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Identification of *Escherichia coli* Recovered from Milk of Sows with Coliform Mastitis by Random Amplified Polymorphic DNA (RAPD) Using Standardized Reagents

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Ramasoota P, Krovacek K, Chansiripornchai N, Pedersen Mörner A, Svenson SB: Identification of *Escherichia coli* recovered from milk of sows with coliform mastitis by random amplified polymorphic DNA (RAPD) using standardized reagents. Acta vet. scand. 2000, 41, 249-259. – A standardized-reagents commercial kit for random amplified polymorphic DNA (RAPD) analysis was used for typing 58 *Escherichia coli* strains that were recovered from the milk of sows, having coliform mastitis, within a single swineherd in Sweden. Previously, the 58 *E. coli* strains were characterized serologically and profiled biochemically. They were also evaluated for their serum resistance and their ability to adhere to fibronectin and bovine fetal fibroblasts. The RAPD analysis was fast, easily performed, and required only a nanogram of DNA. The indistinguishable banding patterns obtained with repeated analyses of 2 isolates from each strain demonstrated that RAPD analysis using standardized beads is a technique that provides reproducible results for typing *E. coli* strains that cause mastitis in sows. The results of the RAPD analyses demonstrated that *E. coli* sow mastitis strains are highly variable in serotype, biochemical profiles, virulence factors, and RAPD type, and that all 58 strains can be differentiated by means of the RAPD technique. The strains grouped into 24 RAPD types by combining the results of 2 primers, and into 38 groups by combining the results of serotype and RAPD type. No relationship between serotypes, virulence factors and RAPD types was found.

E. Coli

Introduction

Mastitis caused by *Escherichia coli* in sows is considered to be part of the agalactia/hypogalactia post partum (APP) syndrome. If not detected early and adequately treated, this lactation failure leads to insufficient colostrum intake by the offspring resulting in their starvation and in economic loss for the owner (*Pedersen Mörner et al.* 1998).

Epidemiological studies have demonstrated the existence of clonal variation in *E. coli* populations throughout the world. This observation was originally based on the association between serotypes and biotypes among strains isolated in different parts of the world (*Ørskov et al.* 1976, *Ørskov & Ørskov* 1977). But clonal relationships are not always clearly demonstrated using serotypes as the discriminating tool. Unrelated clones can have the same serotype, or

strains of different serotypes can show the same enzyme activity (*Caugant et al.* 1985, *Selander et al.* 1986, *Selander et al.* 1987).

Various methods have been used for distinguishing and comparing pathogenic *E. coli* strains for epidemiological investigations. The traditional biochemical and physiological typing methods, including bacteriophage typing, are time consuming and technically demanding. Antibiotic sensitivity testing analysis is of limited value because identical resistance profiles do not prove that the strains are genetically identical. These traditional methods have been replaced by molecular genetic techniques such as pulse field gel electrophoresis (PFGE) (*Lee et al.* 1996) and restriction fragment length polymorphism (RFLP) (*Maurer et al.* 1998), which requires from 1 to 2 microgram of DNA to give informative results.

Also, random amplified polymorphic DNA (RAPD) (*Williams et al.* 1990, 1993), or arbitrarily primed polymerase chain reaction (AP-PCR) (*Mccleland & Welsh* 1994), has been used successfully to discriminate various *E. coli* strains, such as Enterotoxigenic O6:H16 E. coli (ETEC) (*Schultsz et al.* 1997, *Pacheco et al.* 1998), Shigella-like Toxin *E. coli* (STEC O157 *E. coli*) (*Gallien et al.* 1997), Verotoxin (VT)-producing *E. coli* (VTEC) (*Heuvenik et al.* 1995), and Avian Pathogenic *E. coli* (APEC) (*Maurer et al.* 1998). Indeed, there have been claims that RAPD technique for distinguishing *E. coli* is more sensitive than serotyping (*Pacheco et al.* 1997) and multilocus enzyme electrophoresis (*Wang et al.* 1993). And other researchers claim that RAPD is at least as sensitive as the ribotyping technique (*Karkkainen et al.* 1996) or RFLP analysis (*Maurer et al.* 1998).

