Expression of Virulence and Antibiotic Resistance in an *Escherichia coli* Transconjugant Carrying a Large Plasmid pCAT120 of *Shigella dysenteriae* Type I and Its Spontaneous Fragmentations*


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ABSTRACT. The role of a 120-kb plasmid in relation to virulence and drug resistance factor in *Shigella dysenteriae* was studied. For characterization of plasmids, the mating system is a useful and efficient means of transferring both large and small plasmids to a new host. The conjugative transfer of a 120-kb (pCAT120) ampicillin-resistant plasmid of *S. dysenteriae* to *E. coli* K-12 was not successful. Introduction of an *E. coli* fertility factor plasmid F, did not help to mobilize the plasmid. Low transfer frequencies of antibiotic markers to *E. coli* were achieved by treatment of the donor *S. dysenteriae* with N-methyl-N'-nitro-N-nitrosoguanidine. The transconjugants showed resistance to ampicillin, chloramphenicol, tetracycline and cadmium. A transconjugant carrying the 120-kb plasmid of *S. dysenteriae* produced keratoconjunctivitis in guinea pigs. Repeated subculture of Clm r transconjugant (pCAT120) on tryptic soya agar plates became Clm s and showed four distinct DNA bands ranging from 3 to 10 kb in size on agarose gel electrophoresis. Utilization of organic acids, metal resistance (Cd), dye-binding properties (Crb +, Ebr §) and drug resistance (Amp, Tet) were identified on 10, 7, 4 and 3-kb plasmid DNA fragment of pCAT120 respectively. Crb + 4-kb DNA fragment of pCAT120 was isolated, purified and transferred to an avirulent *E. coli* K12 by transformation. However, transformant (pET4) showed poor growth on solid media and its growth in liquid culture was only possible after supplementation of the unknown low-molar-mass thermolabile factor(s) secreted by the recipient strain. A 130-kDa outer membrane protein was synthesized by the transformant (pET4) carrying a 4-kb Congo red binding plasmid DNA fragment of pCAT120. A highly reduced rate of synthesis of a few low-molar-mass outer membrane proteins was also observed among the transformant (pET4) in relation to the recipient strain. Transconjugant carrying four plasmid DNA fragments of pCAT120 and Crb + transformant (pET4) failed to produce keratoconjunctivitis in guinea pigs.

Three-hundred strains isolated from an outbreak of multidrug resistant *Shigella dysenteriae* type I infections in West Bengal, India (Pal 1984) were selected for their drug-resistance pattern and plasmid profiles. They showed similarities in antibiogram and plasmid profiles (Palchoudhuri et al. 1985). Most of the isolates were resistant to ampicillin (Amp), chloramphenicol (Clm), tetracycline (Tet), streptomycin (Stm), trimethoprim (Tmp), sulfonamide (Sul) but were highly sensitive to nalidixic acid (Nal) (Palchoudhuri et al. 1985). The isolates contained six plasmids ranging in size from 120 to 2.5 kb. An Amp resistance determinant was located in the 120-kb plasmid (pCAT120) and a small 2.5-kb non conjugative plasmid encoded for streptomycin-resistant character of our *S. dysenteriae* isolates (Datta et al. 1987, 1988a,b).

The essential virulence property of enteropathogenic bacteria depends on the ability to penetrate and multiply within the colon (LaBrec et al. 1964; Ogawa et al. 1967). Frost et al. (1985) reported that the strains isolated from different global epidemics carried a large plasmid of molar mass of 110 – 120 (MDa). The invasiveness of *Shigella* species has been associated with the presence of a large 120 – 140-MDa plasmid (Sansonetti et al. 1981, 1982, 1983; Sasakawa et al. 1986). Maurelli et al. (1984) and Sasakawa et al. (1986) demonstrated that the loss of Congo red binding ability of *S. flexneri* 2a consistently accompanied loss of virulence, and the majority of them has some deletions in the plasmid, Maurelli et al. (1984) also reported that a few new deletion derivative plasmids originated from the


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140-MDa plasmid of *S. flexneri* 2a. The loss of virulence as determined by mouse Sereny tests (Murayuma *et al.* 1986) and the loss of the ability to bind Congo red have been found to correlate (Sasakawa *et al.* 1986). Watanabe and Nakamura (1985) also found some deletions of plasmid pSS120 in *E. coli* K12 HW80 after transferring it from *S. sonnei*.

