Isolation of *Escherichia coli* Expressing 987P Fimbrial Antigen from Suckling Piglets

Kiyohito TAKAHASHI, Naoji TAKAHASHI, Akiyoshi YAMAMOTO¹⁾, Takashi UEMURA²⁾, and Jun IMOSE
Aburahi Laboratories, Shionogi & Co., Ltd., 1405, Gotanda, Koka-cho, Koka-gun, Shiga 520-34, ¹⁾Animal Health
Products Development Department, Shionogi & Co., Ltd., 1-8, Doshomachi, 3-chome, Chuo-ku, Osaka 541, and
²⁾Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, Mozu-ume-machi 4,
Sakai, Osaka 591, Japan

(Received 12 January 1990/Accepted 31 May 1990)

— *Jpn. J. Vet. Sci.* 52(5): 1113–1115, 1990

KEY WORDS: *Escherichia coli*, fimbrial antigen, pig.

Various infectious agents cause diarrhea in piglets, and enterotoxigenic *Escherichia coli* (ETEC) is one of the important agents causing diarrhea in suckling piglets [1, 9, 18]. Production of enterotoxins and fimbriae contributes to the virulence of ETEC. K88, K99, 987P, F41 and Type-1 among fimbriae of ETEC have been shown involved in the disease [5, 7, 8, 10, 12, 15].

In Japan, enteritic colibacillosis is spreading among suckling piglets, and *E. coli* expressing K88, K99, 987P, F41 and Type-1 fimbriae have been isolated from piglets [13, 14, 17]. With respect to *E. coli* expressing 987P, Uemura *et al.* [17] reported that they were isolated from 27 diarrheal piglets (7.7%) and 25 normal piglets (8.7%), and Nakazawa *et al.* [13] reported the isolation of one strain of *E. coli*. However, there has been relatively little isolation of *E. coli* expressing 987P compared with that expressing the other fimbriae. We therefore attempted to isolate *E. coli* expressing 987P antigen from suckling piglets on three Japanese farms.

It has been reported that *E. coli* grown in liquid medium produced 987P antigen better than when grown on agar plates [2, 4, 6]. Hence, for more effective detection of 987P antigen in the isolates, slide agglutination tests were performed using cells grown in both agar and liquid media.

Diarrheal or normal feces were taken from 60 piglets less than 10 days old on Farm A (Ibaraki Prefecture), and from 89 piglets 5 to 7 days old on Farm B (Kagoshima Prefecture) during the period from July to October, 1987. On Farm C (Kyoto Prefecture), specimens were collected from 14 piglets less than 21 days old in June, 1988.

These specimens were cultured on DHL agar (Eiken Chemical Co., Ltd., Tokyo). After incubation at 37°C overnight, five or ten lactose-

positive colonies were selected from each culture. The isolates were then identified as *E. coli* by their biochemical characteristics with Enterotubes (F. Hoffmann-LaRoche, Nutley, NJ., U.S.A.) and were stored on Dorset egg yolk medium (Nissui Pharmaceutical Co., Ltd., Tokyo) at 4°C.

For detection of fimbrial adhesions, the isolates were cultured in semi-synthetic liquid medium (SSM) [7] at 37°C for two or three days. Two further successive subcultures in fresh SSM were carried out for two or three days each. A loopful of culture in the last SSM was streaked onto Minca Iso-Vitalex (MIS) agar [3] or minimal glucose agar (MGA, Na₃C₆H₅O₇·2H₂O 1.0 g, glucose 2.0 g, KH₂PO₄ 3.0 g, K₂HPO₄ 7.0 g, (NH₄)₂SO₄ 1.0 g, MgSO₄·7H₂O 0.1 g, agar 20.0 g, distilled water 1,000 ml, pH 7.1) and incubated at 37°C overnight. The colonies grown on these agar plates were used to detect K88, K99, 987P and Type-1 antigens in the isolates by slide agglutination tests.

The isolates were cultured in SSM at 37°C overnight and subcultured in fresh SSM. After overnight incubation, the bacterial cells were resuspended with 0.05 M phosphate buffered saline (PBS) equal to 1/10 volume of medium, and these suspensions were used as the antigen in slide agglutination tests for detection of 987P antigen.

Antisera against K88, K99 or 987P fimbrial antigen were commercially purchased (Denka Seiken Co., Ltd., Tokyo) and additionally anti-987P and anti-Type-1 sera used in the experiments were supplied kindly by Dr. H. G. Jayappa (Schering Animal Health, Omaha, NB., U.S.A.).

