Transformation of \textit{Streptomyces granaticolor} with Natural and Recombinant Plasmid Vectors

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\textbf{ABSTRACT.} Protoplasts of \textit{Streptomyces granaticolor} were found to be transformable by the broad-host-range plasmid pIJ350 but no transformants were detected when the narrow-host-range plasmid pIJ2 or the shuttle vector pPM66 (pIJ350 $-$ pBR322) isolated from \textit{E. coli} cells were used. The onset of blue colour granaticin production by \textit{S. granaticolor} cells was used as a marker to prepare protoplasts with a high transformation capacity. The presence of a restriction system is discussed.

\textit{S. granaticolor} producing blue granaticin (Řičícová and Řeháček 1968) has been used in this laboratory for many years to study translation system of streptomycetes (Mikulík et al. 1975; Janda et al. 1981). Recently the finding of inducible $\beta$-D-glucosidase (EC 3.2.1.21) in \textit{S. granaticolor} (Jirešová et al. 1983) and a successful isolation of RNA nucleotidyltransferase (EC 2.7.7.6) from this strain (Spížek et al. 1983) stimulated our effort to investigate a possible gene transfer and gene cloning in \textit{S. granaticolor}. To this end, the information was sought concerning its ability to form protoplasts, sensitivity to PEG treatment, protoplast regeneration, R-M system, replication and stability of vectors in cells of the new strain and sometimes compatibility of the vector molecule with the previously "home made" plasmid or prophage.

\textbf{MATERIAL AND METHODS}

\textit{Bacterial strains and plasmids.} \textit{Streptomyces granaticolor}, wild type (Řičícová and Řeháček 1968) and \textit{Streptomyces lividans} 1326 wild type (Thompson et al. 1982) were used as host strains. Plasmids pIJ350 (encoding resistance to thiostrepton) (Kieser et al. 1982) and pIJ2 (encoding resistance to neomycin) (Thompson et al. 1980) were isolated from \textit{S. lividans} TK128 and TC8, respectively. pPM66 is a recombinant plasmid constructed by \textit{in vitro} ligation of pIJ350 and pBR322 DNAs in this laboratory (Fig. 1). pPM66 was kept in \textit{E. coli} SK1590 gal thi sboB15 endA hsdR4 hsdM $^+$ (Kushner 1978), from which pPM66 DNA was isolated.

\textit{Preparation of plasmid DNAs.} pIJ350 and pIJ2 DNAs were prepared by CsCl$-$ethidium bromide equilibrium gradient centrifugation according to...
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Chater et al. (1982). pPM66 DNA was prepared from E. coli by CsCl—ethidium bromide equilibrium density gradient centrifugation as described elsewhere.

**Media.** Well sporulating cultures of streptomycetes strains on sporulation agar (Tichý et al. 1982), stored at 4 °C, were used as stock cultures. S. grana-
ticolor was grown in the YEP medium of the following composition (g/L): yeast extract (Oxoid) 5, peptone (Spofa) 5, NaCl 5, K$_2$HPO$_4$ 3.5, KH$_2$PO$_4$ 1.5, glucose 10. S. lividans 1326, TK128 and TC8 were cultivated in the YEME medium (Bibb et al. 1977) containing (g/L): yeast extract (Oxoid) 3, bactopeptone (Difco) 5, malt extract (Oxoid) 3, glucose 10, sucrose 340 and 5 mM MgCl$_2$. S. lividans TK128(pIJ350) and S. lividans TC8(pIJ2) were grown in the YEME medium containing thiostrepton (30 μg/mL) and neoc-
mycin (5 μg/mL), respectively. E. coli SK1590 was cultivated in the M9 minimal medium, to which the required growth factors were added. A high copy number of pPM66 was achieved by adding chloramphenicol (to 150 μg/mL) to the exponential culture with a subsequent overnight incubation of cells at 37 °C.

**Preparation of protoplasts.** The original procedure for preparation of protoplasts and their reversion to intact cells was described by Okanishi et al. (1974). Mycelium grown in 80 mL of YEP (S. grana-
ticolor) or YEME (S. lividans) in a 500-mL flask was washed twice in 10.3 % sucrose and the pel beat was suspended in medium P (Chater et al. 1982). Mycelia were converted to protoplasts by digesting the cell wall with lysozyme (EC 3.2.1.17; Calbiochem, 14 870 U/mg; 1 mg/mL) in medium P at 32 °C. Although the protoplasts of S. grana-
ticolor were formed within 30 min, the incubation proceeded for 60 min to ensure complete protoplasting. Under the conditions used the mycelium of S. lividans 1326 was completely converted to protoplasts in 60—90 min. The residual intact mycelium was removed by cotton filtration, protoplasts were collected by low-speed centrifugation at room temperature (2000 g, 10 min, 20 °C) and washed once with the P medium. The pellet was then suspended in 2 mL of the P medium.

**Transformation of protoplasts** was performed according to Thompson et al. (1982). The transformation mixture contained: 30 μL of plasmid DNA (2.3 μg) and 200 μL of protoplasts suspended in the medium P. Transformation was started by adding 0.5 mL of polyethylene glycol 1500 (Lachema)