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High yield preparation of genomic DNA from *Streptomyces*

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Streptomyces species produce important drugs such as antibiotics, immunosuppressants, and antitumor compounds. The isolation of genomic DNA is imperative for the understanding of the biosynthesis of these compounds and has led to the rational design of new analogs (1–5). *Streptomyces* are Gram-positive bacteria, making DNA isolation difficult due to their resistance to cell lysis (6,7). Most methods use lysozyme and sodium docecyl sulfate (SDS) for cell disruption. To further increase lysis, glycine is often incorporated into media to minimize peptidoglycan cross-linking; muramidases such as mutanolysin or grinding of mycelia are also commonly used (1,8–10). Compared with DNA isolation methods for *Escherichia coli*, most methods are time-consuming or low yielding, or give low-quality DNA (1). This report details an improved method for DNA isolation from *Streptomyces* species using achromopeptidase, lysozyme, and SDS for cell lysis that results in higher yield compared with current standard methods.

The addition of achromopeptidase was prompted by its use in protoplast generation in *Streptomyces*, suggesting that it interacts with the cell wall of the bacterium (11). It has also been used in the lysis of other Gram-positive organisms (12,13). Presumably the mode of action of the protease in *Streptomyces* is to cause disruption of the peptidoglycan layer through cleavage of *N*-acetylmuramoyl-L-alanine amide bonds together with D-Ala-Gly and Gly-Gly bonds as reported for *Staphylococcus aureus* (14). When incubated simultaneously with lysozyme disrupting glycosidic linkages in the polymer, the resulting bacterial structures are more susceptible to SDS lysis. The increase in cell lysis would lead to an increase in DNA concentration for purification in the later stages of the protocol.

Streptomyces nodosus (ATCC 14899; American Type Culture Collection, Manassas, VA, USA), *S. noursei* (ATCC 11455), *S. avermitilis* (NRRL 3165; Agricultural Research Service Culture Collection, Peoria, IL, USA)

Table 1. Comparison of Yield and Purity of DNA Obtained from *Streptomyces nodosus*

Method (Reference)	Yield of DNA (mg/g wwt)	DNA Purity (A_{260}/A_{280})
This study	4.3 ± 0.1 (*n* = 4)	1.90 ± 0.15
Hunter (2)	1.0 ± 0.1 (*n* = 2)	1.75 ± 0.07
Kutchma et al. (7)	0.9 ± 0.3 (*n* = 3)	1.61 ± 0.43

wwt, wet weight.

S. coelicolor (NRRL B-16638), and *Streptomyces* sp., an uncharacterized soil isolate, were cultured in 30 mL of YMG medium (yeast extract 4 g/L, malt extract 10 g/L, glucose 4 g/L) or tryptone soya broth (Difco, Detroit, MI, USA) supplemented with 0.5% (w/v) glycine for 46 h with shaking at 28°C. Cells were harvested by centrifugation (5 min, 4000× *g*), washed [2× 10 mL of 10% (w/v) sucrose] and either freeze-dried for dry weight measurements or resuspended in 10 mL of lysis solution (0.3 M sucrose, 25 mM EDTA, 25 mM Tris-HCl, pH 7.5, containing 2 U of RNase) in a 50 mL Falcon tube™ (Becton Dickinson, Franklin Lakes, NJ, USA).

Lysozyme (10 mg) and achromopeptidase (5 mg; Sigma, St. Louis, MO, USA) were added as crystalline solids to the bacterial suspension and incubated at 37°C for 20 min. Ten percent (w/v) SDS (1 mL) and proteinase K (5 mg; Sigma) were then added with further incubation at 55°C for 1.5 h. After addition of 5 M NaCl (3.6 mL) and chloroform (15 mL), the sample was rotated end-over-end for 20 min at 6 rpm. After centrifugation (20 min, 5000× *g*), the aqueous phase was transferred with wide bore pipet into a clean tube. DNA was precipitated by addition of 1 volume of isopropanol and spooled using a sealed Pasteur pipet before being transferred into a microcentrifuge tube and rinsed with 1 mL 70% (v/v) ethanol. The air-dried DNA was dissolved in a minimal volume of prewarmed buffer containing 10 mM Tris-HCl, pH 7.4, and 10 mM EDTA at 60°C. Quantity and quality of DNA were determined by spectrophotometry and agarose gel electrophoresis.

Using both characterized and uncharacterized *Streptomyces* isolates, the method reported here yielded 3.4 ± 0.5 mg genomic DNA per gram wet weight (wwt) of mycelia [10.6 ± 1.6 mg/30 mL of stationary phase culture or 53 ± 8 mg/g dry weight (dwt) of mycelia]. This is significantly higher than that reported for most genomic DNA isolation procedures that typically yield 0.5–1 mg DNA per gram of wet mycelia (1).

To directly compare the yield of our method with other published protocols, we performed side-by-side DNA extractions from *S. nodosus* mycelia. As shown in Table 1, our method produced DNA of higher yield than two other commonly used procedures reported by Hunter et al. (2) and Kutchma et al. (7). Our yield for DNA obtained from mycelia using the method of Kutchma et al. corresponded well to the values originally reported (7). Although Kutchma et al. described yields of up to 5 mg/g wwt from spore samples, we obtained a yield of up to 2.4 mg/g wwt using their method—an amount that is still less than that obtained using our method on mycelial samples.