Unfortunately, the results obtained with RAPD are generally not reproducible within and between laboratories (*Meunier et al* 1993, *Tyler et al.* 1997). This may be a result of DNA quality, reagent concentrations, DNA polymerase or annealing temperature (*Meunier et al.* 1993, *Power et al.* 1996). However, by using pre-formulated RAPD analysis beads that contain all PCR reaction buffers, nucleotides and 2 DNA polymerases (Ampli Taq and Stoffel fragment), *Vogel et al.* (1999) achieved stable banding patterns and within-laboratory reproducibility, and *Grundmann et al.* (1997) achieved inter-laboratory reproducibility. Therefore, since suitable use of these standardized RAPD beads provides reproducible results, and as they are inexpensive and fast to use, we employed them to identify *E. coli* strains obtained from mastitis milk of sows within a single swineherd in Sweden.

Materials and methods

Bacterial strains, serotypes, and biochemical tests

Fifty eight *E. coli* strains were isolated from samples of mastitis milk of sows within a single herd in Sweden. The sows were clinically examined with special reference to APP for 6 consecutive lactations (*Persson et al.* 1989, 1996), and all 58 milk samples were tested previously for their O-antigens, for K88, K99, 987P antigens using 1-163 O-antigen, and for fimbriae-specific antisera; and also for their biochemical profiles using API 20E (Bio Merieux SA, Marcy I'Etoile France), serum resistance, and ability to adhere to fibronectin and bovine fetal fibroblasts, as previously described (*Pedersen Mörner et al.* 1998).

Chromosomal DNA Preparation

All strains were cultured on horse blood agar. Loopfuls of cultured bacteria were transferred to conical tubes containing 0.5 ml sterile water, one loopful per tube. The tubes were then vortexed for 1 min before being ultrasonicated for 1 min, and then centrifuged at 13,000 RPM for 5 min in an Eppendorf centrifuge 5042.

To purify the DNA from the supernatant in each

Table 1. The random amplified polymorphic DNA primers screened for this study.

Primer	Sequence (5' to 3')	No. of Bands	Banding Molecular size range (bps)
RAPD Analysis Primer 1.	5'-d[GGTGCGGGAA]-3'	11	350-1500
RAPD Analysis Primer 2.	5'-d[GTTTCGCTCC]-3'	4	350-850
RAPD Analysis Primer 3.	5'-d[GTAGACCCGT]-3'	9	200-1000
RAPD Analysis Primer 4.*	5'-d[AAGAGCCCGT]-3'	12	200-2000
RAPD Analysis Primer 5.	5'-d[AACGCGCAAC]-3'	9	350-1250
RAPD Analysis Primer 6.*	5'-d[CCCGTCAGCA]-3'	13	200-2000

*RAPD Analysis Primer 4 and RAPD Analysis Primer 6 were later used to examine each of the 58 *E. coli* strains.

tube, an equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) was added, mixed 10 times by inversion, then centrifuged at 10,000 RPM for 15 min. The top layer was removed and placed in a new microcentrifuge tube, and then an equal volume of Chloroform:Isoamyl Alcohol (24:1) was added, mixed 10 times by inversion, and the content of the tube centrifuged at 10,000 RPM for 15 min. Again the top layer was removed and placed in a new microcentrifuge tube. The DNA in the supernatant of each tube was precipitated by adding 1/10 volume of 3 M sodium chloride, and then by adding 2 volumes of 95% ethanol, before leaving the tube overnight at 4°C and then centrifuging it at 10,000 RPM for 15 min. The supernatant was removed and the DNA pellet washed with 70% ethanol, and then dried at room temperature. The remaining DNA was then dissolved in 100 ml sterile distilled water. The concentration and purity of DNA was determined spectrophotometrically at 260 nm and 280 nm using the Gene Quant RNA/DNA calculator (Pharmacia Biotech, Uppsala, Sweden).

Selection of RAPD primers

DNA samples of 4 chosen *E. coli* strains; 2 *E. coli* reference strains, BL21 and C1a; and 2 strains that were randomly selected from the set of 58 *E. coli* sow-mastitis strains, were initially amplified with 6 different primers from a RAPD commercial kit (Amersham Pharmacia Biotech, Uppsala, Sweden) (Table 1). Because 2 of the 6 primers, namely, primer 4 (5'-AAGAGCCCGT-3') and primer 6 (5'-CCCGTCAGCA-3'), yielded the largest number of amplicons over a broad range of molecular sizes, they were selected for further RAPD analysis of the 58 strains.

