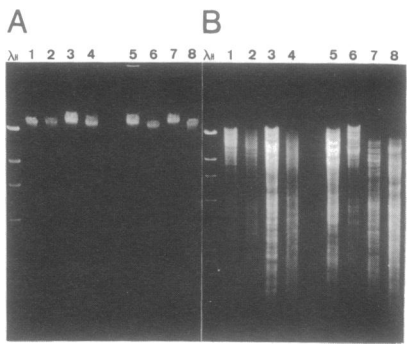

Figure 2. (**A**) Gel electrophoresis of uncut genomic DNAs prepared from different bacteria. Land 1—*E.coli*, Land 2—E. *chrysanthemi*, Land 3—X. *campestris* pv. *citri*, Land 4—P. *solanacearum*, Land 5—V. *parahaemolyticus*, Land 6—V. *damsela*, Land 7—A. *tumefaciens*, Land 8—R. *fredii*. (**B**) Lane 1−8 — EcoRI-digested DNAs in above order.