Preparation of Actinomycete DNA for Pulsed-Field Gel Electrophoresis

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A procedure for preparing actinomycete DNA samples for pulsed-field gel electrophoresis is described. When this method was used, DNAs from strains belonging to the genera Streptomyces, Kutzneria, Dactylosporangium, Microtetraspora, Actinoplanes, Saccharothrix, and Micromonaspora were successfully restriction digested with infrequently cutting enzymes, such as DraI and Asel.

Low-frequency restriction fragment analysis, a relatively new method for studying bacterial genomes, became possible with the advent of pulsed-field gel electrophoresis (PFGE). This technique has been found to be useful in taxonomic studies of various bacterial genera (3, 5, 6), including identification of Frankia and Streptomyces to species (1, 2). Currently under investigation in our laboratory are the DNA contents of actinomycete strains classified as members of the genera Kutzneria, Dactylosporangium, Microtetraspora, Actinoplanes, Saccharothrix, and Micromonaspora. When we used the original procedures (1, 2, 10), we experienced difficulty in releasing undergraded DNAs from some strains, as indicated by smearing of the restriction bands on the gels. In this paper we describe a revised protocol for processing actinomycete cells that results in the release of intact DNA and leads to clear restriction patterns.

In our initial experiments, we used the method of Smith and Cantor (10) for isolation and restriction analysis of large DNA molecules. Intact cells were initially immersed in low-melting-point agarose to protect the long, fragile DNA molecules from shearing. Once imbedded, the cells were treated with EC lysing buffer and proteinase K to release the DNA (10). When we used this method to prepare DNAs from members of various genera of actinomycetes, we encountered two technical problems.

The first problem was partial to total degradation of the DNAs isolated from some Streptomyces, Frankia, and Microtetraspora strains, as indicated by smearing of the restriction bands on the gels (Fig. 1, lanes 3 and 4). One possible cause for this smearing was the presence of nucleases, either nucleases from the actinomycete DNA samples or nucleases from contaminated restriction enzymes and other enzymes used in the procedure. To eliminate actinomycete nucleases, we pretreated the cells with various solvents before we imbedded them in the low-melting-point agarose. Frankia strains were extracted with acetone, ethanol, and chloroform, which resulted in clearer restriction patterns in some, but not all, strains (1). Other actinomycete strains were treated with warm (56 to 75°C) nonbuffered water-saturated phenol and sodium dodecyl sulfate (SDS) solutions (9), which resulted in the release of excellent-quality DNA from the majority of the strains (Fig. 2). Contamination of the restriction enzymes did not appear to be a significant cause of smearing since nonrestricted DNA from Streptomyces canescens NRRL 2419T (T = type strain) smears during PFGE (Fig. 1, lane 5).

The DNAs from some Streptomyces and Microtetraspora strains were still partially degraded even after phenol extraction was incorporated into the protocol. Evans and Dyson (4) reported that they obtained PFGE restriction patterns for Streptomyces lividans 1326 DNA by using HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer. The substitution of HEPES for Tris (4) resulted in removal of the residual partial degradation products from the restriction bands obtained with the Streptomyces and Microtetraspora strains (Fig. 1, lane 2, and Fig. 2, lane 3).

A second possible cause of the smearing of the restriction bands on the gels was degradation that was not related to the enzymes and occurred during electrophoresis, as reported by Kieser et al. (7). We may have encountered degradation of this type when DNA from Streptomyces canescens NRRL 2419T was prepared according to the original protocol (1, 2). PFGE of DraI-digested, Asel-digested, or nondigested samples of this DNA resulted in smearing of the bands on the gels (Fig. 1, lanes 3 through 5). In this case, it is likely that nonenzymatic degradation occurred during electrophoresis (7). The addition of thiourea to all running buffers eliminated the nonspecific degradation caused by Tris during PFGE (8). When we used the latest protocol, Asel restriction digestion of Streptomyces canescens DNA resulted in eight clear restriction bands (Fig. 1, lane 2), and the background of degraded DNA observed with nonrestricted or DraI-restricted DNA was not present. (Streptomyces canescens has no DraI restriction sites at 90 to 1,500 kb.)