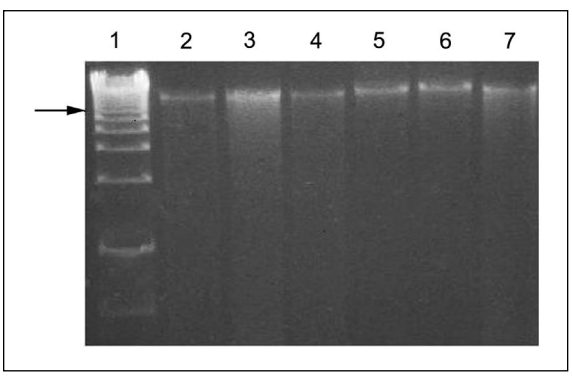

Figure 1. Total DNA isolated from *Streptomyces* species and separated on a 0.4% Tris-acetate-EDTA agarose gel. Lane 1, 5 kb marker; arrow represents 30 kb. Lanes 2–7 are genomic DNA from *S. nodosus*, *S. noursei, S. avermitilis, S. lividans, S. coelicolo*r, and *Streptomyces* sp., respectively.

In addition to being high yielding, our method allows efficient preparation of high-quality DNA. The protocol of Kutchma et al. takes a similar amount of time as our protocol (approximately 3 h), but produces DNA of inferior quality as assessed by A_{260}/A_{280} ratio (Table 1). The method of Hunter et al. produces DNA of more comparable purity to our method but is much more time-consuming (taking approximately 1–2 days).

The size of the DNA fragments isolated using this new method was assessed by agarose electrophoresis to be over 30 kb, with very little fragmentation even after storage at -20°C for 3 months (Figure 1). This should allow the DNA to be used for library construction where cloning of large gene fragments is required. The large fragment size obtained can probably be attributed to the rapidity of the method, which would be expected to limit the exposure of DNA to the many endonucleases known to exist in *Streptomyces* species and shearing forces associated with multiple handling steps (15–17).

We have also successfully tested the DNA in PCR amplifications. We have amplified 16S rRNA fragments for taxonomic studies and have also generated polyketide gene fragments (data not shown). Furthermore, other studies have shown that the DNA is amenable to digestion using normal concentrations of restriction enzymes and incubation times (data not shown).

In summary, we have described a new method for isolating high-quality DNA from *Streptomyces* species. The procedure can be completed in 3 h using standard laboratory equipment. Yields are higher than those obtained by other commonly used protocols, and the DNA is of high molecular weight, which is important for genetic studies into the biochemistry of bioactive molecule synthesis.

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Improved transfection technique for adherent cells using a commercial lipid reagent

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A variety of techniques have been developed for cell transfection including chemical [e.g., calcium phosphate (1) and lipid-based methods (2)], physical [e.g., electroporation (3)], and viral [e.g., retrovirus (4)] approaches. Nonviral techniques tend to be relatively safe and simple but also tend to be relatively inefficient compared with viral techniques. Lipid-based reagents, including liposomal and nonliposomal lipids, have become increasingly popular for in vitro and in vivo gene transfer (5). Despite the success of lipid-based methods, existing approaches may not be sufficient when an experiment requires transfection of the majority of cells in a population. We have developed an improved method using a commercial nonliposomal lipid reagent (Effectene™; Qiagen, Valencia, CA, USA) to transfect cultured adherent cells that results in improved transfection efficiencies. We transfected C2C12 skeletal myoblasts and NIH-3T3 fibroblasts immediately after trypsinization, while the cells were in suspension; the standard approach is to transfect adherent cells several hours after they have attached to the culture dish. The transfection efficiency of the new method (70%–80% of cells transfected) may obviate the need for time-consuming stable transfections in many situations.

For the standard transfection procedure, cells were transfected following attachment to plastic culture dishes according to the manufacturer's protocol. C2C12 myoblasts and NIH-3T3 fibroblasts were obtained from American Type Culture Collection (Manassas, VA, USA). Cells were seeded at 2×10^5 cells per well in 6-well plates in 2 mL of growth medium composed of Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (GIBCO/Invitrogen, Grand Island, NY, USA). Cells were incubated overnight at 37°C and 5% CO_2. The following morning, lipid-DNA complexes were prepared according to manufacturer's instructions using a 1:8 DNA-to-En-

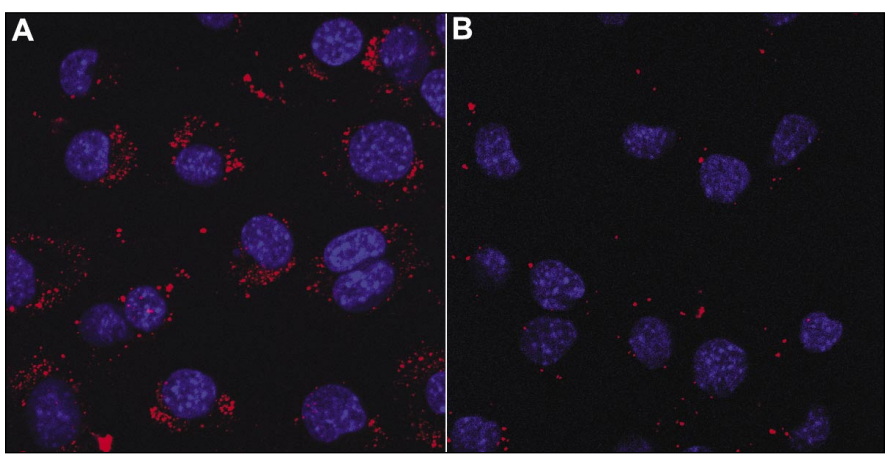

Figure 1. C2C12 myoblasts transfected with labeled plasmid DNA. C2C12 myoblasts transfected with rhodamine-labeled plasmid DNA showed greater DNA uptake when using the new transfection procedure (A) compared to the standard procedure (B). For both procedures, 2×10^5 cells were transfected with 1 µg DNA, and cells were viewed using confocal microscopy 24 h after transfection.