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DNA SEQUENCE OF A SMALL, UNIDENTIFIED PLASMID ISOLATED FROM A *HAEMOPHILUS SOMNUS* STRAIN: SHORT COMMUNICATION

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One of the plasmids present in a *Haemophilus somnus* strain isolated from nasal discharge of a cattle with respiratory disease was purified and cloned for DNA sequencing. The plasmid was found to be 1065 base pairs long with 39.2% G+C content, and showed no homology to any DNA sequenced so far. It has no capacity to code any protein longer than 43 residues. It is not clear yet if this plasmid codes *Haemophilus somnus* specific factors.

Key words: DNA, sequencing, plasmid, cattle, *Haemophilus somnus*

Haemophilus (*H.) somnus*, a small, pleomorphic, Gram-negative, pathogenic coccobacillus was isolated for the first time from a case of thromboembolic meningoencephalitis in cattle (Griner et al., 1956). The taxonomic position of *H. somnus* is uncertain (Kilian and Biberstein, 1984). On the basis of the DNA relatedness proven by DNA-DNA hybridisation, it was suggested that *H. somnus*, *Histophilus ovis* and *Haemophilus agni* can be considered as a single species (Walker et al., 1985; Piechulla et al., 1986).

H. somnus is associated with pneumonia, infertility, abortion, mastitis, septicaemia, arthritis and thromboembolic meningoencephalitis in cattle (Panciera et al., 1968; Andrews et al., 1985; Widders et al., 1986; Higgins et al., 1987; Harris and Janzen, 1989), abortion in ewes and epididymitis in rams (Kennedy et al., 1960; Lees et al., 1994).

Bulls may carry *H. somnus* asymptomatically in the preputial epithelium and cows in the vaginal epithelium, and it is supposed that in the presence of predisposing factors, *H. somnus* may cause severe diseases (Humphrey and Stephens, 1983; Andrews et al., 1985; Harris and Janzen, 1989; Corbeil, 1990; Rusvai and Fodor, 1998; Rusvai et al., 1999). However, with the help of chromoso-

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mal DNA analysis and ribotyping, the strains isolated from the genital tract and from pathologic samples could be differentiated (Fussing and Wegener, 1993).

Despite the economic importance of this bacterium, its biology is not well understood. Although *H. somnus* is usually susceptible to most antibiotics, resistant strains are emerging (Humphrey and Stephens, 1983), several potential virulence factors have been identified which are expressed on the cell surface, and most of the investigations were done on the outer membrane proteins and related genes. Since in some members of this genus plasmids may encode drug resistance or may carry virulence factors (Huether et al., 1989; Ishii et al., 1991), the DNA sequence of a small plasmid found in an isolate from Hungary was investigated and GenBank homology search was performed to find similarity to any sequences coding virulence or resistance factors in other (related) bacteria. The results are described in the present paper.

Nasal swabs collected from cattle with respiratory disease symptoms were inoculated on chocolate agar, and the agar plates were incubated at 37 °C for 48 h in the presence of 10% CO_2. The nine isolated *H. somnus* strains were identified on the basis of cultural and biochemical characteristics (Humphrey and Stephens, 1983). Till further examination, the isolates were stored at –80 °C.

Brain heart infusion broth (DIFCO) supplemented with calf serum was inoculated with the *H. somnus* strains and cultured at 37 °C with slight agitation. Plasmid preparation was carried out using the alkaline lysis method from 2 ml of liquid culture of all the nine *H. somnus* strains.

Two plasmids could be detected in one strain (57/98) out of the nine examined *H. somnus* strains by agarose gel electrophoresis. Since the applied isolation method is for high-copy-number cloning vectors of *E. coli* (Sambrook et al., 1989), it can be supposed that the examined *H. somnus* plasmid present in its host is in high copy number as well.

The smaller plasmid was recovered from agarose gel and digested with *Bam*HI, *Eco*RI, *Hin*dIII, *Pst*I, and *Xho*I enzymes to determine the physical maps. *Bam*HI, *Pst*I, and *Xho*I did not cut the plasmid, while *Hin*dIII had one and *Eco*RI had two recognition sites (Fig. 1). The two *Eco*RI fragments with an approximate size of 200 and 800 base pairs were cloned into the plasmid pBluescript SK and strain XL1 Blue of *E. coli* was transformed with the ligates.

The cloned DNA fragments were sequenced from both ends using T3 and T7 primers on an ABI 373 automated DNA sequencer at the Biological Research Centre of the Hungarian Academy of Sciences (Szeged, Hungary). The sequences were analysed on six possible reading frames (both strands in both directions) by the LASERGENE program package (DNASTAR Inc., Madison, Wisconsin). Homology search was performed using the BLASTN and BLASTX programs on the non-redundant data bases of the National Center for Biotechnology Information (NCBI, USA).

The exact size of the complete *H. somnus* plasmid proved to be 1065 base pairs with a G+C content of 39.2%. The two restriction cleavage sites of the *Eco*RI endonuclease enzyme were found to be 821 base pairs apart (Fig. 2).

