PREPARATIVE METHOD FOR THE ISOLATION OF SUPER-COILED DNA FROM A CHLORAMPHENICOL-PRODUCING STREPTOMYCETE

Sir:

A preparative method for the isolation of plasmid DNA which is applicable to several streptomycetes is described.

Chloramphenicol is derived by an unusual diversion of intermediates in the shikimic acid pathway of aromatic amino acid biosynthesis in streptomycetes[1]. Genetic maps involving auxotrophic markers of chloramphenicol-producing streptomycetes have been constructed[2,3], and significant information has also been obtained concerning the biogenesis and regulation of this antibiotic[4].

Although genes for chloramphenicol biosynthetic enzymes have not been mapped, AKAGAWA, *et al.*[2], predicted involvement of plasmid-borne genes in biogenesis of chloramphenicol based on genetic analysis of *Streptomyces venezuelae*. I here report a preparative scale method for the isolation of a plasmid from a chloramphenicol-producing *Streptomyces* sp. 3022a (UC® 2374) which was isolated from soil at The Upjohn Company. This plasmid may be a useful vector for cloning foreign genes in streptomycetes.

The streptomycete used here is a clone selected for good chloramphenicol production after mutagenesis of *Streptomyces* sp. 3022a with nitrosoguanidine. One hundred ml of medium per 500-ml flask was always used. Primary inoculum was grown at 28°C for 24~36 hours, pH 7.2, in tryptone (0.5%), molasses (1.0%), glycerol (1%), and yeast extract (0.25%). This was used to seed 10 flasks of glycerol-serine-lactate medium at the rate of 1%[5] (glycerol 2%; 60% sodium lactate 2.8%; DL-serine 0.3%; sodium chloride 0.6%; Difco casamino acids 0.025%; KH_2PO_4 0.14%; K_2HPO_4 0.2%; $MgSO_4 \cdot 7H_2O$ 0.05%, $MnSO_4 \cdot H_2O$ 0.0008%; $CuSO_4 \cdot 5H_2O$ 0.0006%; $ZnSO_4$ 0.0012%; pH adjusted to 7.0). After 3 days of growth, cells were harvested by centrifugation ($20,000 g \times 20$ min), the pellet was resuspended in 200 ml TES buffer (30 mM Tris-HCl, pH 8.0; 5 mM EDTA; 50 mM NaCl) and centrifuged again. Supernatant was discarded and the washed cells were frozen above liquid nitrogen.

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Frozen cells were thawed and resuspended in 200 ml of 25% sucrose, 50 mM Tris, pH 8.0, with the help of a Potter-Elvejhem Teflon homogenizer. This cell suspension was transferred to an Erlenmeyer flask and 40 ml of freshly prepared lysozyme (20 mg/ml in 0.25 M Tris, pH 8.0) were added and mixed thoroughly. After incubation for 30 minutes at 25°C, 100 ml of 0.25 M EDTA (pH 8.0) was added and mixed thoroughly. After another 30 minutes, 320 ml of DOC-Brij solution (1% w/v Brij, 0.04% w/v DOC; 0.06 M EDTA and 50 mM Tris, pH 8.0) was added and incubation was continued for 30 minutes at room temperature; then for an additional 30 minutes at 50°C.

The viscous lysate was centrifuged (60 min, 20 K rpm) in 50-ml Sorvall RC-2 centrifuge tubes using the SS-34 anglehead rotor at 20°C. Supernatant was collected and the pellet was again extracted with 320 ml of DOC-Brij solution. The combined supernatants were extracted once with an equal volume of freshly distilled phenol which was equilibrated with TES. These phenol-lysate mixtures were centrifuged in a Sorvall GSA head (30 minute, 5,000 K rpm) using 150-ml Corex bottles and the top aqueous phase was extracted once with chloroform. The aqueous phase was thoroughly mixed with 2 volumes of Gold Shield ethanol at −30°C, and stored at −30°C overnight, after which the precipitate was collected by centrifugation (GSA head, 5,000 rpm, 5°C, 30 minutes) and the supernatant decanted. The Corex bottles were inverted to let the precipitate drain (10~20 minutes) and the material was dissolved in a total of 50 ml of TES buffer. Extensive pipetting was avoided. The following procedures were carried out in the dark. Ten ml of ethidium bromide solution in TES (10 mg/ml) was added and the mixture vortexed gently. The red precipitate was removed by centrifugation (10,000 rpm, 30 minutes) and the supernatant (5.5 ml) was mixed in a polyallomer centrifuge tube with 6.5 ml of CsCl solution in TES buffer (2 g/ml). Mineral oil was layered on top to fill the empty space and the tubes were centrifuged in a 50 Ti rotor at 35 K for at least 35 hours at 20°C. Two DNA bands were visible on excitation with 545 nm light or long wave UV light. The lower fluorescing band (plasmid DNA) was collected with an 18 gauge needle connected to a 2-ml syringe. The tube was punctured about 10 mm below the lower fluorescing band and the needle

Fig. 1. Electron micrograph of plasmid. 45,000 × magnification.

