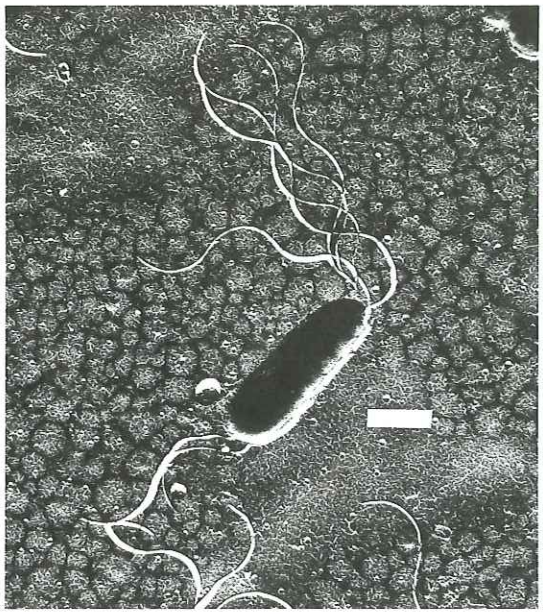

FIG. 1. *Pseudomonas putida* biovar A, strain WRF (ATCC 35546). Shadowed transmission electron micrograph. Cells are 1 × 2.5 to 1 × 3 μm and are motile with 2–5 flagella. Scale bar = 1 μm.

histidine. Amino acids incapable of supporting growth included norvaline, norleucine, methionine, cysteine, cystine, phenylalanine, tyrosine, tryptophan, α-amino-*n*-butyrate, and β-aminoisobutyrate in Frisell's unpublished work. A melanin-like pigment accumulated in the medium when the organism was grown on tyrosine.

It is concluded that *Pseudomonas* strain WRF is most closely related to *P. putida* biovar A.

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Clarification of the structure of the ampicillin-resistance plasmid RSF0885 from *Haemophilus influenzae* HR-885 serotype *b*

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The nonconjugative ampicillin-resistance plasmid RSF0885 has been reported to be as small as 2.9 MDa and as large as 4.1 MDa with at least two restriction enzyme maps reported. In addition, the source of the original plasmid has been reported to be *Haemophilus influenzae* and *Haemophilus parainfluenzae*. Characterization of the source strains and sequencing data of the plasmids revealed that *H. influenzae* serotype *b* was the original source strain and that IS*1*-K in the larger plasmid was presumably acquired when the smaller plasmid was maintained in *Escherichia coli* in S. Falkow's laboratory during the late 1970s.

Key words: *Haemophilus influenzae*, *Haemophilus parainfluenzae*, RSF0885, ampicillin resistance, IS*1*-K

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Le plasmide non-conjugatif de résistance à l'ampicilline RSF0885 a été décrit de façon contradictoire soit comme un plasmide plus petit de 2,9 MDa ou plus gros de 4,1 MDa et deux cartographies par restriction enzymatique ont été proposées. De plus la souche d'origine du plasmide a été identifiée comme *Haemophilus influenzae* et *Haemophilus parainfluenzae*. La caractérisation des souches supposées d'origine et les résultats de séquence des plasmides ont révélé que RSF0885 provenait d'une souche d'*H. influenzae* de sérotype *b* et que la présence de IS*1*-K dans le plus gros plasmide était probablement le résultat d'une acquisition lorsque le petit plasmide était maintenu dans une souche d'*Escherichia coli* dans le laboratoire de S. Falkow à la fin des années 1970.

Mots clés : *Haemophilus influenzae, Haemophilus parainfluenzae*, RSF0885, résistance à l'ampicilline, IS*1*-K.

[Traduit par la rédaction]

