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Transformation and expression of a staphylococcal plasmid in *Escherichia coli*

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Abstract: A multiple antibiotic-resistant *Staphylococcus aureus*, was found to possess three plasmid bands in agarose gel electrophoresis. A plasmid of approximately 4.3 kb (pMC790/2) was found to code for ampicillin and tetracycline resistance and to have one *Eco*RI site when transformed into *S. aureus* RN 4220. pMC790/2 in unmodified form was transformed into a recA$^-$ *E. coli* at a frequency of 1.2×10^4 transformants/μg of plasmid DNA. Plasmid (pMC790/2) replicated, maintained itself stably and expressed far better in the *E. coli* host than in *S. aureus*.

Key words: Plasmid; *S. aureus*; Transformation; *Escherichia coli*

Introduction

We were interested in studying the antibiotic resistance plasmids in *Staphylococcus aureus*, the cause of more than 10% of all nosocomial infections [1]. It has been found that more than 90% of clinical isolates of *S. aureus* obtained from Calcutta hospitals are resistant to the penicillin group of antibiotics [2]. A multiply-resistant clinical strain of *S. aureus* MC790 was found to have three plasmid bands [3]. One of these bands has been found to correspond to the covalently-closed circular (CCC) conformer of an approximately 4.3 kb plasmid encoding β-lactamase production and tetracycline resistance (unpublished).

Due to a variety of limitations, data on the existence, replication and expression of plasmids in different organisms is restricted. Although staphylococcal genes have been expressed in *E. coli* as recombinant molecules [4,5], there are very few reports of *E. coli* being transformed by unmodified plasmid DNA isolated from other bacterial sources, especially from Gram-positive organisms. In all these cases plasmid copy number was low and expression poor in *E. coli*. [6,7].

In this paper we report the transformation of *E. coli* with a 4.3 kb *S. aureus* plasmid, pMC790/2. The transformed plasmid appeared to be unmodified and unaltered and replicated and expressed itself better in *E. coli* than in *S. aureus*.

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Materials and Methods

Bacterial strains

S. aureus MC790, a phage 95 (RTD) strain, was isolated from sputum at the Calcutta Medical College and Hospital, Calcutta. *S. aureus* RN834 containing plasmid pSN3, 30 kb; pSN1, 4.2 kb and pSN2, 1.3 kb and *S. aureus* RN4220, sensitive to all antibiotics and plasmid-free, were gifts from R.P. Novick, New York, USA. *E. coli* HB101 recA$^-$ plasmid-free and streptomycin-resistant were gifts from Dr. N.C. Mandol, Bose Institute, Calcutta.

Determination of antibiotic susceptibility pattern and preliminary detection of β-lactamase

Susceptibility to different antibiotics was determined by the disc diffusion technique [8] and the minimum inhibitory concentration (MIC) by the broth dilution method [9]. β-Lactamase production was detected with the chromogenic cephalosporin, nitrocefin [10].

Plasmid isolation and electrophoresis

Plasmid DNA was isolated and purified as described previously [3].

Restriction endonuclease digestion

Plasmid DNA was dissolved in reaction buffer (100 mM Tris HCl, 50 mM NaCl, and 10 mM $MgCl_2$, pH 7.5) and digested with *Eco*RI. Double digestion with *Eco*RI and *Pst* was performed in 50 mM Tris HCl, 50 mM NaCl, and 10 mM $MgCl_2$, pH 7.5 at 37°C.

Transformation

E. coli HB101 was made competent following the method of Maniatis et al. [11]. The optimum period of incubation in 50 mM $CaCl_2$, 10 mM Tris HCl, pH 8.0 was found to be 14 h. Plasmid DNA (30–40 ng) was added to 0.2 ml of competent cell suspension and transformants selected on nutrient agar plates containing 40 μg/ml ampicillin.

Hybridisation

DNA from an agarose gel was transferred to a Hybond-N membrane and probed with *Eco*RI linearised pMC790/2 labelled with randomly primed digoxigenin dUTP (Boehringer Mannheim). Hybridisation was carried out by standard techniques [11]. Hybridisation was detected by the enzyme-linked immuno-assay using antibody coujugate and subsequent enzyme catalysed colour reaction with 5-bromo-4-chloro, 3-indolyl (X-phosphate) and Nitroblue tetrazolium salt.

