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Veterinary Microbiology 86 (2002) 303–311

veterinary microbiology

www.elsevier.com/locate/vetmic

DNA sequences coding for the F18 fimbriae and AIDA adhesin are localised on the same plasmid in *Escherichia coli* isolates from piglets

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Received 11 June 2001; received in revised form 3 January 2002; accepted 26 January 2002

Abstract

The adhesin-involved-in-diffuse-adherence (AIDA) afimbrial adhesin is produced by human, but not by animal, *Escherichia coli*, with the exception of German porcine verotoxigenic *Escherichia coli* (VTEC) [Clin. Diagn. Lab. Immunol. 8 (2001) 143]. Presence and localisation of DNA sequences (*aidA*) coding for and production of an AIDA adhesin were investigated in a collection of Belgian VTEC and non-VTEC *E. coli* isolated from piglets at weaning time. The 174 isolates were also studied by colony hybridisation for the presence of DNA sequences coding for the Stx2e verocytotoxin and the F18 fimbrial adhesin (*fed*): 71 were Stx2 + F18 + AIDA+, 26 were F18 + AIDA+, 12 were AIDA+, two were Stx2 + AIDA+, and one was Stx2+ only. Fifty-four of the 58 (F18+)AIDA+ isolates tested positive in a western blotting assay with an immune serum raised against the AIDA protein. Hybridisation with the AIDA gene probe on plasmid DNA profiles identified a probe-positive plasmid band in the 10 AIDA+ and in 24 of the 25 F18 + AIDA+ isolates studied. Moreover in F18 + AIDA+ isolates, only one plasmid band hybridised with both F18 and AIDA probes. These results confirm the presence of *aidA*-related genes in not only VTEC, but also non-VTEC, isolates from piglets and the production of an antigenically AIDA-related protein by the majority of probe-positive *E. coli*. Moreover the plasmid DNA hybridisation results suggest a localisation on the same plasmid of the *aidA*- and *fed*-related DNA sequences.

Keywords: *Escherichia coli*; Pig-bacteria; F18 fimbriae; AIDA adhesin; Plasmid DNA

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0378-1135/02/$ – see front matter
PII: S0378-1135(02)00019-6

1. Introduction

Human pathogenic *Escherichia coli* can adhere to epithelial cells in culture (HeLa, HEp-II) following three different patterns: localised, diffuse and aggregative (Scaletsky et al., 1984; Levine, 1987; Nataro et al., 1987). Different adhesins can confer diffuse adherent properties to *E. coli*. Examples are the Afa family of afimbrial adhesins (Le Bouguénec and Bertin, 1999) and the adhesin-involved-in-diffuse-adherence (AIDA) afimbrial adhesins, coded by the *aah* (previously *orfA*) and *aidA* (previously *orfB*) genes (Benz and Schmidt, 1989, 2001; Suhr et al., 1996).

aidA-like sequences have been identified in a very few bovine, canine, feline and avian *E. coli* (Mainil et al., 1993, 1998a; Niewerth et al., 2001; Stordeur et al., 2001). Nevertheless, preliminary colony hybridisation results suggest that *aidA*-like sequences are more frequent amongst porcine *E. coli* (Mainil and Benz, unpublished data), more especially verotoxigenic *E. coli* (VTEC) which cause the edema disease syndrome in recently weaned piglets and produce the Shiga toxin 2 porcine variant (Stx2vp or Stx2e) and the F18 fimbriae, as putative colonisation factor (Imberechts et al., 1992a; Mainil, 1999; Nagy and Fekete, 1999; da Silva et al., 2001). Identical results on German porcine VTEC were recently published (Niewerth et al., 2001).

The purpose of the present study was to confirm these associations in Belgian porcine VTEC and non-VTEC by DNA colony hybridisation, to investigate the production of AIDA protein by the probe-positive strains, and to precise the localisation of the *fed*- and *aidA*-related DNA sequences by plasmid DNA hybridisation.