RSF0885, a nonconjugative ampicillin-resistance plasmid, was originally described in 1975 from a serotype *b* strain of *Haemophilus influenzae* (HR-885) sent by S. Cohen at the University of California San Francisco Medical Center to S. Falkow, then at the University of Washington in Seattle (Elwell et al. 1975). The original description of this plasmid included molecular mass estimates of about 3 MDa (5 kilo base pairs (kbp)) based on DNA contour lengths from electron microscopy studies. Subsequent work in the same laboratory reported similar mass and size estimates using agarose gel electrophoresis and ethidium bromide – CsCl density gradient centrifugation (Meyers et al. 1976; de Graaff et al. 1976). Discrepancies with the original reports as to the identification of the source strain and the size of the plasmid appeared in the literature as early as 1977. Gromkova and Goodgal reported that the source strain was not *H. influenzae* serotype *b* but rather *Haemophilus parainfluenzae* (Gromkova and Goodgal 1977). Investigators in S. Falkow's laboratory recalculated the mass by means of electron microscopy, sucrose gradient centrifugation, and agarose gel electrophoresis with internal standards and reported the plasmid to have a mass of 4.1 MDa (Roberts et al. 1977). A similar mass was reported by Laufs et al. (1979). Investigators in J. Setlow's laboratory, who received the plasmid in 1981 from Falkow's laboratory as a transformant in *H. influenzae* Rd, reported RSF0885 to have a mass of 3.7 MDa and to have only a single restriction site for *Pst*I (Setlow et al. 1981, McCarthy et al. 1982). J. Brunton, who received the plasmid from Falkow's laboratory as a transformant in *Escherichia coli* J53, reported RSF0885 to have two *Pst*I restriction sites (Brunton et al. 1981).

Besides historical interest in this plasmid as one of the earliest ampicillin-resistance plasmids described in the genus *Haemophilus*, this plasmid has been used to develop several shuttle vectors for use in the *Pasteurellaceae* and *Enterobactereaceae* (McCarthy et al. 1982; Danner and Pifer 1982; Trieu and McCarthy 1990) and we were interested in clarifying both the source and structure of this plasmid.

The original HR-885 strain was obtained in 1992 as a lyophile from M. Roberts in S. Falkow's old laboratory in Seattle. This strain was identified in the Provincial Laboratory of Public Health in Edmonton as a cross-reacting, nonserotypable, biotype II strain of *H. influenzae*, using conventional methods. This identification was confirmed by R. Weaver (personal communication) at the Centers for Disease Control and Prevention in Atlanta, Georgia. The strain was also confirmed as a capsule-deficient, spontaneous b^- mutant by D. Crook (personal communication) at the Public Health Laboratory, John Radcliffe Hospital, Oxford, using previously described methods (Falla et al. 1993).

On the basis of the discrepancies in size reported for RSF0885, we hypothesized that the difference was due to an insertional element being present in the larger plasmid, referred to as RSF0885*. Both RSF0885 and RSF0885* were electroporated into *E. coli* W3350 and maintained on Luria–Bertani (LB) plates containing 100 $\mu g \cdot L^{-1}$ ampicillin. Plasmid DNA from *E. coli* was prepared from 24-h cultures in 1 L of "terrific broth" (Tartof and Hobbs 1987) containing 100 $mg \cdot L^{-1}$ ampicillin and incubated at 37°C. Cells were harvested by centrifugation and lysed by alkali, and the plasmid DNA was isolated after CsCl – ethidium bromide gradient centrifugation or by precipitation with polyethylene glycol (Sambrook et al. 1989). Plasmid recovery from *H. influenzae* HR-885 was performed in a similar manner except that the cells were grown for 72 h on chocolate agar plates containing 75 $mg \cdot L^{-1}$ of ampicillin in an atmosphere of 5–7% CO_2.

TABLE 1. Susceptibility of isogenic strains of *Escherichia coli* and *Haemophilus influenzae* containing RSF0885 and RSF0885::IS*1* to ampicillin

	Minimum inhibitory concentration ($\mu g \cdot mL^{-1}$)
E. coli ATCC 25922	4
E. coli W3350	4
E. coli W3350 (RSF0885)	128
E. coli W3350 (RSF0885::IS*1*)	>1024
H. influenzae ATCC 49247	4
H. influenzae Rd*nov*	<0.5
H. influenzae Rd*nov* (RSF0885)	64
H. influenzae Rd*nov* (RSF0885::IS*1*)	256

Agarose gel electrophoresis of plasmid DNA revealed RSF0885 from HR-885 to have the same size as the smaller plasmid described in the literature and received by us from J. Setlow (data not shown). Thus, the larger plasmid must have acquired the insertional element during subsequent transformation of other strains. To map the insertion site, both plasmids were digested with *Pst*I, *Pvu*II, and *Taq*I following the manufacturer's specifications and the digested DNA was separated by electrophoresis in 1% agarose gels. The data shown in Fig. 1 showed the larger plasmid, RSF0885*, to have an additional *Pst*I and *Pvu*II site when compared with RSF0885, as reported by Brunton et al. (1985), and that the proposed insertional element was in the 2.3-kb *Taq*I fragment.