RAPD-Polymerase chain reaction conditions

"Ready-To-Go RAPD Analysis Beads" commercial kit (Amersham Pharmacia Biotech) consists of standardized dry beads that are room-temperature-stable. The beads contain thermostable polymerases (AmpliTaq DNA polymerase and Stoffel fragment), dNTPs (0.4mM; each, dNTPs in a 25 μl reaction volume), bovine serum albumin (2.5 μg) and buffer (3mM $MgCl_2$, 30 mM KCl and 10 mM Tris pH 8.3 in a 25 μl reaction volume). We added 10 μl of primer (2.5 pmol/μl) (Table 1), 8 μl of template DNA (10ng/μl), and then 7 μl of sterile water to a total volume of 25 μl. The reaction mixture was incubated at 94 °C for 5 min, and then run in a Gene Amp PCR system 2400 thermocycler (Perkin Elmer, USA) for 45 cycles (1 min at 94 °C, 1 min at 36 °C, and 2 min at 72 °C). The amplicons were visualized by ethidium bromide staining after electrophoresis in a 2% Nusieve agarose gel. Photographs were digitalized and stored in Tiff file format. To assure

Table 2. The O-antigens found in the 58 *E. coli* strains, and for each antigen, the number of times it was identified within the 58 strains and its frequency of being identified.

O-antigen	Number of strains	(%)
5	1	(1.7)
8	3	(5.2)
9	2	(3.5)
45	5	(8.6)
60	1	(1.7)
62	1	(1.7)
68	2	(3.5)
91	1	(1.7)
99	2	(3.4)
110	2	(3.4)
111	1	(1.7)
127	2	(3.4)
149	1	(1.7)
Not typeable	34	(59)

reproducibility of the results, PCR was done at least twice on all samples.

RAPD Data analysis

The RAPD patterns for the individual strains were scored based on band presence or absence. The Reference strain *E. coli* BL21 was used as an internal control for measuring the variability of RAPD patterns among experiments. The RAPD was recognized as variable if different RAPD patterns were produced for *E. coli* BL21. The similarity index (F) between samples was calculated using the following formula (*Nei and Li* 1979)

$$F_{xy} = 2n_{xy} /(n_x + n_y),$$

where n_{xy} is the number of RAPD bands shared by 2 samples x and y, and n_x and n_y are the number of RAPD bands scored in each sample. The genetic distance (*d*) was calculated using the formula of *Hillis & Moritz* (1990),

$$d = 1 - F.$$

The degree of relationship between serotypes, biochemical profiles, virulence factors and RAPD types was calculated by means of Chi-square and Fisher's Exact Test using the statistical software SAS.

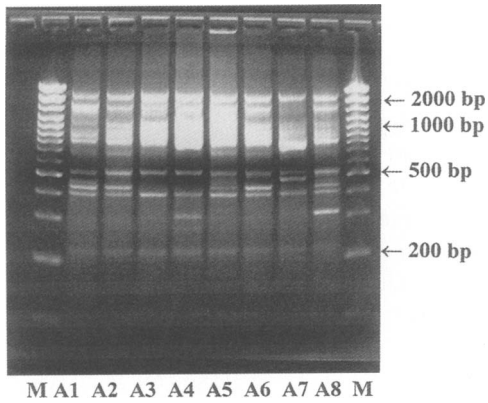

Figure 1. 8 RAPD profiles (using RAPD primer 6) of *E. coli* isolated from mastitis sows in Sweden, Lanes 1-8, and RAPD profiles A1- A8, Lane M: a 100 bp DNA ladder. [Note that A2 and A6 differs by a band of 500 bp (present in A6)].

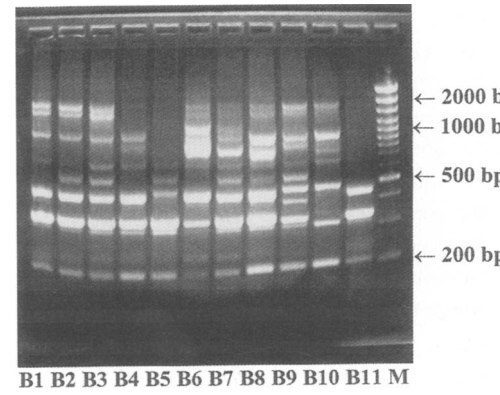

Figure 2. 11 RAPD Profiles (using RAPD primer 4) of *E. coli* isolated from mastitis sows in Sweden, Lanes 1-11, and RAPD profiles B1- B11, Lane M: a 100 bp DNA ladder.

