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Short communication

A novel plasmid (pEMCJH03) isolated from *moraxella catarrhalis* possibly useful as a cloning and expression vector within this species☆

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Abstract

A preliminary screening study of six *Moraxella catarrhalis* isolates from primary school children in the Netherlands identified a small 3.5 kb plasmid (pEMCJH03), containing four open reading frames, which encoded three mobilizing and one replicase protein. Insertion of a kanamycin containing transposon (yielding pEMCJH04) allowed selection and isolation of the plasmid in *Escherichia coli*. Natural transformation of pEMCJH04 into M. catarrhalis was successful for 25% (3/12) of non-isogenic isolates, with no link between (lack of) transformability and genetic lineage or (lack of) transformability and complement phenotype, though the transformation efficiency was found to be rather low at approximately 615 CFU/μg (range = 60–1040 CFU/μg). This is only the second publication detailing a plasmid isolated from this important respiratory pathogen, and the ability to clone and express foreign proteins in *M. catarrhalis* using pEMCJH04 could help in the development of a vaccine expression vector, as well as providing a useful tool for studying promoter activity and in complementation studies of gene knockout mutants.

Keywords: *Moraxella catarrhalis*; pEMCJH03; pEMCJH04; Mobilizable plasmid

☆ The complete DNA sequences for plasmid pEMCJH03 and pEMCJH04 (pEMCJH03ΩEZ::TN⟨KAN-2⟩) are available at GenBank Accession Nos. AY167745 and AY453632, respectively.

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Moraxella catarrhalis is a common commensal of the human upper respiratory tract which has been associated with a number of disease states including acute otitis media in children (Catlin, 1990; Faden et al., 1994a,b) and both acute and chronic bronchitis in adults (Hager et al., 1987; Nicotra et al., 1986). Nosocomial spread and outbreaks of this pathogen have been reported (Cook et al., 1989; Patterson et al., 1988) as well as cases of near fatal pneumonia (Dyson et al., 1990). Moreover, there exists a very high prevalence of β-lactamase production within the species (Thornsberry et al., 2002). Vaccines against *M. catarrhalis* are currently being developed (Chen et al., 1996; McMichael, 2000), though a suitable universal cloning vector for the expression of *M. catarrhalis* proteins within this species and other genera has yet to be fully described and developed. Several studies have investigated the carriage of plasmids in non-*Moraxella catarrhalis* species including *M. bovis* (McDonald and Pugh, 1986), and environmental *Moraxella* species (Kawasaki et al., 1992; Rani et al., 1996; Vasudevan and Paulraj, 1994), though only one plasmid has been fully characterized and sequenced within *the M. catarrhalis* species (plasmid pLQ510). This 12 kb plasmid pLQ510 (Beaulieu et al., 1988; Liu and Hansen, 1999) has previously been suggested as a possible cloning and expression vector, though the size of the plasmid (even without the presence of inserted genes) could reduce its ability to be efficiently transformed. Here, we report on the finding of a small 3.5 kb plasmid in *M. catarrhalis* which could possibly fulfill such a role.

Initial plasmid screening efforts utilized 6 *M. catarrhalis* isolates (three colonizing isolates originally isolated in 1989 from primary school children in Nieuwegein, The Netherlands, and three colonizing isolates originally isolated in 1993 from primary school children in Heerenveen, The Netherlands). The isolates were retrieved from −80 °C storage and grown overnight (approximately 16 h) on 5% sheep blood agar at 37 °C until semi-confluent, whereby 1/4 plate of growth was removed and embedded in agarose blocks according to established pulsed field gel electrophoresis (PFGE) protocols (Vu-Thien et al., 1999), but without the addition of a restriction enzyme. PFGE was performed using a CHEF Mapper (Bio-Rad) with a ramping protocol of 6 V/cm constant voltage at 15 °C, and a pulse time increased from 2 to 10 s for 10 h. After electrophoresis, the presence of any extrachromosomal plasmid DNA was detected using ethidium bromide staining. PFGE testing indicated the presence of at least three different extra-chromosomal elements within the range of isolates tested (Fig. 1), ranging in size from approximately 5–60 kb in size. At this point, the smallest plasmid (pEMCJH03) was isolated from its host (*M. catarrhalis* isolate 6.12) using the SV Wizard plus minipreps system (Promega), and recovered by insertion of a kanamycin encoding transposon using the EZ::TN⟨KAN-2⟩ insertion kit (Epicentre). The resultant pEMCJH03 plasmid containing the EZ::TN⟨KAN-2⟩ transpo-

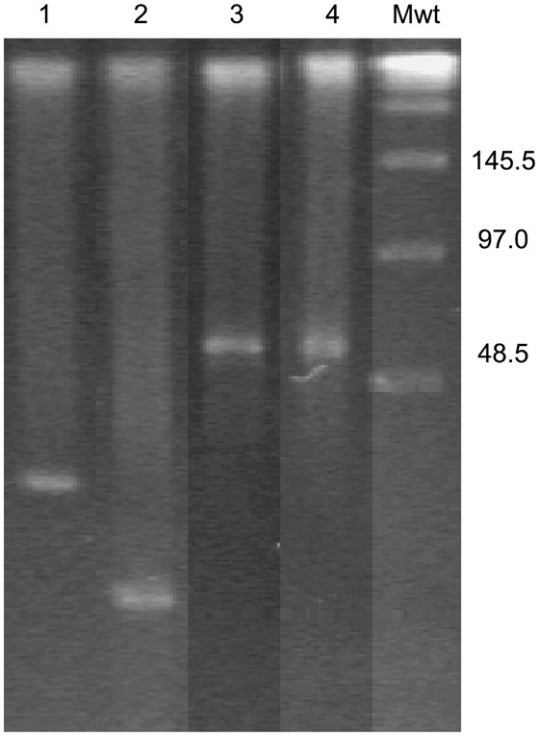

Fig. 1. Composite picture of extra-chromosomal elements observed in 4 *M. catarrhalis* isolates recovered from children living in The Netherlands during the years 1989 and 1993 using native pulsed field gel electrophoresis. 1–4 = *M. catarrhalis* isolates 3.21, 5.12N, 6.12K, F6.92, and F5.82, respectively. Mwt, lambda ladder molecular weight marker (kbp).