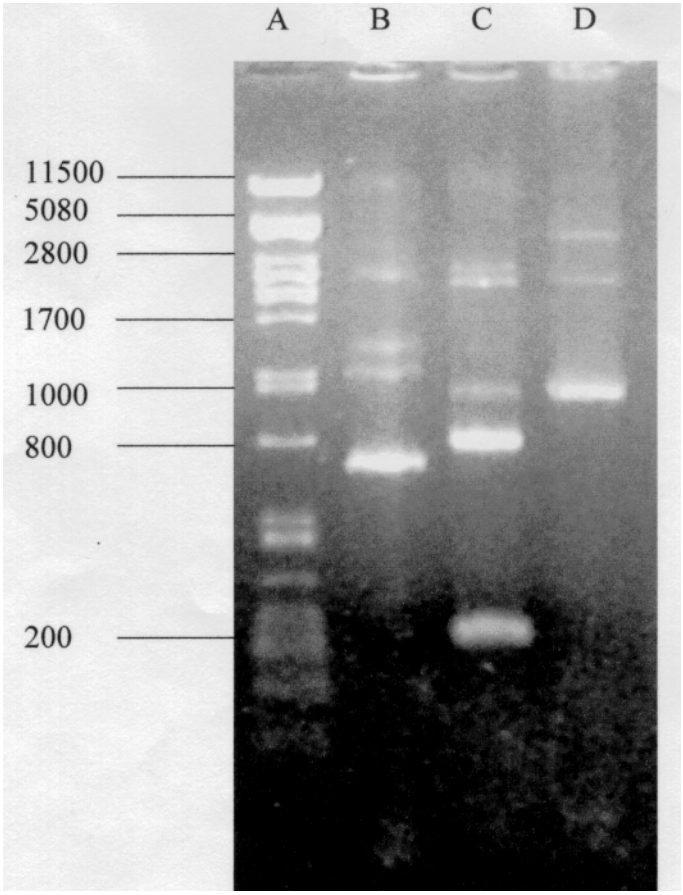

Fig. 1. Plasmids isolated from a *H. somnus* strain. *Lane A:* λ phage DNA digested with *Pst*I, used as marker (size shown in bp.). *Lane B:* Intact *H. somnus* plasmids. *Lane C: H. somnus* plasmids cleaved by *Eco*RI. *Lane D: H. somnus* plasmids cleaved by *Hin*dIII. The smallest plasmid in *Lane B* is supercoiled and was cleaved at two sites by *Eco*RI resulting in two fragments with approximate sizes of 200 and 800 bp, and at one site by *Hin*dIII resulting in a linear form of the plasmid

Homology search failed to find any similar DNA in the nucleic sequence data bases. The longest putative open reading frame (ORF) was found to be 261 pairs long. Based on the location of possible translation initiation codons, there is a theoretical possibility that this ORF codes for a protein of 43 amino acids. The homology search, however, showed no significant similarity with the currently available protein data either.

The presence of plasmids in *H. somnus* was detected by some authors, but neither the function nor the DNA sequence or genetic relatedness of these plasmids was examined (Fussing and Wegener, 1993; Appuhamy et al., 1998). The size of the plasmids isolated by Fussing and Wegener (1993) ranged from 1.5 to 3.4 kb which is comparable to the size of the plasmid described in the present a rticle.

Since the homology search did not help to connect the plasmid to specific virulence or drug resistance factors, transformation of a plasmid-free *H. somnus* strain using the isolated plasmid is planned to investigate the role of the plasmid.

GAATTCGTTTATCAAGTCAAATCAAATAAACCGCAAAAAAGAGAGCGGTTTTT
TTATGCCTATTTTTCCGCAATCCAAATAAAAAGCCTTCTAAACGAGTGGTAGG
AACAAGCGTCACTGGCGTTTAGTCGTATTAATCTGTTACCAGCGGAAACAAGC
AAAGCAGAAACGCTATGAGTGGTTTTAAATCGAGTGCAGTGCATTTATATTGG
GTTAGTCCCCTTGAGTGCGTAGTCAGCGATTTAGAGCGGTTATGAGCGTGCTT
GTGCGGTGAGCTATACCGTAATCATAACAGCGAGAGAGATAGCCCTTGATCTC
GTCCAAAGGCGACACGGCTCGGCTCCGTCGGCGATGTCCATCTTTGGCAATGC
ACTAGGCTTTGTTTTTTTAACAAGGTCTAAGGGTGAGTGTTGCCAAAAACTCT
CCAACTCTCACCTTCAGCGATGAGCCTTATCTCCCCACTTTTCAAAAAGAAGA
GAAGAAAAAAAAAAAGACAGAGAACGTTAGATCAGTGAATGTAAGAATGTTGA
GCTGTACTCAATTCTATTCACTACTCTTAATTCGGAGGACGAACTTCATTATG
GCAACTCAAAAAACGGCTCAAAACTTGCTTTTGAGTTGGTTTTTGAGTTCAAC
AACCGTAGGGCGTTAGTTTTCTAGTGCAGGATAGGGATTTATCCGATATTGTA
TGACAATATCAGCTAAATCCCCCTGTACTTGCAGTAGGCTCACGGCTACGCCG
TAACTACCGCACTTCACTTCGTTCCGTTTGCTCAATTCAAAAAATCGATTAAA
AGACTTCGCTATTTAATCCATTTTTT**GAATTC**TCAAATTAGAAAACTAACGCC
CTAAAAAACGAAAAAGCCAACATTTTTTTTATGTTGGCTCTTTACGTTTTAAT
TCATTTAAATTAAATTATCGGCGTGGTTTATTTGCCCCCAGCAAATAAACGAA
TTTCTCACAACAGATTTTA***AAGCTT***CTCAGGTTTTAAAACTGTCATAAATAAA
GTTTCTCAGGCTTTATTTTAGTGTCGAAATTCCCGATAAGGTGAATGATAAGA
GAAAA

Fig. 2. Nucleotide sequence of the *H. somnus* plasmid GenBank/EMBL/DDBJ accession number AF 318175. *Eco*RI sites are shown by bold letters, *Hin*dIII site is shown by bold italics letters, the longest ORF is underlined, and the possible methionine codon in the reading frame is double underlined

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