

Genetic characterization of an *Escherichia coli* isolate from piglet with post-weaning diarrhea in Bali - Indonesia

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Abstract

Here we report various loci of a Porcine Pathogenic *Escherichia coli* isolate from post-weaning colibacillosis in a piggery from Bali, Indonesia. The isolate, named BPOS4, poses fimbriae F18 subtype and triple toxins of heat-labile enterotoxin A, heat-stable enterotoxin, and shiga toxin 2e subunit A.

Keywords: Porcine Pathogenic *Escherichia coli*; Bali Indonesia; F18 subtype; Heat-labile enterotoxin A; Heat-stable enterotoxin; and Shiga toxin 2e subunit A.

1. Introduction

The Porcine Pathogenic *Escherichia coli* (PPEC) causes diarrheal diseases in piglets and is responsible for substantial losses of pig industry due to high mortality following septicemia and reduced growth rate (1-6). Report on PPEC from Indonesia in international journals is scarce. Here we described genetic sequence of some loci of an isolate from post-weaning diarrhea case in Bali, Indonesia.

2. Methods

The case occurred in a sow-to-fattening piggery in Bangli District of the provinces in May 2019. The case affected piglets of 30 – 40 days of age. The number of just weaned piglets were 180. The number of sick piglets were 60. The clinical signs were appetite loss, white-yellowish scour, pale, and wasting. Bacteria were cultured from 10 fecal samples in the Eosin Methylene Blue Agar (EMBA) medium. The colonies were black or dark color with metallic green center, and suspected to be *E. coli* as previously described (7). Three colonies from each sample was cultured in Luria Broth Medium (Sigma-Aldrich, South Korea) overnight in 37 °C. Whole DNA was isolated using chelax 10% (Biorad-Laboratories, CA). The following gene loci were amplified, namely 16S ribosomal RNA, the fimbriae of F4, F5, F6, F17, and F18, and F41 subtypes, as well as intimin (*eae*), heat-stable enterotoxin EAST1, heat-labile enterotoxin A (ETXA), heat-stable

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enterotoxin STa, and shiga toxin 2e subunit A (STx2eA) using GoTaq Green Master Mix (Promega Corporation, WI). The primer sets and sources of primers for amplification of those loci are available in Table 1.

Table 1 List of primer sets applied in the study Genetic characterization of *Escherichia coli* isolate of piglet with post-weaning diarrhea in Bali - Indonesia

Target Locus	Primer pair sequence	Reference
16S-rRNA	5'-CAGGCCTAACACATGCAAGTC-3' and 5'-GGGCGWGTGTACAAGGC-3'	(10)
Fimbriae subtype F4	5'-GGTCGCGAGTGACGGCTTTGT-3' and 5'-CCACTGAGTGCTGGTAGTTACAGCC-3'	(3)
Fimbriae subtype F5	5'-TGCGACTACCAATGCTTCTG-3' and 5'-TATCCACCATTAGACGGAGC-3'	(11)
Fimbriae subtype F6	5'-TCTGCTCTTAAAGCTACTGG-3' and 5'-AACTCCACCGTTTGTATCAG-3'	(11)
Fimbriae subtype F17	5'-GGGCTGACAGAGGAGGTGGGGC-3' and 5'-CCCGGCGACAACCTTCATCACCGG-3'	(3)
Fimbriae subtype F18	5'-GTGAAAAGACTAGTGTTTATTTTC-3' and 5'-CTTGTAAGTAACCGCGTAAGC-3'	(12)
Fimbriae subtype F41	5'-GAGGGACTTTCATCTTTTAG-3' and 5'-AGTCCATTCCATTTATAGGC-3'	(11)
Intimin (eae)*	5'-ATGATTACTCATGGTTKTTATDCCCGGAC-3' and 5'-TTATTTTACRCAAGTKGCRRTARGCATT-3'	This study
Heat-stable enterotoxin EAST1*§	5'-ATGCCATCAACACAGTATATCCG-3' and 5'-TCAGGYCGMGAGTGACGGCYTTGTAGTCC-3'	This study
Heat-labile enterotoxin (ETXA)* A	5'-ATGAAAAATATAACTTTTCATTTTTKTTATT-3' and 5'-TCATAATTCATYCCGAATTCYGYTATA-3'	This study
Heat-stable enterotoxin STa*§	5'-ATGAAAAAGYTAATRYTGGCAATTTTTATYTCT-3' and 5'-GGATTACAACAAAGTTCACAGCAGTAAAA-3'	This study
Shiga toxin 2e subunit (STx2eA)**	5'-ATGAAGTGTATAWTRTTWAAATGG-3' and 5'-TTATTCTCMGGATGTATTTAAAGAGTG-3' or 5'-TTATTCACCCGTTGTATATAAAGACTGTGA-3'	This study