The second technical problem which we encountered was the lack of release of DNA from members of the genus Actinoplanes, as shown by our inability to detect restriction patterns on gels. The Actinoplanes strains produced no patterns with DraI (Fig. 3, lane 3) or with Asel or SpeI, and there was no degradation of nondigested DNA on the gels when the samples used for PFGE were obtained by using the original protocol (1, 2). This problem was solved by substituting HEPES for Tris (4) in the buffers used for the protocol (Fig. 3, lane 2).

The revised procedure for isolation and restriction analysis of intact actinomycete DNA is as follows: (i) cultures are grown on shakers at 250 rpm and 28°C in Trypticase soy broth (Difco Laboratories, Detroit, Mich.) for 2 to 3 days (younger cells are more susceptible to the subsequent treatments and yield abundant DNA), harvested, and frozen at −80°C in the presence of 10% glycerol; (ii) cells are thawed, washed twice with 0.5 M EDTA, ground with Brock tissue grinders, extracted with water-saturated phenol at 56°C for 15 min, and washed three times with chloroform-isoamyl alcohol (24:1) at...
room temperature; (iii) the cells are then treated with 1% SDS at 56°C for 15 min and washed five times with HE buffer (10 mM HEPES, 1 mM EDTA; pH 8) (4); (iv) the cells are suspended in HE buffer, the cell concentration is adjusted to an equal volume of 2% low-melting-point agarose (Beckman), and the resulting preparations are poured into molds and allowed to solidify at 4°C; and (v) the imbedded samples are treated with lysozyme (1 mg/ml in HE buffer) for at least 2 h and then with subtilisin (1 mg/ml in HE buffer; Carlsberg) for at least 2 h and then washed twice with HE buffer at 56°C (at least 2 h per wash). The DNA samples imbedded in agarose are stored in HE buffer at 4°C and are stable for at least 18 months. Restriction digestions with the rare cutters DraI (TT T'AAA), AseI or AsnI (AT'TAAT), and SpeI (A'CTAGT) are performed as recommended by the manufacturer's (New England Biolabs, Bethesda Research Laboratories, Boehringer-Mannheim). A GeneLine TAFE I system (Beckman Instruments, Inc., Fullerton, Calif.) is the gel electrophoresis system used. The conditions used for PFGE are modified as described by Evans and Dyson (4); thiourea (final concentration, 50 mM) is added to 1 liter of the running buffer (1 X TBE [0.1 M Tris, 0.1 M boric acid, 0.2 M EDTA] or 1 X TAFE [10 mM Tris, 0.5 mM EDTA, 4 mM glacial acetic acid]) Finally, the DNA bands are visualized by soaking the gels in ethidium bromide.

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REFERENCES


FIG. 1. PFGE of Streptomyces canescens NRRL 2419i DNA. Lane 1, Saccharomyces cerevisiae standard; lane 2, AseI digest of a DNA sample prepared by the method described in this paper; lane 3, AseI digest; lane 4, DraI digest; lane 5, nonrestricted DNA sample prepared by a previously described method (1, 2).

FIG. 2. PFGE of restriction digestes of DNAs of actinomycete strains belonging to different genera. Lane 1, Saccharomyces cerevisiae standard; lane 2, Saccharothrix araneoligenes ATCC 39243T DraI digest; lane 3, Microtetraspora sp. strain SCC 1776 AsnI digest; lane 4, Kutzneria vitiodigita ATCC 25242T DraI digest; lane 5, Micromonospora purpurea ATCC 15835T AseI digest; lane 6, Dactylosporangium thailandense ATCC 23490T SpeI digest.

FIG. 3. Restriction digestion of Actinoplanes sp. strain ATCC 55600 DNA. Lane 1, Saccharomyces cerevisiae standard; lane 2, DraI digest of a DNA sample prepared by the method described in this paper; lane 3, DraI digest of a DNA sample prepared by a previously described method (1, 2).