Table 3. Similarity coefficients, *F* (top-right diagonal) and genetic distance, *d* (bottom-left diagonal) of RAPD profiles of *E. coli* from mastitis sows, using a) primer 6 (profiles A1, A2, ..) and b) primer 4 (profiles B1, B2, ..).

a

RAPD Profile	A1	A2	A3	A4	A5	A6	A7	A8	Ac*
A1	-	0.95	0.90	0.86	**0.96**	0.91	**0.82**	**0.96**	0.56
A2	0.05	-	0.95	0.90	0.91	0.95	0.90	0.91	0.58
A3	0.10	0.05	-	0.95	0.86	0.90	0.90	0.86	0.52
A4	0.14	0.10	0.05	-	**0.82**	0.86	0.95	0.91	0.58
A5	0.04	0.09	0.14	0.18	-	**0.96**	0.87	0.92	0.53
A6	0.09	0.05	0.10	0.14	0.04	-	0.91	0.87	0.56
A7	0.18	0.10	0.10	0.05	0.13	0.09	-	0.87	0.56
A8	0.04	0.09	0.14	0.09	0.08	0.13	0.13	-	0.56
Ac*	0.44	0.42	0.48	0.42	0.47	0.44	0.44	0.44	-

b

RAPD profile	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	Bc*
B1	-	0.86	0.93	0.61	0.66	0.61	0.82	0.87	0.53	0.46	0.60	0.40
B2	0.14	-	0.93	0.61	0.66	0.61	0.82	0.87	0.53	0.31	0.46	0.53
B3	0.07	0.07	-	0.71	0.77	0.71	0.89	0.94	0.62	0.43	0.54	0.37
B4	0.38	0.38	0.29	-	0.73	0.67	0.62	0.67	0.71	0.67	0.67	0.28
B5	0.33	0.34	0.23	0.27	-	0.73	0.67	0.71	0.61	0.36	0.75	0.31
B6	0.38	0.39	0.29	0.33	0.27	-	0.75	0.67	0.57	0.33	0.44	0.28
B7	0.18	0.18	0.11	0.38	0.33	0.25	-	**0.95**	0.55	0.53	0.50	0.47
B8	0.12	0.13	0.06	0.33	0.29	0.33	0.05	-	0.71	0.53	0.50	0.59
B9	0.47	0.47	0.38	0.29	0.39	0.43	0.45	0.29	-	0.71	0.54	0.25
B10	0.54	0.69	0.57	0.33	0.64	0.67	0.47	0.47	0.29	-	**0.22**	0.14
B11	0.40	0.54	0.46	0.33	0.25	0.56	0.50	0.50	0.46	0.78	-	0.18
Bc*	0.60	0.47	0.63	0.72	0.69	0.72	0.53	0.41	0.75	0.86	0.82	-

* RAPD profiles Ac and Bc were obtained from the reference strain, *E. coli* BL21.
Values shown in bold are minimum and maximum similarity coefficient.

Results and discussion

The 58 sow-mastitis *E. coli* strains from the swineherd in Sweden could be differentiated from one another on the basis of their serotypes and RAPD types. A total of 14 O-antigens, 2 K88, and 34 (59%) untypable strains were identified (Table 2). All strains could be differentiated by the presence and size of their RAPD products. RAPD profiles for each sample were produced using the 2 RAPD Analysis Primers, 4 and 6. Amplification reactions generated informative arrays of bands composed of a minimum of 4 and a maximum of 13, with the molecular size ranging from 200 to approximately 2,000 bps (Figs. 1 and 2).

RAPD amplification with RAPD primer 4 was found to be more discriminating than with RAPD primer 6. With RAPD primer 6, eight different profiles were found in the 58 strains, and each profile had a very high genetic similarity, between 82% (minimum) and 96% (maximum). When compared with RAPD profile Ac

Table 4. Characterization of the 58 *E. coli* strains isolated from the milk of mastitis sows within a swineherd in Sweden.