2. Materials and methods

2.1. E. coli isolates

The 174 *E. coli* were isolated from piglets with suspicion of edema disease (Pohl et al., 1989). Porcine verotoxigenic *E. coli* (VTEC) strain 107/86 and human DAEC strain 2787 were the reference strains for the F18 (F107) fimbrial adhesin and for the AIDA afimbrial adhesin, respectively (Benz and Schmidt, 1989; Imberechts et al., 1992b). *E. coli* K12 clones contain the recombinant plasmids pNN111-19 (Newland and Neill, 1988), pIH120 (Imberechts et al., 1992b), pIH16 (Imberechts et al., 1992b), pIB6 (Benz and Schmidt, 1989) and pBA2 (Iglesias et al., 1983) carrying the genes coding for the Stx2 Shiga toxin, the F18 fimbrial adhesin (*fed* gene cluster), the F18 fimbrial major subunit (*fedA*), the AIDA adhesin (*aah* and *aidA*), and the rRNA of *Bacillus subtilis*, respectively.

2.2. Gene probe derivation

The recombinant plasmids were purified using a Qiagen KitII (Qiagen) and the gene probes were derived by digestion with appropriate restriction endonucleases (Table 1) following the manufacturer's instructions (BRL). Three probes, which cover the whole gene (Fig. 1), were derived from the *aidA* gene (Table 1). The restriction DNA fragments were separated by migration for 30 min in 2% agarose gel and the probe fragments were

Table 1
Derivation of the gene probes

Probe name	Recombinant plasmid	Endonuclease[a]	Size[b] (bp)	Reference
Stx2	pNN111-19	*Pst*I	900	Newland and Neill (1988), Mainil et al. (1989)
F18	pIH16	*Pst*I	914	Imberechts et al. (1992b)
AIDA	pIB6	*Eco*RI	696	Benz and Schmidt (1989)
		*Eco*RI + *Sau*3AI	873	This study
		*Eco*RI	1765	This study
16S	pBA2	*Pst*I + *Hin*dIII	1300	Iglesias et al. (1983)

[a] Restriction endonuclease used to derive the gene probe fragment.
[b] Size of the probe fragment.

eluted using a Geneclean II kit (Bio 101). Probes were radioactively labeled with dATP α-^{32}P (Amersham) using a Random Primed Hexanucleotide Labelling kit (Boehringer Mannheim). The positive controls were strain 107/86 for the Stx2 and F18 probes and strain 2787 for the AIDA probe.

2.3. Colony and plasmid DNA hybridisation

The DNA colony hybridisation assay was performed as previously described (Mainil et al., 1993). Plasmid DNA was extracted according to an adaptation of the original method of Kado and Liu (Kado and Liu, 1981; Broes et al., 1988). The different plasmids were separated by an overnight electrophoresis in 0.5% agarose gel. Plasmid DNA hybridisation with the F18 and AIDA gene probes was performed within the dehydrated agarose gels as previously described (Broes et al., 1988).

2.4. Plasmid DNA purification and southern blotting

Plasmid DNA was purified by ultracentrifugation in cesium chloride gradient with ethidium bromide, after alkaline extraction, from three porcine VTEC and from the human DAEC reference strain 2787 (Maniatis et al., 1982). Both the plasmid and chromosome

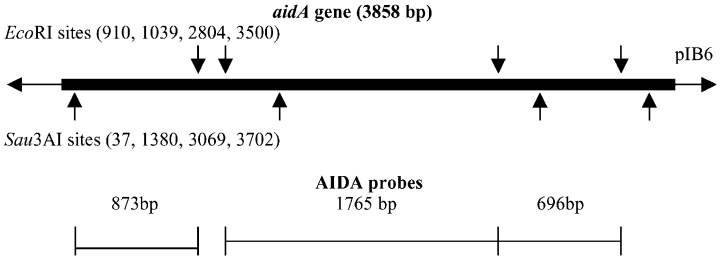


Fig. 1. *Eco*RI and *Sau*3AI partial restriction map of the *aidA* gene of human reference DAEC strain 2787 on recombinant pIBH6 plasmid (Benz and Schmidt, 1989) and derivation of the three AIDA probes.

bands were recovered and digested with *Eco*RI restriction endonuclease following the instructions of the manufacturer (BRL). After overnight electrophoresis in 0.8% agarose gel, the restriction fragments were transferred onto nylon membrane (Hybond-N+, Amersham) by southern blotting (Maniatis et al., 1982). The nylon membranes were subsequently hybridised with the F18, AIDA and 16S probes.

