A rapid method for the analysis of plasmid content and copy number in various Streptomyces grown on agar plates

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The techniques available for the identification of plasmid DNA in gram-positive bacteria such as mycelium-forming Streptomyces are time consuming. Nearly all methods described require the cultivation of the bacteria in liquid medium (1). Simple plasmid isolation techniques have been developed for gram-negative bacteria (2) but they are not applicable to gram-positive prokaryotes. Here, we present a new rapid and reproducible method by which the bacteria to be lysed can directly be taken from agar plates. For the cell lysis a part of a single colony (1—2 mg) is suspended nearly homogeneously in 20 μL TSE solution (50 mM Tris, 10 mM NaCl, 5 mM EDTA pH 8.0) and gently mixed with 20 μL L-solution consisting of 25% sucrose, 3% Ficoll 400, 1 Unit RNase A and 1 mg/ml lysozyme (freshly dissolved) in TB electrophoresis buffer (90 mM Tris, 90 mM boric acid, 2.5 mM EDTA pH 8.2). Immediately 30 μL of the suspension is filled into the slot of a submerged 0.2% SDS-containing agarose gel. Depending on plasmid size and separation desired the gel concentrations may vary from 0.5—2.0% agarose in TB buffer. Complete cell lysis is achieved by electrophoretic transfer of the negatively charged SDS into the wells for 40 min at 1 V/cm. Electrophoresis is continued for 2—4 h at 10 V/cm. Before staining with EtBr the gel is rinsed in water to remove SDS.

This lysis technique can also be used for the determination of the plasmid copy number by scanning the negatives of the gel photographs with CHROMOSCAN 3 (Yoice Loebl, Transmission Reflection 0.5A, Aperture Width 0.1mm, Filter 626nm). By this way the copy numbers of pSG5 and pSVH1 (3) based vectors in S. lividans TK23 (1) were calculated to be 55 and 75 (average values) per chromosome, respectively. For pU101, SLPl.2 and SCP2* derivatives (1), plasmids used as control, copy number values of 65, 2.1 and 1 could be determined, respectively.

The procedure has successfully been applied to various morphologically different Streptomyces strains such as S. venezuelae, S. ghanaensis, S. viridochromogenes, S. coelicolor 'Müller', S. prasinas and S. cattleya. The method can also be used for the lysis of the gram-positive bacterium Corynebacterium glutamicum by increasing the SDS concentration to 0.3% and an incubation of the cells in L-solution (30 min at room temperature) before loading the gel (data not shown).

Figure 1 shows an example for the migration pattern of different hybrid plasmids based on pSVH1 (6.0—20.0 kb in length) isolated from S. lividans TK23 in a 0.2% SDS/0.8% agarose gel running for 2.5 h. As indicated by an arrow also linear chromosomal DNA fragments corresponding a plasmid size of about 13.0—16.0 kb migrate into the gel. Since there is only little mechanical shearing during loading the gel the majority of these fragments probably arise from the action of bacteria specific nucleases. The identification of plasmids of 13.0—16.0 kb is possible by decreasing the agarose concentration. Also plasmids of different sizes in C. glutamicum could be demonstrated by this method (results not shown).

REFERENCES

Figure 1. Analysis of recombinant plasmids in transformants of S. lividans TK23. The lanes represent some of the resulting hybrid plasmids after a shotgun cloning experiment of Sau3A digested S. coelicolor total DNA using pEB15 (4) as high copy number vector. The plasmid lengths vary from 6.0—20.0 kb.

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