Strain[1]	O-group[2]	Presence[3] Of K88 or K99	Lactation[4]	RAPD profile		RAPD Type	Combination of Serotype & RAPDtype
				Primer6	Primer4		
213V6-A	45	-	-	A1	B1	1	Group 1
213 V6-B	45	-	-	A1	B1	1	
242 V4I	60	-	-	A1	B1	1	Group 2
249 H4 I-B	110	-	A	A1	B1	1	Group 3
194V5 II	NT	-	-	A1	B1	1	
213H5 I-A	NT	-	B	A1	B1	1	
213H5 I-B	NT	-	-	A1	B1	1	Group 4
226H6 -II	NT	-	-	A1	B1	1	
187 V4I	68	-	A	A1	B2	2	Group 5
201 H2I	NT	K88+	B	A1	B2	2	Group 6
201H 2	NT	-	-	A1	B2	2	
201V5 I	NT	-	A	A1	B2	2	
201V7-A	NT	-	-	A1	B2	2	Group 7
201V7-B	NT	-	-	A1	B2	2	
236H3 I	NT	-	-	A1	B2	2	
249H4 II	NT	-	-	A1	B2	2	
223 H7-I	45	-	-	A1	B3	3	Group 8
201V5-B	NT	-	-	A1	B3	3	Group 9
221H5-I	NT	-	-	A1	B3	3	
237H6 I-A	NT	-	-	A1	B3	3	
270V4 I	NT	-	-	A1	B5	4	Group 10
270H2 II	NT	-	-	A1	B5	4	
211H3 I	NT	-	A	A1	B7	5	Group 11
211H3 II	NT	-	A	A1	B7	5	
1542 V4	149OX 46	-	-	A1	B11	6	Group 12
249H4 I-A	110	-	A	A2	B1	7	Group 13
249V2 II	NT	-	-	A2	B1	7	
201V5-A	NT	-	-	A2	B1	7	Group 14
237V5 I	NT	-	-	A2	B1	7	
284H5 II	NT	K88+	B	A2	B3	8	Group 15
211V3 I	5	-	A	A2	B3	8	Group 16
211V3 II	NT	-	-	A2	B3	8	Group 17
284H7 I	NT	-	B	A2	B3	8	
217H6 I	45	-	-	A2	B4	9	Group 18
264V5 I	NT	-	-	A2	B4	9	Group 19
237H6 I-B	NT	-	-	A2	B5	10	Group 20
264H2 I	NT	-	-	A2	B10	11	Group 21
240V4 I	8	-	-	A2	B11	12	Group 22
211V1 I	99	-	A	A3	B1	13	Group 23
211V1 II	99	-	A	A3	B1	13	
275H3 I	91	-	-	A3	B1	14	Group 24
187H5 I	8	-	B	A3	B4	15	Group 25
283H3 I	9	-	-	A3	B4	15	Group 26
284H5 I	9	-	A	A3	B4	15	
214H6I	NT	-	-	A3	B4	15	
283V5 I	NT	-	-	A3	B4	15	Group27
201H6 I	NT	-	-	A3	B4	15	
199H5 I	8	-	-	A3	B8	16	Group 28
66	68	-	-	A3	B9	17	Group 29
229V5 I	NT	-	-	A3	B9	17	Group 30
220H4 II	62	-	C	A4	B6	18	Group 31
259H4 I	NT	-	-	A4	B6	18	Group 32
270V4	NT	-	-	A5	B5	19	Group 33
214H4I	NT	-	-	A5	B8	20	Group 34
211H5 II	111	-	B	A6	B3	21	Group 35
255V4 I	NT	-	-	A7	B6	22	Group 36
238V5 II	127	-	B	A8	B5	23	Group 37
238V5 I	127	-	B	A8	B6	24	Group 38

of internal control E. coli BL21, the average genetic similarity was 56%, with a genetic distance of 0.44 (Table 3 a). With RAPD primer 4, eleven different profiles were found in the 58 strains, and each profile had a genetic similarity level between 22% (minimum) and 95% (maximum). When compared with RAPD pattern Bc of internal control E. coli BL21, the average genetic similarity was 35%, but with a high genetic distance of 0.66, when compared to that for primer 6 (Table 3 b).