2.5. Western blotting

The production of an AIDA-like protein was investigated by western blotting using an immune serum raised against the AIDA protein produced by the recombinant *E. coli* K12 (pIB4) after cloning from the human DAEC strain 2787 and preabsorbed against *E. coli* K12 (pBR322). The mature and active AIDA adhesin of strain 2787 migrates as a ca. 100 kDa protein (Benz and Schmidt, 1992).

3. Results

3.1. Colony hybridisation

The porcine reference VTEC strain 107/86 hybridised with the F18 and with the three AIDA probes, while the human reference DAEC strain 2787 hybridised only with the three AIDA probes. Similarly 96 wild-type porcine VTEC and non-VTEC hybridised with the F18 and the three AIDA probes and 14 others with the AIDA, but not the F18, probes (Table 2).

Both *E. coli* K12 harboring the pIH16 and pIH120 recombinant plasmids tested positive with the F18 probe and neither with the AIDA probes; the *E. coli* K12 harboring the pIB6 recombinant plasmid tested positive with the AIDA probes and negative with the F18 probe.

3.2. Western blotting

Western blotting was performed on 58 AIDA+ (including reference strain 107/86) and on 29 AIDA-negative porcine VTEC and non-VTEC. Strain 2787 was the positive control (Fig. 2). Fifty-four of the AIDA probe-positive (including reference strain 107/86) and

Table 2
Colony hybridisation results of the 174 porcine VTEC and non-VTEC isolates

Pathotype	No. of isolates	Isolates (%)
Stx2 + F18 + AIDA+	71[a]	41
Stx2 + AIDA+	2	1
Stx2+	1	<1
F18 + AIDA+	26	15
AIDA+	12	7

[a] Including VTEC reference strain 107/86.

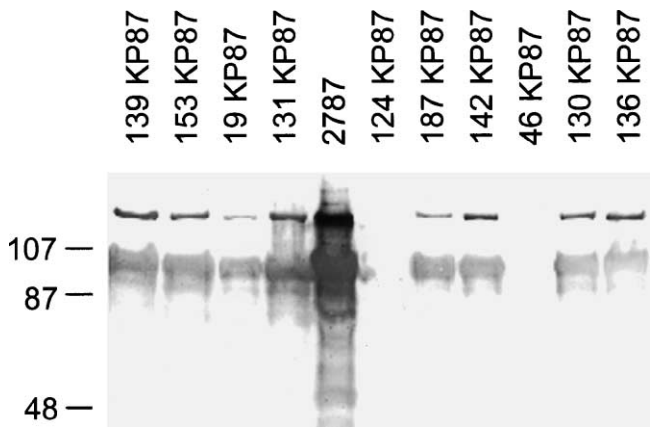


Fig. 2. Western blotting results on porcine *E. coli* isolates with an anti-AIDA immunserum (Benz and Schmidt, 1992). Human DAEC strain 2787 and porcine isolates 139KP87, 19KP87, 131KP87, 187KP87, 142KP87, 130KP87 and 136KP87 were AIDA+ by colony hybridisation; porcine isolates 153KP87, 124KP87 and 46KP87 were AIDA-negative by colony hybridisation; 107, 87 and 48 are molecular weights (kDa).

three of the AIDA probe-negative isolates gave positive results in western blotting (Table 3).