Production of heat-stable enterotoxin (ST) was determined by using suckling mouse method [16]. The isolates were cultured in CAYE medium [11] at 37°C for 18 hr with continuous shaking. Bacterial cells were removed by centrifugation, and the supernatant fluid was passed

through 0.45 μm filters and used for the test. For heat-labile enterotoxin (LT) assay, the isolates were cultured on heart infusion agar containing 90 $\mu\text{g/ml}$ of lincomycin at 37°C overnight. Bacterial cells grown on agar medium were suspended in saline to which was added 1,000 units/ml polymyxin B, and the suspension was incubated at 37°C for 30 min. The supernatant fluid obtained by centrifuging was assayed for LT by latex agglutination using a commercial kit (VET-RPLA, Denka Seiken).

When the colonies grown on agar plates were agglutinated by anti-987P serum, the fimbrial antigen of the isolate was called A-987P. Though the colonies agglutinated by anti-987P serum were not found in some isolates, the cells grown in SSM responded to this antiserum. Such a 987P antigen was named L-987P to distinguish it from A-987P.

K88, K99, 987P or Type-1 antigens were found among *E. coli* isolated from piglets on Farms A and B, but only *E. coli* expressing Type-1 or K88

were isolated from piglets on Farm C (Table 1). *E. coli* expressing Type-1 were isolated most frequently among the isolates in three farms.

E. coli expressing A-987P or L-987P were also isolated from diarrheal and normal piglets. Though *E. coli* expressing A-987P were found in only 19 strains isolated from 15 piglets, those expressing L-987P could be found in 39 strains isolated from 21 piglets. As a result, the number of piglets from which *E. coli* expressing 987P were isolated was nearly equal to the number from which *E. coli* expressing K88 were isolated.

E. coli expressing A-987P could not be isolated from 16 of 21 piglets from which *E. coli* expressing L-987P were isolated (Table 2). All of 32 isolates expressing 987P produced ST, but not LT.

Type-1 or 987P fimbriae of *E. coli* undergo phase variation [6]. In the laboratory, depending upon the methods of culture, cultures can be grown containing cells in either phase. Guinée *et al.* [4] reported that when *E. coli* were cultured in

Table 1. Isolation of *Escherichia coli* expressing 987P or other fimbriae from diarrheal and normal piglets on three farms

Fimbrial antigen	Farm A		Farm B		Farm C	
	Diarrheal	Normal	Diarrheal	Normal	Diarrheal	Normal
A-987P ^{a)}	4(7.8) ^{c)}	0	5(12.8)	6(12.0)	0	0
L-987P ^{b)}	8(15.7)	2(11.1)	8(20.5)	3(6.0)	0	0
K88	14(27.5)	2(22.2)	12(30.8)	1(2.0)	5(55.5)	1(20.0)
K99	1(2.0)	0	5(12.8)	2(4.0)	0	0
Type-1	42(82.5)	9(100)	17(43.6)	16(32.0)	6(66.7)	4(80.0)
Total	51(100)	9(100)	39(100)	50(100)	9(100)	5(100)

a) Isolates cultured on agar medium were agglutinated by anti-987P serum.

b) Isolates cultured in broth (semi-synthetic medium) were agglutinated by anti-987P serum, but those on agar were not agglutinated.

c) Number of piglets from which *E. coli* expressing fimbriae were isolated(%).

Table 2. Isolation of *E. coli* expressing A-987P or L-987P from diarrheal and normal piglets

Fimbrial antigen		Farm A		Farm B	
A-987P	L-987P	Diarrheal	Normal	Diarrheal	Normal
○ ^{a)}	× ^{b)}	0	0	5 ^{c)}	5
×	○	4	2	8	2
○	○	4	0	0	1

a) Isolated.

b) Not isolated.

c) Number of piglets.

static broth cultures for about a week and then were subcultured on nutrient agar with 5% sheep blood, 987P piliated colonics increased. Francis *et al.* [2]. reported that more 987P tested strains were agglutinated by anti-987P serum after being cultured in MIS broth or a synthetic broth containing glucose and citrate (E medium) than after being cultured MIS or E agar. Therefore, slide agglutination tests for the detection of 987P antigen were performed using the isolates grown in broth.

We were able to find many isolates expressing 987P when they cultured in SSM. Isolates grown in some liquid media like SSM were useful to detect 987P antigen by slide agglutination tests.

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要 約

987P 線毛保有大腸菌の哺乳豚からの分離(短報): 高橋清人・高橋直治・山本昭義¹⁾・植村 興²⁾・妹背 醇(塩野義製薬油日ラボラトリーズ, ¹⁾動植薬開発部, ²⁾大阪府立大学農学部獣医公衆衛生学教室)——3 農場の哺乳豚99頭の下痢便および64頭の正常便から987P 保有大腸菌の分離を試みた。寒天培地培養菌を用いたスライド凝集反応においては, 15頭から分離された19株に987P の保有が認められ, 半合成液体培地培養菌を用いた場合には, 21頭から分離された39株に認められた。987P 保有大腸菌が分離された子豚の数は, K88保有菌が分離された子豚とほぼ同数となり, 987P の検出には半合成液体培地培養菌を用いるのが有用であった。