*The primer sets were designed spanning from start codon to stop codon of respective locus based on database in GenBank. The IUPAC code was applied if there were mismatches in the primer sites; †Two backward primers for STx2e were selected to accommodate excessive mismatches in the priming sites to cover all possible existing field sequences; §There is an ambiguity in assigning the heat-stable enterotoxin as EAST1 and STa in the literature and GenBank, although both have completely different sequence. Therefore, we differentiate as heat-stable enterotoxin EAST1 and STa;

3. Result and discussion

All colonies were positive in F18 subtype, but not in other subtypes. Three colonies from one sample, denoted as BPOS4, were positive ETXA, STa, and STx2e, while others were positive in one of those toxins only. We reported the sequences of the BPOS4 only, that was deduced using Automatic Big Dye Termination Method.

The list of gene loci, accession number, and BLAST result of each sequence found in this study is presented in Table 2. All loci are closely related, with percentage of identity (PID) of over 98%, to the sequence of respective gene or gene fragments of *E. coli* dated 1985-2017. Stated origins were Asia and Europe, while declared isolation sources were pig and meet sausage. Some isolates were without information on origin and isolation source. Other toxins, namely eae and EAST1, were negative from all samples.

Table 2 Information on the sequence of each locus of *E. coli* BPOS4 and its BLAST result summary

No.	Gene Locus	Length of readable sequence (bp)	Acc. No.	BLAST Result (<i>E. coli</i> strain; gene name with the highest PID; Acc. No; PID)*	Species and Country; Year of isolation or submission or publication; Origin; Ref; PID)
1	16S-rRNA	1257	MN414475	PYK9;16S ribosomal RNA gene; MF582337; 99.92;	Species unknown; South Korea; 2017; Unpublished;
2.	Fimbriae	513	MN414476	85; FedA gene or Major subunit of F18 fimbriae; GQ325631; 98.44;	Pig; Germany; 2009; (13).
5	Heat-labile enterotoxin A (ETXA)	777	MN414477	EcoPV-173; Heat-labile enterotoxin A (LTA) subunit; EF057802; 99.74;	Pig; Vietnam; 2017; Unpublished
4	Heat-stable enterotoxin STa*	219	MN414478	Strain unknown; Heat-stable enterotoxin I gene; M25607; 99.54;	Species bovine or avian or pig; Origin unknown; 1985; (14);
3	Shiga toxin 2e subunit A (STx2eA)	960	MN414479	TS07/08; Stx2eA; FM998849; 99.58;	Meat sausage; Germany; 2009; (15);

*Upon BLAST, we selected the sequence with highest percentage identity (PID) annotated as specific gene locus; Full genome or full plasmid sequences are not selected;

The finding of this study expands the knowledge that PPEC might express multiple toxins in Bali, Indonesia. The virotype of the BPOS4 isolate is F18 – ETXA – STa – STx2eA. Virotypes of PPEC with various combinations of fimbriae subtypes and multiple toxins have been reported in Mexico (8). An *E. coli* producing F18 fimbriae and STx2e found in our study has been implicated as major health problems in young piglets resulting in poor health and death (9). An isolate with multiple toxins found in this study should be promising to be developed as a vaccine seed to prevent economic loss due to *E. coli* infection in pig industry in Indonesia.

3.1. Data availability

The accession numbers of sequence data from this study are MN414475, MN414476, MN414477, MN414478, and MN414479. The flat files of those are available temporarily for reviewing purpose in Google Drive with the link as the following: MN414475, MN414476, MN414477, MN414478, and MN414479. The Accession number of secondary data are MF582337, GQ325631, EF057802, M25607, and FM998849. Link to List of primer sets applied in the study is <https://drive.google.com/file/d/1o8jvrY9tIcouWrlVvpzPw5llamg0r0ZW/view>

4. Conclusion

We conclude that the porcine pathogenic *Escherichia coli* isolate from post-weaning colibacillosis in a piggery from Bali, Indonesia so called BPOS4 is of fimbriae F18 subtype and poses triple toxins of heat-labile enterotoxin A, heat-stable enterotoxin, and shiga toxin 2e subunit A.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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