To obtain sequence data, the 2.3-kb *Pst*I fragment of RSF0885* was subcloned into the multiple-cloning site of pUC119 and transformed into *E. coli* MV1193. Nucleotide sequences were obtained using double-stranded sequencing in an automated cycle-sequencing device (Applied Biosystems). Sequences were extended by primer walking into the cloned fragment and the original plasmids, using oligonucleotides prepared in a PCR-MATE 391 DNA synthesizer (Applied

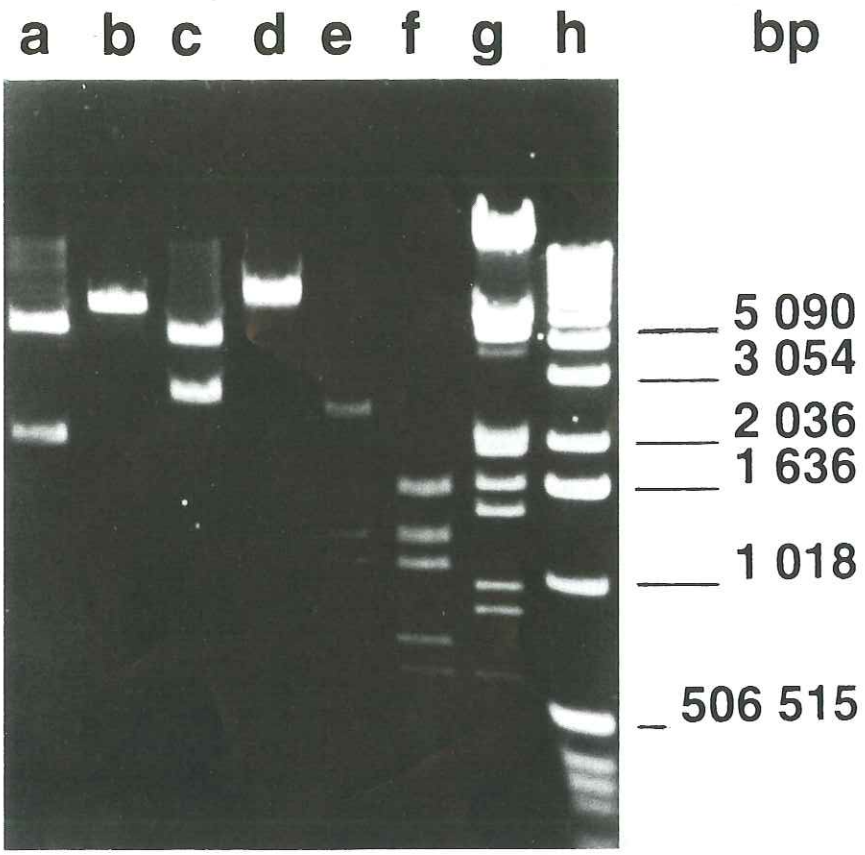

FIG. 1. Agarose gel electrophoresis of plasmids RSF0885 (lanes *b*, *d*, and *f*) and RSF0885* (lanes *a*, *c*, and *e*) digested with *Pvu*II (lanes *a* and *b*), *Pst*I (lanes *c* and *d*), and *Taq*I (lanes *e* and *f*). Lane *g* contains lambda *Eco*RI and *Hin*dIII molecular weight standards. Lane *h* contains a 1-kb