Combining the data obtained with the 2 primers, 24 genomic RAPD types were found among the 58 analyzed strains, with the following groups being predominant: RAPD type 1 and RAPD type 2, represented by 8 strains (14%) each, followed by RAPD type 15, represented by 6 strains (10%) (Table 4). These data suggest that using 2 different primers to generate RAPD profiles can substantially improve the flexibility and sensitivity of the RAPD typing technique.

The results of O-antigen typing and RAPD typing in Tables 2 & 4 make clear that the 58 strains are a heterogeneous group, which corroborates other reports (e.g., *Sanchez-Carlo et al.* 1984) on the heterogeneity of *E. coli* strains from bovine mastitis. Accordingly, there seems to be no special pathogenic serotype / RAPD type of *E. coli* that causes mastitis, which is consistent with the fact that sows can suffer from multiple infections (e.g., sow number 211 became infected with serotype O99 and O5/ RAPD types 5, 8, and 13; and sow number 284 became infected with serotype O9 and K88 / RAPD types 8 and 15) (Table 5). Moreover, sows can also suffer from recurrent infections (e.g., the third right teat of sow number 211 was infected with RAPD type 5 on the first and third day of lactation, and the first left teat of the same sow was infected with serotype O99 / RAPD type 13 on the first and third day of lactation) (Table 5). We also found that sows can be infected with different strains at different lactation (e.g., the fifth left teat of sow number 284 was infected with serotype O9 / RAPD type 15 on the first day of lactation A, and infected with serotype K88/ RAPD type 8 on the third day of lactation B, the strain with RAPD type 8 was also found in the seventh left teat on the first day of lactation B) (Table 5).

The diversity observed in serotypes and RAPD types confirms the well-known route of a galactogenous infection of mastitis in sows, which is transmitted to the teats of sows from their contaminated environments (feces or straw) rather than from sow to sow.

With this RAPD technique, it was possible to differentiate strains of the same serotype (Table 5). For example, the RAPD technique subdivided *E. coli* strains with the O45 profile into RAPD types 1, 3 and 9; and strains with the O8 profile into RAPD types 13, 15 and 16; and finally, strains with the K88 profile into RAPD types 2 and 8. However, in reverse, strains with same RAPD type could be subtyped by serotyping, such as RAPD type 13, which included strains with the O8 and O99 profiles (Table 5).

Table 4 (continued)

[1] Strains with the same number indicate that they came from the same sow.
Letters following the strain number indicate isolation from a left (V) or right (H) teat of the mammary gland. Roman numeral I designates those strains isolated from mastitis sows on the first day of lactation, and Roman numeral II, strains isolated on the third day of lactation, when infection was still persistent in the gland.

[2] Serological investigations based on the identification of the O-group were performed according to O. Söderlind (1971) and F. & I. Ørskov (1977). NT, Not typable with the available standard typing sera.

[3] Investigations on the presence of K88 or K99 were performed at the same time as the serological tests were carried out.

[4] Letters A-C indicate isolations from different lactations.

Table 5. Comparison of serotypes and RAPD types of selected *E. coli* strains from milk of mastitis sows

Strain	O-group	Presence Of K88 or K99	Lactation	RAPD profile		RAPD Type
				Primer6	Primer4	
211H3 I	NT	-	A	A1	B7	5
211H3 II	NT	-	A	A1	B7	5
211V1 I	99	-	A	A3	B1	13
211V1 II	99	-	A	A3	B1	13
211V3 I	5	-	A	A2	B3	8
211V3 II	NT	-	-	A2	B3	8
238V5 I	127	-	B	A8	B6	24
238V5 II	127	-	B	A8	B5	23
249 H4 II	NT	-	-	A1	B2	2
249V2 II	NT	-	-	A2	B1	7
264H2 I	NT	-	-	A2	B10	12
264V5 I	NT	-	-	A2	B4	9
270V4	NT	-	-	A5	B5	19
270V4 I	NT	-	-	A1	B5	4
283V5 I	NT	-	-	A3	B4	15
283H3 I	9	-	-	A3	B4	15
284H5 I	9	-	A	A3	B4	15
284H5II	NT	K88+	B	A2	B3	8
284H7I	NT	-	B	A2	B3	8
201H2I	NT	K88+	B	A1	B2	2
213V6-A	45	-	-	A1	B1	1
213V6-B	45	-	-	A1	B1	1
217H6I	45	-	-	A2	B4	9
223H7I	45	-	-	A1	B3	3
187H5I	8	-	B	A3	B4	15
199H5I	8	-	-	A3	B8	16
240V4I	8	-	-	A2	B11	13

This means that the RAPD technique can not replace serotyping in epidemiological studies, and that the best way to identify *E. coli* mastitis strains is a combination of serotyping and RAPD typing. Thus, by combining the information from both typing techniques, we were able to group more informatively, and with greater detail (in to 38 groups), the 58 *E. coli* strains of our study (Table 4).