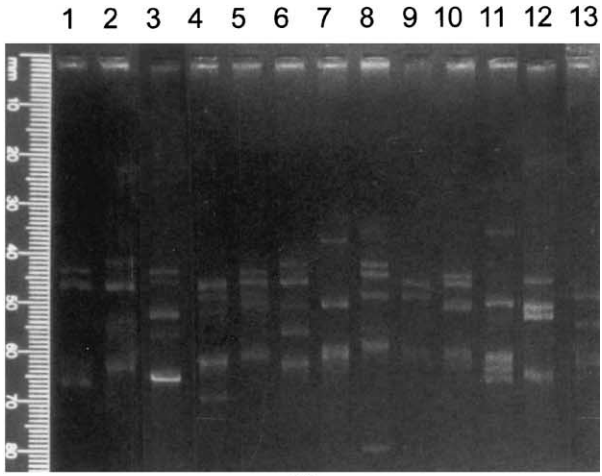
3.3. Plasmid and total DNA hybridisation

Plasmid DNA was extracted by the method of Kado and Liu from 24 F18 + AIDA+ and 10 AIDA+ porcine VTEC and non-VTEC and from the reference strains 107/86 (F18 + AIDA+) and 2787 (AIDA+) (Fig. 3). The gels were hybridised with the F18 probe and one AIDA probe (0.696 kb; Table 1). All strains harbored two to five high-molecular weight plasmid bands (higher than 30 MDa). In 24 of the 25 F18 + AIDA+ porcine isolates, one plasmid band (molecular weight between 40 and 100 Mda) tested positive with both F18 and AIDA probes (Fig. 3). In one isolate no positive reaction was observed with either probe. In the 10 AIDA+ porcine isolates and in the human reference strain 2787 one plasmid band hybridised with the AIDA probe and none with the F18 probe.

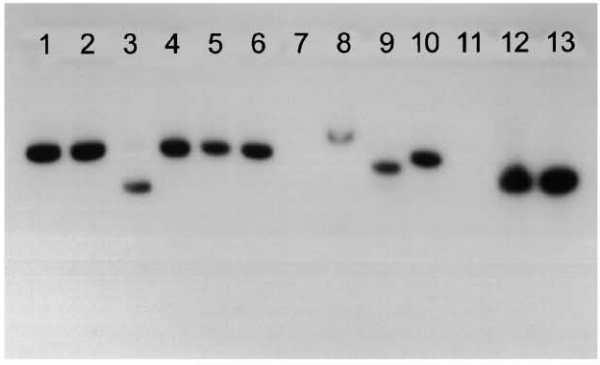
Table 3
Western blotting results on 58 AIDA+ and 29 AIDA-porcine VTEC and non-VTEC isolates

Pathotype	Western blotting results	
	Positive	Negative
Stx2 + F18 + AIDA+	36[a]	2
F18 + AIDA+	14	2
AIDA+	4	–
–	3	26

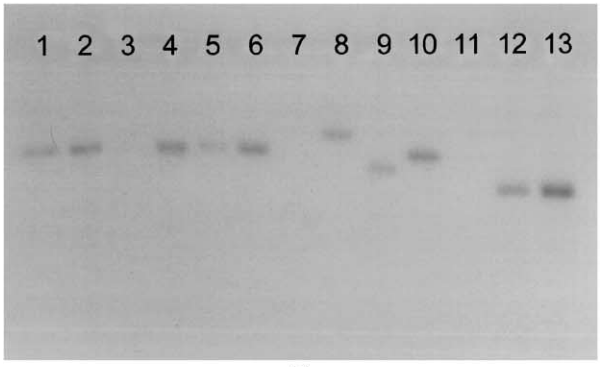
[a] Including VTEC reference strain 107/86.



(a)



(b)



(c)

Fig. 3. Plasmid profiles and plasmid DNA hybridisation results with AIDA and F18 probes of porcine *E. coli* isolates after extraction by the method of Kado and Liu (Kado and Liu, 1981; Broes et al., 1988). Lanes 1, 2, 4–6, 8–10, 12, 13: porcine VTEC and non-VTEC F18 + AIDA+ isolates (lane 13 is strain 107/86). Lane 3: human DAEC AIDA+ strain 2787. Lane 7: plasmid size markers (98, 42 and 24 MDa). Lane 11: porcine F18- and AIDA-negative isolate.

The F18 and AIDA probes tested positive with one restriction fragment each of both plasmid and total DNA from three F18 + AIDA+ porcine VTEC (107/86, E57 and 749S87) after purification by ultracentrifugation in CsCl gradient and digestion by the restriction endonuclease *Eco*RI. The AIDA probe also tested positive with one restriction fragment of both plasmid and total DNA from the human F18-AIDA+ DAEC 2787. Conversely, the 16S probe hybridised with restriction fragments of the total DNA and not of the plasmid DNA of the four strains.