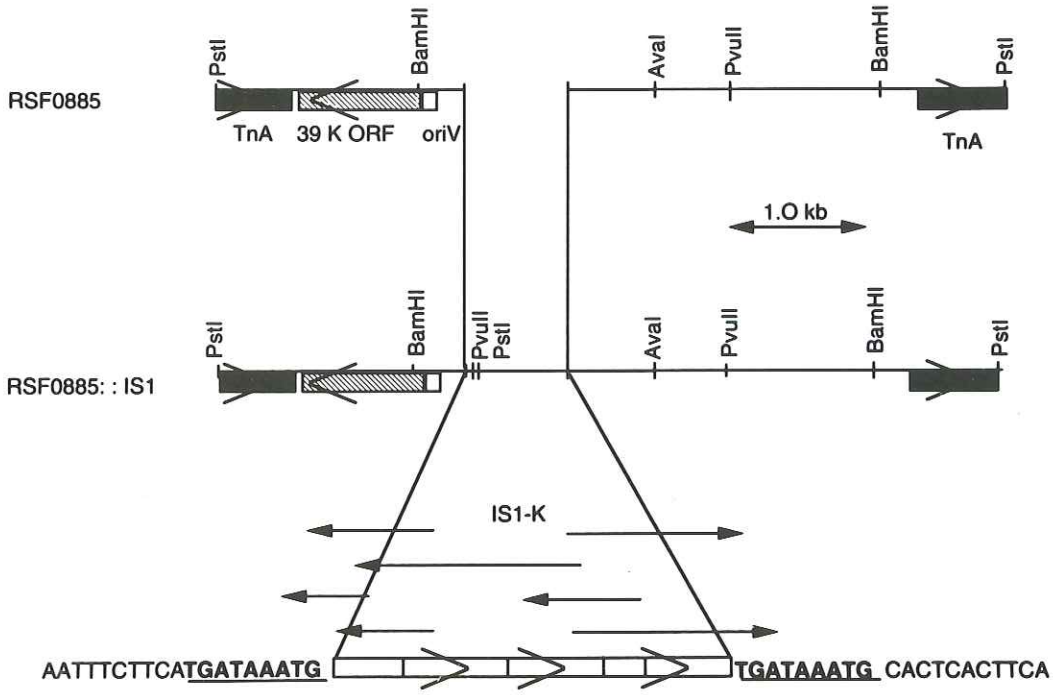

FIG. 2. Restriction endonuclease map of RSF0885 and RSF0885::IS*1*-K (RSF0885*) showing the sequencing strategy and orientation of IS*1*-K. Bold-faced and underlined bases refer to the 9-bp sequence duplicated at the insertion site of IS*1*-K. Large arrows indicate orientation of transcription for the indicated genes.

Biosystems) according to the manufacturer's instructions. Sequencing data revealed that the insertional element had 100% homology with IS*1*-K (Ohtsubo et al. 1984). The insertion site was located 636 bases upstream of the *Ava*I site (J.R. Dillon, personal communication) and 2752 bases upstream of the β-lactamase gene (Fig. 2). This site is approximately 400 bases from the insertion site of the element inserted in the 3.2-MDa ampicillin-resistance plasmid to create the 4.4-MDa ampicillin-resistance plasmid found in both *H. influenzae* and *Neisseria gonorrhoeae* (Laufs et al. 1979). In our hands, RSF0885 is identical to the 3.2-MDa plasmid also found in both *H. influenzae* and *N. gonorrhoeae*. Since IS*1*-K elements have not been reported in the *Pasteurellaceae*, transposition to RSF0885 most likely occurred when RSF0885 was introduced into *E. coli* by transformation. Most *E. coli* strains, including J53, have more than one copy of IS*1* (Sawyer et al. 1987).

Since RSF0885 has been reported to be unstable in an *E. coli* host (Trieu and McCarthy 1990), we determined whether or not the insertion of IS*1* into the plasmid conferred some survival value for RSF0885 in *E. coli*. RSF0885 and RSF0885::IS*1* (RSF0885*) were introduced into *E. coli* W3350 and *H. influenzae* by electroporation. When compared with the isogenic plasmidless strains and standard strains (*E. coli* ATCC 25922, *H. influenzae* ATCC 49247) used for susceptibility testing, RSF0885::IS*1* containing the insertion sequence conferred significantly greater resistance to ampicillin than RSF0885 without the insertion sequence (Table 1). This enhanced resistance is presumably due to the IS*1* sequence increasing the copy number and (or) stability of the plasmid, or to the IS*1* sequence enhancing expression of the β-lactamase gene. In any event, the increased resistance conferred by the IS*1* element could easily explain the selective isolation of a strain containing RSF0885::IS*1* from a strain containing a multicopy RSF0885 plasmid pool.

In summary, our data suggest that RSF0885 was indeed originally described in a serotype *b* strain of *H. influenzae* (HR-885) and that subsequent reported variations in size and restriction enzyme digestion patterns were due to the introduction of IS*1*-K during maintenance of the plasmid in a laboratory strain of *E. coli*.

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