

SHORT COMMUNICATION

Complete Lysis of Glutamic Acid-producing Bacteria by the Use of Antibiotics which Inhibit the Biosynthesis of Cell Walls

By YASUHIKO KOMATSU

*Fermentation Research Institute, Agency of Industrial Science and Technology,
Ministry of International Trade and Industry, Inage, Chiba 281, Japan*

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A novel method for obtaining complete lysis of coryneform bacteria has been devised. Bacteria in the late-exponential phase of growth are treated with antibiotics which inhibit biosynthesis of the cell wall. Organisms treated in this way show increased sensitivity to lysis by added lytic enzymes. The preparation of DNA from this group of organisms is thus greatly facilitated.

INTRODUCTION

In the course of experiments on DNA homology in glutamic acid-producing bacteria, it became necessary to find an efficient method for complete bacterial lysis in order to prepare DNA. Among the coryneform bacteria used in these experiments, many were lysed only to a small extent by the standard treatments in current use. Treatment with lysozyme and sodium dodecyl sulphate (SDS) (Guerry *et al.*, 1973) or resuspension of harvested organisms in sucrose/Tris buffer followed by treatment with lysozyme (Huber & Godfrey, 1978) were generally ineffective. Schrepff *et al.* (1975), and earlier Yamada & Komagata (1970), reported that some micro-organisms grown in the presence of high concentrations of glycine showed increased sensitivity to lysis by lysozyme, but the use of this method for the isolation of DNA is restricted by the marked lowering in the yield of organisms. Sonication was ineffective for some of the bacteria used in the current experiments, and the procedures involved in the purification of fragmented DNA from French-press cell extracts proved cumbersome and time-consuming.

This communication describes a method which has proved particularly convenient for the preparation of DNA from this group of organisms.

METHODS

The glutamic acid-producing bacteria used were *Corynebacterium glutamicum* ATCC 13032, *Brevibacterium helvolum* IAM 1637, *Microbacterium ammoniophilum* ATCC 15354, *Arthrobacter tumescens* IAM 1458, *B. lactofermentum* ATCC 13655, *B. divaricatum* ATCC 14020, *Nocardia erythropolis* IAM 1484 and *B. flavum* ATCC 14067. They were grown in a medium (pH 7.0) containing (g l⁻¹): meat extract, 10; polypeptone, 10; NaCl, 5; yeast extract, 2. Overnight cultures (100 ml) were inoculated into 1 l of fresh medium and incubated for a further 3.5 h at 30 °C with shaking, by which time all the bacteria tested had reached the late-exponential phase of growth. Cultures of *C. glutamicum*, *M. ammoniophilum*, *B. lactofermentum*, *N. erythropolis* and *B. flavum* were then supplemented with crystalline potassium penicillin G (200000 units; Meiji Seika Co., Kyobashi 2-4-6, Tokyo, Japan 104) and incubation was continued for 0.5 h. For *B. divaricatum*, 250 mg sencephalin [7-(D-2-amino-2-phenylacetamido)-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-carboxylic acid; Takeda Yakuhin Co., Higashiku 2-27, Osaka, Japan 541] was

added after 3.5 h incubation, and incubation was continued for 2.0 h. *Brevibacterium helvolum* and *A. tumescens* were harvested after 3.5 h incubation, since they could be lysed without antibiotic treatment. Cultures were chilled in ice/water immediately after the termination of incubation, and the bacteria were harvested by centrifugation at 3 °C and washed twice with ice-cold 50 mM-Tris/HCl, pH 8.5, before lysis.

The conditions for lysis were modified from those of the manufacturer (Kyowa Hakko Kogyo Ltd, 1969). Kyowa's lytic enzyme no. 2 (40 mg), which has *N*-acetylmuramoyl-L-alanine-amidase, peptidase and endopeptidase activities, and lysozyme (10 mg) were dissolved in 10 ml of 50 mM-Tris/HCl containing 50 mM-EDTA, pH 8.5. After the addition of this solution to 4.0 g (wet wt) of the harvested bacteria, the mixture was homogenized at 0 °C and then incubated for 50 min at 50 °C. In general, bacterial suspensions became extremely viscous following lysis, which was assessed by microscopic observation. After the termination of the enzymic reaction, 1.0 ml of 5% (w/v) SDS solution was added and the mixture was incubated for 10 min at 60 °C. This SDS treatment usually completed lysis, as judged by clearing of the bacterial suspension.

RESULTS AND DISCUSSION

Schneider & Schlegel (1977) obtained lysis of certain corynebacteria after pretreatment of the organisms by repeated cycles of freezing and thawing. In the present study, lysis and the release of DNA from *C. glutamicum* and *M. ammoniaphilum* was obtained after two or three such cycles followed by treatment of the organisms with lytic enzymes and detergent. This method proved to be cumbersome and time-consuming. Moreover, organisms frozen quickly to -80 °C did not lyse after thawing and to obtain satisfactory lysis it was necessary to freeze slowly at -20 °C.

As an alternative method of lysis, the effect of β -lactam antibiotics, compounds known to inhibit wall biosynthesis, was examined. All the organisms treated with penicillin G, with the exception of *B. divaricatum*, showed increased sensitivity to lysis by added lytic enzymes. In general, antibiotic treatment for 0.5 h proved most effective; with longer periods some lysis of the cultures was observed.

It seemed possible that *B. divaricatum* was resistant to penicillin treatment because of the production of a β -lactamase although this was not tested. Lysis of this organism was obtained after incubation of growing cultures with sencephalin for 2 h. Under these conditions, subsequent treatment with lytic enzymes and detergent proved effective in releasing DNA. Thus, using a combination of treatments involving the inhibition of wall biosynthesis by incubation of growing organisms with the appropriate antibiotics and lysis of the residual wall, it has proved possible to isolate DNA suitable for use in DNA homology experiments from a range of glutamic acid-producing bacteria.

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