A rapid DNA isolation procedure from Petri dish grown clinical bacterial isolates

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Submitted September 19, 1990

Gardnerella vaginalis, a pleomorphic Gram variable bacteria (1), with a Gram positive cell wall (2), that stains predominantly as Gram negative is associated in bacterial vaginosis (1). We have described a lysozyme-SDS-proteinase K method for the isolation of DNA from 250 ml Casman broth cultures requiring about 60 to 72 hours (3). However, this was not convenient for comparative restriction endonuclease analysis (REA) studies involving a large number of clinical isolates. Here, we describe a procedure that has been modified from broth cultures to petri dish cultures and the time required has been shortened to less than 4 hours. This DNA preparation spooled from 33% ethanol was better than reported for the large scale procedure (3), in that, besides being of high molecular weight (50-80 kbp) and suitable for REA, it was free of RNA contamination (Figure).

A 36 to 48 h G. vaginalis plate culture grown on human bilayer agar (a semiselective medium) and incubated under anaerobic conditions was collected by adding 1.7 ml of an ice cold suspension buffer (0.15 M NaCl, 1 mM EDTA pH 8.0) and scraping off with a 5 ml pipette. The cell suspensions from three plates were pooled in a 15 ml Corex tube, adjusted to 5 ml and the bacterial concentration was determined from absorbancy measurements at 600 nm (an absorbancy of 1 corresponded to about 40 million cells in a Petroff—Hauser counter). The 5 to 10 mm diameter bacterial pellet collected by centrifugation at 1k g (4°C) for 7 min was washed by resuspension in 5 ml of the same buffer and recentrifugation. The washed pellet was placed on ice and was loosened to a homogeneous slurry by first shaking it repeatedly over a vortex mixer and then with a glass rod. The glass rod was kept in the tube in all subsequent steps (till centrifugation) and has been utilized in mixing the contents thoroughly. The bacterial slurry was taken in 1.0 ml of a freshly prepared 50 mg/ml of lysozyme in 50 mM glucose, 100 mM Tris.Cl, 10 mM EDTA, pH 8.0 and incubated at 37°C for 30 min. It was then supplemented with 10 μl of a heat treated pancreatic RNase (10 mg/ml) and after 30 min at 37°C with 1 ml of 4% SDS, 10 mM EDTA, pH 8.0. After another 30 min the translucent solution was supplemented with 40 μl of 25 mg/ml Proteinase K. It was incubated at 48°C for 30 min (when the solution became clear) and then at 65°C for 10 min. It was brought to room temperature and was mixed with a 3 ml mixture of phenol:chloroform:isoamyl alcohol (50:48:2) to milky white using the glass rod. The aqueous phase was collected after centrifugation at 5k g (20°C) for 10 min and was reextracted with 3 ml of chloroform:isoamyl alcohol (24:1). The final aqueous phase was incubated on ice in a 30 ml Corex tube and the DNA was spooled around a glass rod after overlaying with 6 ml of cold absolute alcohol (−20°C). The spooled DNA was washed with 75% alcohol, dried in air and dissolved in 500 μl of 0.1×SSC (0.15 M NaCl, 0.015 M Na Citrate, pH 7.0).

The DNA yield was 20.1 ±3.4 μg (n=11) per A600 or 144 ± 56 μg (n=11) per plate. The total yield from 3 plates was 0.4 to 0.5 mg DNA from a bacterial concentration of 21.7 ± 8.3 A600 (n=17).

The critical feature of this procedure is the prevention of bacterial aggregate formation by employing low centrifugation speeds and a thorough mixing with a glass rod as well as incubation with higher concentrations of lysozyme, SDS and proteinase K. In cases where the cells were not fully dispersed, an overnight digestion with proteinase K was required for a comparable DNA yield. The prior status of proteinase K (autodigestion for 0, 1, 2 or 4 h at 37°C) made no difference. The 1 h RNase digestion (1/2 h in the presence of SDS) and spooling of DNA in alcohol (without additional salt) resulted in a RNA free, high molecular weight DNA preparation. This method has proven useful in the REA studies and in the screening of not only a large number of G. vaginalis isolates (GVP) but also several other fastidious Gram negative bacteria.

ACKNOWLEDGEMENT

This work was supported in part by a C.W. Post Research Committee grant and by funds provided by the Dean of the College of Arts & Sciences (M.Schmidt-Raghavan) and the Biology Department (M. Hertz, Chairperson).

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