4. Discussion

The results of this study confirm the presence of complete *aidA*-related genes in not only VTEC, but also non-VTEC, isolates from piglets at weaning, and the production of an antigenically AIDA-related protein by the majority of probe-positive *E. coli*. Moreover the *aidA*-related genes are located on a plasmid along with the *fed*-related genes coding for the F18 fimbrial adhesin.

If the association between the verotoxin produced by porcine VTEC, Stx2e, and AIDA is relatively high (Niewerth et al., 2001; this study), the association between their fimbrial colonisation factor, F18, and AIDA is even higher (Table 2). The non-VTEC isolates may well produce other toxins, such as classical heat-stable (STa and STb) or heat-labile (LT) enterotoxins of enterotoxigenic *E. coli* and be associated with post-weaning diarrhea (Nagy and Fekete, 1999). Several isolates of this study were previously assayed for the presence of genes coding for these enterotoxins, but only a few tested positive for the STb toxin, and none for the STa or LT toxins (data not shown). The actual meaning of F18+ toxin-negative isolates in disease is still unclear (Nagy and Fekete, 1999). The few strains with discordant colony hybridisation and western blotting results may (i) harbor *aidA*-like but not *aah*-like genes (Benz and Schmidt, 2001); (ii) harbor unexpressed *aidA*-like genes; (iii) have given false positive or negative results in either the western blotting or the colony hybridisation assay.

As in human DAEC and in German porcine VTEC (Benz and Schmidt, 1989; Niewerth et al., 2001), the *aidA*-related sequences are located on high molecular weight plasmids in Belgian porcine VTEC and non-VTEC. Though the F18 fimbriae gene clusters were originally thought to be located on the chromosome (Imberechts et al., 1992b), the *fed*-related sequences are also located on high molecular weight plasmids in Belgian porcine VTEC and non-VTEC, according to the hybridisation results on plasmid DNA. Transfer by conjugation of F18-positive plasmids has actually been observed although rarely (Dean-Nyström et al., 1993; Fekete et al., 2002).

Moreover, according to our results the *aidA*-related sequences are located on the same plasmid than the *fed*-related sequences coding for the F18 fimbriae. The presence of two plasmids of identical size cannot be formally excluded, but would be surprising in all the strains tested. The negative plasmid DNA hybridisation results in one non-VTEC isolate is most probably the consequence of a plasmid or gene loss, as can happen in vivo and in vitro with other virulence plasmids (Casey and Moon, 1990; Mainil et al., 1992, 1998b). The alternative hypothesis of plasmid integration into the chromosome was not tested.

Besides the F18 fimbriae, the AIDA adhesin may represent an alternative or complementary colonisation factor of the porcine intestine before the production of the toxin(s) by the *E. coli* isolates. If the toxin is Stx2e, the piglets may develop edema disease; if the toxin is one of the classical enterotoxins, the piglets will develop post-weaning diarrhea (Mainil, 1999; Nagy and Fekete, 1999; da Silva et al., 2001). In humans, however, only a few % of the DAEC are positive for the AIDA adhesin and their actual involvement in enteritis and diarrhea is still controversial (Garcia and Le Bouguénec, 1996; Nataro and Kaper, 1998; Le Bouguénec and Bertin, 1999). In vivo experiments with wild-type F18 + AIDA+ porcine isolates and their allelic mutants in the *aidA* and *fed* genes are clearly needed to assess the respective role of each adhesin in the colonisation of the porcine gut. These experiments shall also define the adherence pattern of AIDA+ porcine *E. coli* to cells in culture in comparison to human AIDA+ isolates.

Acknowledgements

The authors thank Miss Pascale Rémy, Mr Sébastien Dester, and Miss Chiara Graceffa, trainees from the ''Institut Provincial d'Enseignement Supérieur Pédagogique et Agricole—Haute Ecole Rennequin Sualem''. This work was supported by a grant (Crédits aux chercheurs) from the ''Fonds National de la Recherche Scientifique (FNRS)''.

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