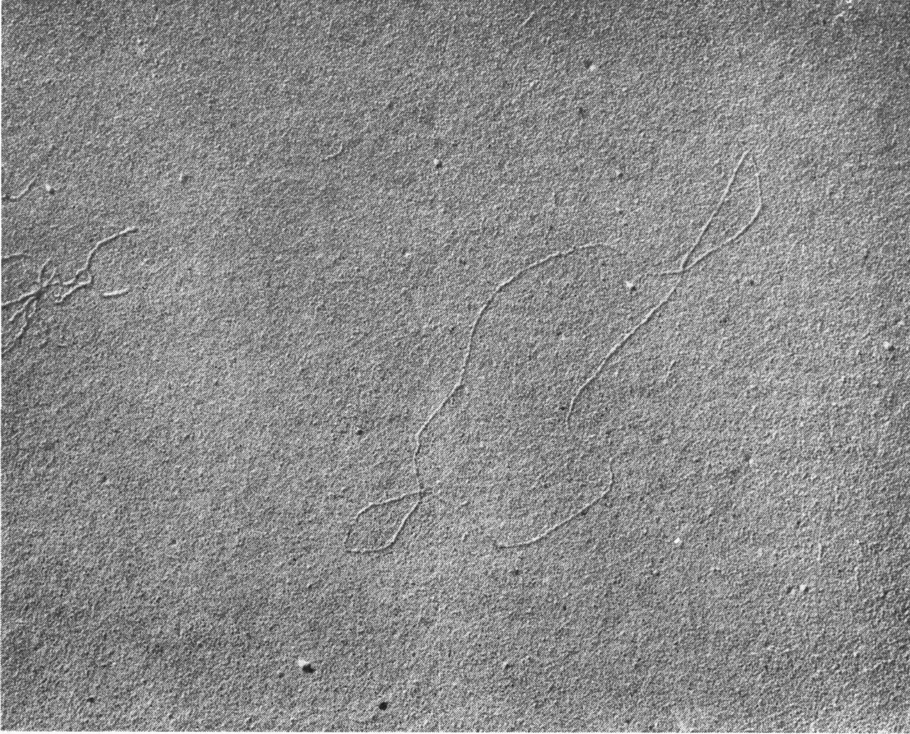
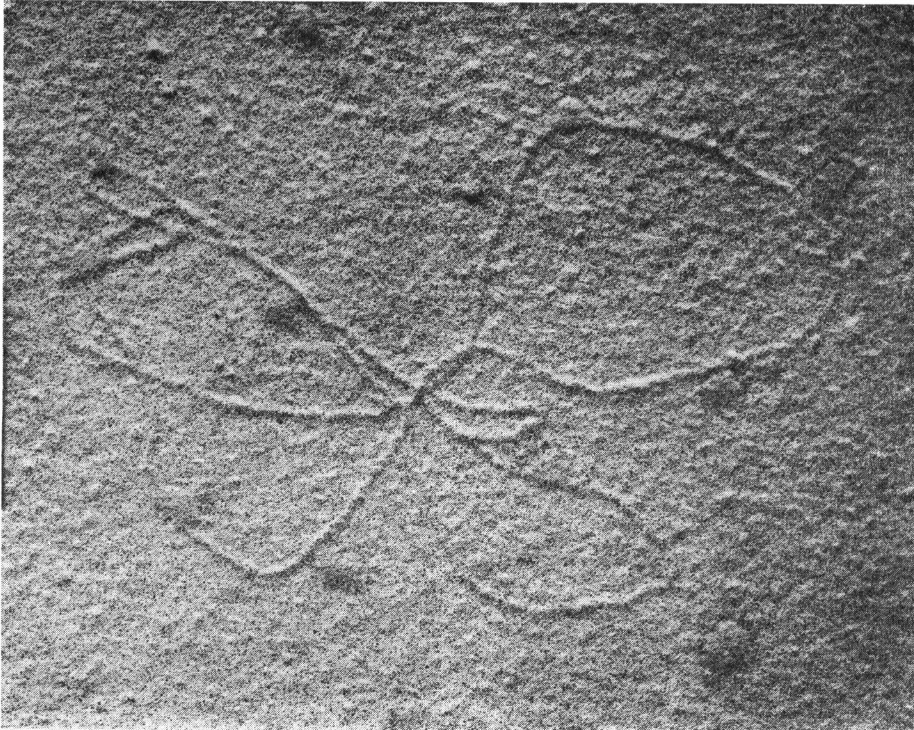


Fig. 2. Electron micrograph of plasmid. 135,000 × magnification.



lifted until it was just below the band with its beveled side up. About one ml of fluid containing the lower fluorescent band was pulled out of the tube. Fractions from all the tubes were pooled and extracted three times by gentle mixing with an equal volume of CsCl-saturated isopropanol or 1-butanol. The aqueous phase was then dialyzed against two changes, 4 liters each, of TES buffer, and the DNA purified once more by banding in CsCl-EtBr. The DNA which was centrifuged twice in CsCl-EtBr gradients was examined by electron microscopy after dialysis in TES buffer. Circular structures of approximately 18×10^6 daltons were seen and photographed (Figs. 1~2). The DNA was stored over liquid nitrogen in chromic acid washed glass vials that were heated one hour at 120°C to inactivate nucleases.

Using this method, plasmid DNA bands were also seen in the lysates of *S. spectabilis* (UC 2472), *S. espinosus* (UC 5371), *S. vellosus* (UC 5656), *Streptoverticillium flavopersicus* (UC 5066), *S. pseudogriseolus* chemovar. *linmyceticus* (UC 5462) and *S. variabilis* chemovar. *liniabilis* (UC 5484). An analytical method for the isolation of closed circular DNA from *Streptomyces coelicolor* has been published by SCHREMPF, *et al.* (1975), but the advantage of the procedure reported here is that a large amount of purified plasmid can be prepared without using any radioactive isotope.

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