In the present study, we examined the role of the large plasmid pCAT120 of *S. dysenteriae* in relation to drug resistance and virulence factor (invasive). These genes are located in the intact plasmid pCAT120. A 130-kDa Crb\(^+\) outer membrane protein is synthesized by a 4-kb plasmid DNA fragment of pCAT120 which reduces cell growth and production of a few low-molar-mass OMP in comparison with wild-type recipient *E. coli* K12. Unknown low-molar-mass thermolabile substance(s) secreted by the wild type recipient *E. coli* K12 is required for the growth of the transformant (pET4). Both transconjugant (fragmented pCAT120) and transformant (pET4) showed a negative Sereny reaction.

**MATERIALS AND METHODS**

*Strains and media.* *Shigella dysenteriae* type I (Dt66) strains were isolated from cases admitted at the Infectious Diseases Hospital, Calcutta, during April to July, 1984. The strains were identified according to their antigenic properties and with the API-20E identification system (Smith *et al.* 1972). *S. dysenteriae* contained six plasmids ranging in size from 120 kb (pCAT120) to 2.5 kb. A large plasmid pCAT120 of donor *S. dysenteriae* was used for conjugal transfer to an avirulent recipient *E. coli* strain. Strains of *E. coli* K12, Tn903, pWS7 (F'Km \(\text{r}\)) (carrying Tn903 in the fertility factor plasmid F) and KL318 (F-, pro-48, trpA9605, his-85, metA9605, his-85, nalA19, rpsL171, \(\lambda^{-}\)) were obtained through the courtesy of Dr. S. Palchaudhuri of Wayne State University. Strain *E. coli* K12 V517 was used for the plasmid DNA marker (Macrina *et al.* 1978). Clm\(^{\text{r}}\) transconjugant (fragmented pCAT120) and transformant pET4 (4-kb Crb\(^{\text{r}}\)) were also used in this study.

Organisms were routinely cultured on bacto-tryptic soya broth (TSB) and agar (TSA) (Difco). Minimal M9 (Miller 1972) medium supplemented with vitamins and amino acids (modified medium) was used to determine the utilization of organic acid as a sole carbon source. The concentrations used for growth in presence of cadmium and ethidium bromide (Ebr) were 100 and 50 mg/L, respectively.

**Determination of resistance pattern.** Tryptic soya agar (4 %) was sterilized in an autoclave for 15 min at 103 kPa. Amp and Kan were dissolved in sterile phosphate buffer (0.1 mol/L) and sterile NaOH (1 mol/L) was used for nalidixic acid. Tet and Clm were dissolved in sterile water and ethanol, respectively. The antibiotics were mixed with sterile TSA (below 50 °C) before plating. The final concentration (mg/L) of antibiotics used for the selection of transconjugant was as follows: Tet 20, Amp and Clm 30 each, Kan and Nal 100 each. The resistance pattern was also verified by the disc diffusion technique.

**Mating conditions.** Donor and recipient strains were grown separately to exponential phase (100–200 CFU/mL). In all matings 0.1 mL of a solution of deoxyribonuclease I (EC 3.1.21.1) (1 g/L; Sigma) was added to both donors and recipients 10 min before mixing. Samples (0.1 mL) of the donor cultures of *S. dysenteriae* wild type (UV or MNNG treated) or *E. coli* K12 pWS7 were gently mixed with 0.8 mL recipient *E. coli* K12 KL318 or *S. dysenteriae* and the mating mixtures were allowed to stand for 2 h at 37 °C. At the start of mating diluted samples of donor and recipient were plated on TSA plates with and without antibiotics. At the end of mating, samples of the mating mixtures were washed, diluted and plated on to TSA plates containing suitable antibiotics (Amp−Nal, Clm−Nal, Tet−Nal and Kan−Nal).

**Isolation of derepressed mutants.** *S. dysenteriae* was grown at 37 °C with shaking in TSB to an absorbance of 0.3 at 590 nm. The cells were then chilled on ice, centrifuged and resuspended in an equal volume of MgSO\(_4\) (10 mmol/L). A 5-mL portion of this suspension was irradiated (254 nm, 1 W/m\(^2\)) in a Petri dish (100 mm diameter) while being stirred. Cells to be mutagenized chemically were grown to exponential phase and washed in citrate buffer (0.1 mol/L, pH 5.5) and treated with MNNG (50 mg/L) as described by Miller (1972). The UV-irradiated or MNNG-treated *S. dysenteriae* cells were directly used as donors in mating experiments.

**Plasmid isolation.** Cells of the donor, recipient, transconjugants (Clm\(^{\text{r}}\) and Clm\(^{\text{s}}\)), *E. coli* K12 V517 were grown in 5 mL TSB medium with constant shaking for 18 h. Transformant pET4 was grown