Measurement of DNA concentration

Plasmid DNA concentration was measured by the ethidium bromide method [11]. Petri-plates containing 10 ml of 1% agarose and 0.5 μg/ml ethidium bromide in TE were spotted with equal volumes of a known concentration of DNA. The DNA concentration of the unknown was determined by comparing their fluorescence with the controls.

Results and Discussion

E. coli transformants were selected on nutrient agar containing 40 μg/ml ampicillin and exam-

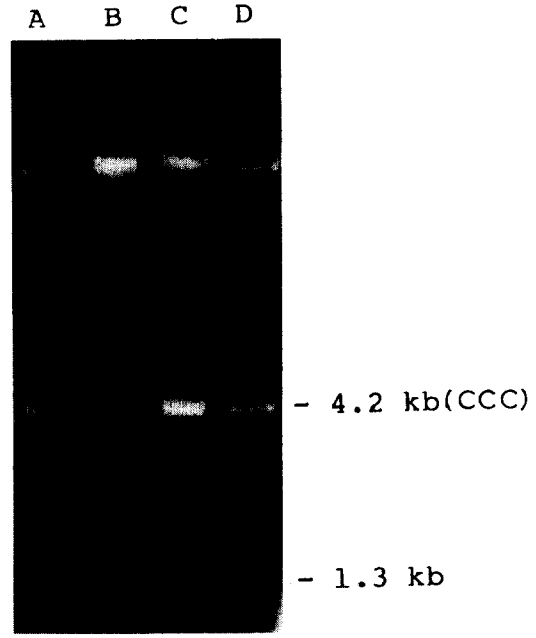

Fig. 1. Agarose gel electrophoresis of cleared lysates for plasmid DNA. Horizontal electrophoresis was performed with 0.8% agarose in TBE buffer (pH 8.2), for 6 h. Lane A, *S. aureus* MC790; lane B, untransformed *E. coli* HB101; lane C, *S. aureus* RN834 containing the plasmid size standards. Only pSN1 (4.2 kb) and pSN2 (1.3 kb) are visible. Lane D, transformed *E. coli* HB101.

ined for plasmid DNA by agarose gel electrophoresis. Plasmid mobility was compared with plasmids isolated from *S. aureus* MC790 containing pMC790/2, and *S. aureus* RN834 containing plasmid molecular weight standards. Competent *E. coli* HB101 incubated without DNA was used as a negative control. The plasmid in the transformed *E. coli* had the same mobility as pMC790/2 in *S. aureus* MC790 (Fig. 1). The transformation frequency for plasmid pMC790/2 into *E. coli* HB101 was 1.2×10^4 transformants/μg DNA. When tested for antibiotic susceptibility pattern, by disc diffusion, the transformed *E. coli* cells were found to be resistant to ampicillin and tetracycline. Plasmid pMC790/2 as well as the plasmid isolated from the transformed *E. coli* both had one *Eco*RI restriction site (Fig. 2a, 2b) and also one *Pst*I restriction site. Double digestion with *Eco*RI and *Pst*I revealed the same pattern of bands (Fig. 2c). pMC790/2 from *S. aureus* MC790 linearised with *Eco*RI and dig-labelled, hybridised with the plasmid from the transformed *E. coli* (Fig. 3).