Although we found that all 58 strains were *E. coli*, based upon biochemical tests using the API20 E kit, no uniform pattern of biochemical reactions could be identified among the strains. We proceeded then to use Chi-square and Fisher's Exact Test to see if our data might include some conceivable relationships between types and profiles for the 58 strains, but without success. For example, no statistically significant relationship ($p > 0.05$) was found between the API20 E biotypes, O antigens and RAPD types (data not shown), nor between RAPD types and virulence factors, such as fibronectin-binding and attachment to fibroblasts (Table 6), when we examined some selected strains ($p > 0.05$; data not shown).

Finally, unlike another study of *E. coli* serogroups O6 (*Pacheco et al.* 1997), which found a relationship between RAPD types and H antigen serotypes, we found no relationship between RAPD types and O-antigen types

Table 6. O-group, fibronectin-binding, attachment to fibroblasts, and RAPD profiles of selected *E. coli* strains isolated from mastitis sow milk.

Strain	O-group[a]	Fibronectin Binding (%)	Attachment to fibroblast[b]	RAPD profile		RAPD type
				primer 6	primer4	
249H4 I-B	110	49.5	#	A1	B1	1
236H3 I	NT	38.2	#	A1	B2	2
221H5 I	NT	13.4	(#)	A1	B3	3
223H7I	45	8.2	0	A1	B3	3
217H6 I	45	4.3	0	A2	B4	9
211V1I	99	47.0	#	A3	B1	13
66	68	44.0	#	A3	B9	17
270V4	NT	6.2	0	A5	B5	19
238V5II	127	7.7	0	A8	B5	23

[a] NT, not typeable
[b] #, >100; (#), 0-100; and 0, no. of bacteria bound per fibroblast.

($p > 0.05$), nor a relationship between RAPD types and the K88 or K99 profile (data not shown). But these results prompt further investigation. To begin with, the H antigen serotypes of the 58 E. coli strains that caused mastitis in sows need to be examined. In addition, in order to determine if, for those 58 *E. coli* strains, the relationship found by *Pacheco et al.* (1997) again holds (i.e., between RAPD types and H antigen types), another RAPD analysis of those strains needs to be run using the same primers employed in their study.

The indistinguishable banding patterns obtained with repeated analyses of 2 isolates from each strain included in the study demonstrated that RAPD analysis using Ready-To-Go standardized beads is a reproducible technique for typing *E. coli* strains that cause mastitis in sows (result not shown). All 58 strains in the study could be typed by RAPD, in contrast to the O-antigen typing technique for which 34 of the strains (59%) could not be typed, despite the use of all 163 of the available O-antigen antisera for serotyping.

The RAPD technique, using standardized beads, is simple, cheap and fast, and moreover, requires only nanogram amounts of DNA, needs no radioisotope probes, and easily handles large numbers of samples. However, for larger-scale molecular genetic epidemiological surveillance of *E. coli* that cause mastitis in sows, further investigations are needed to establish the feasibility of this simple and fast method.

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Sammanfattning

Karakterisering av E.coli stammar isolerade från mjölk från suggor med coliform mastit med hjälp av en Random amplified Polymorphic DNA teknik.

En "random amplified polymorpic DNA" (RAPD) teknik utvärderades för subtypning av 58 E. coli isolat från mjölk från suggor med coliform mastit. Resultaten visade en mycket hög variabilitet och alla isolat kunde differentieras genom RAPD tekniken. Detta överenstämmer också med en hög variabilitet i såväl serotyp som biokemiskt mönster hos samma stammar. Sammanfattningsvis förefaller det som E. coli stammar förorsakande mastit hos gris inte är beroende på specifika virulensfaktorer utan snarare på omgivningssmitta sannolikt huvudsakligen förorsakade av E. coli av fekalt ursprung.

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