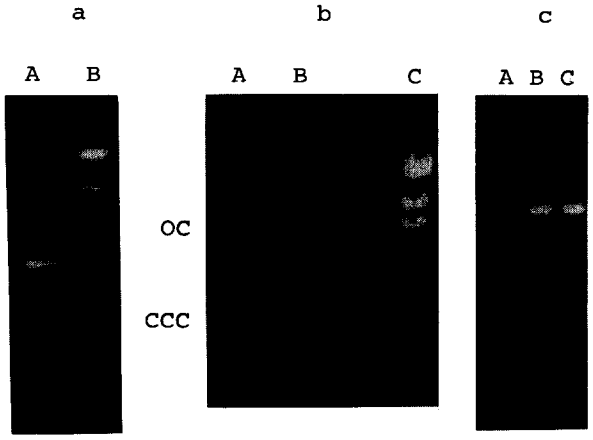

Fig. 2. Agarose gel electrophoresis of plasmids digested with *Eco*RI and/or *Pst*I. (**a**) Lane A, plasmid pMC790/2 from *S. aureus* MC790 and digested with *Eco*RI. Lane B, *Hin*dIII digested λ DNA. (**b**) Lane A, pMC790/2 from transformed *E. coli* HB101 uncut; Lane b, digested pMC790/2 from *E. coli* HB101 with *Eco*RI; Lane C, *Hin*dIII digested λ DNA. CCC, covalently closed circular DNA; OC, open circular. (**c**) Lane A, *Hin*dIII-digested λ DNA. Lane B, pMC790/2 from *S. aureus* digested with *Eco*RI and *Pst*I. Lane C, pMC790/2 from *E. coli* digested with *Eco*RI and *Pst*I.

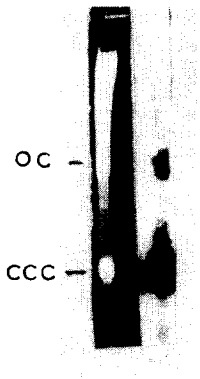

Fig. 3. Plasmid DNA from transformed *E. coli* hybridised with pMC790/2 from *S. aureus*. a, agarose gel of plasmid DNA from transformed *E. coli*. b, Southern blot of the DNA transferred from a. OC, open circular, CCC, covalently closed circular DNA.

Quantitation of the pMC790/2 DNA from *S. aureus* MC790 and pMC790/2 from the transformed *E. coli* revealed that transformed *E. coli* had three times more pMC790/2 than *S. aureus*. The yield of plasmid pMC790/2 from approximately 1×10^{12} CFU/ml of *S. aureus* was 32 μg/100 ml and from the same number of *E. coli* approximately 95 μg/100 ml. When the MIC for ampicillin was determined, it was found that the transformed *E. coli* cells had a much higher MIC (4000 μg/ml) than that of *S. aureus* (500 μg/ml).

The differences in the MIC values would appear to be due to more than just an increase in the copy number of pMC790/2 in *E. coli* and suggests that the plasmid is expressed better in *E. coli* than in *S. aureus*.

The results indicate that the staphylococcal plasmid, pMC790/2, was successfully transformed, unmodified, into *E. coli* and that it is expressed more efficiently in *E. coli* than in *S. aureus*.

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References

1 Ruben, F.I. and Norden, C.W. (1982) In: Bacterial Infections of Humans: Epidemiology and control (Evans, A.S. and Feldman H.A., Eds.), pp. 511–524, Plenum Medical Book Company, New York.
2 Ray, B. and Bal, M. (1982) Proc. VI Nat. Congr. Ind. Assoc. Med. Microbiol. pp. 154–158.
3 Saha, B., Saha, D., Niyogi, S. and Bal, M. (1989) Anal. Biochem. USA 176, 344–349.
4 Chang, A.C.Y. and Cohen, S.N. (1974) Proc. Natl. Acad. Sci. USA 71, 1030–1034.
5 Goze, A. and Ehrlich, S.D. (1980) Proc. Natl. Acad. Sci. USA 77, 7333–7337.
6 Verma, V., Qazi, G.N., Parishad, R. and Chopra, C.L. (1989) Plasmid 22, 265–267.
7 Patricia, G., Sera, K., Patricia, M. and Jorge, Z. (1991) FEMS Microbiol. Lett. 80, 147–150.
8 Acar, J.F. (1980) In: Antibiotic in Laboratory Medicine (V. Lorian Ed.), pp. 24–54. Williams and Wilkins, Baltimore.
9 Ray, B., Panja, K. and Bal, M. (1984) Indian J. Med. Res. 79, 482–486.
10 O'Callaghan, C.H., Morris, A., Kirby, S. and Shingler, A.H. (1972) Antimicrob. Agents Chemother. 1, 283–288.
11 Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.