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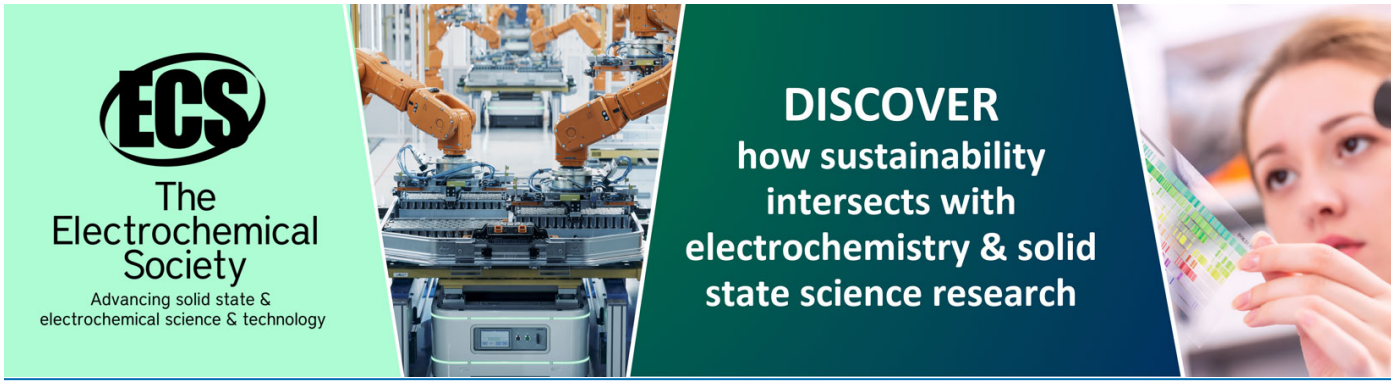

Molecular subtyping and dna sequencing homology of *Escherichia coli* O157:H7 isolated from Aceh cattle

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Abstract. *Escherichia coli* (*E. coli*) O157:H7 is one of bacteria that recognized as pathogenic agent, both in animals and human. Several isolates of this bacteria were obtained from local livestock, such as in bali cattle and aceh cattle. However, the bacteria found in aceh cattle has not been confirmed through molecular technique. The current study was aimed to confirm the finding of *E. coli* strain O157:H7 in aceh cattle using molecular analysis. Three isolates of *E. coli* O157 (MA-35, MA-44, and MA-58) obtained from aceh cattle were extracted for the DNA using Presto^{TM} Mini gDNA *bacteria kit* (Geneaid) through several processing stages of sample preparation, lysis, DNA binding, washing and elution. Then, it was continued with DNA Amplification Using Polimerase Chain Reaction (PCR). DNA sequencing homology was conducted using BLASTN program. Data of this study were analysed descriptively. The results showed that one of isolate (MA-58) was detected for the presence of genes of *rfb* (339 bp), *fli*C (401 bp), and *stx*2 (115 bp). Then, this isolate has a homology of 100% to 16 different strains of *E. coli* O157:H7 sequence in the GenBank. In conclusion, the molecular analysis of the local isolate of bacteria of MA-58 obtained from aceh cattle confirmed that it is *E. coli* O157:H7 and detected with the presence of genes of *rfb*, *fliC*, and *stx2*.

1. Introduction

Escherichia coli O157 (*E. coli* O157) is a very important food-borne pathogen and the case spread world-wide in human. In particular, this bacteria has been identified as the main cause of infection in humans in developing countries. The route of infection to human is possibly through the contamination of food by animal feces [1, 2, 3, 4]. The bacteria will release shiga toxin that acts on kidney, intestine and other parenchymatous organs, and, in turn could cause hemorrhagic colitis and hemolytic uremic syndrome [5, 6].

Many studies have been conducted to isolate and identify this bacteria in cattle herds, since this animal was established as the natural reservoir of *E. coli* O157 [5, 7, 8].. The infection in cattle is without clinical sign, but shed the bacteria intermittently [9, 10]. Several epidemiological studies

conducted in many countries, including in Asia, have reported a wide range of prevalence estimates ranging from 0.1% to 62% of *E. coli* O157 in cattle [11, 12, 13, 14]. In Indonesia, in addition of the isolation of the bacteria in feces and meat of cattle, it was found also in feces and meat of sheep as well as in chicken and human feces [13, 15, 16]. In particular, the finding of *E. coli* O157 in bali cattle has convinced the hypothesis that the serotype of this bacteria could be isolated from local breed animals in Indonesia [13].

In addition to bali cattle, several local breeds are also exist in Indonesia. For example, in the Province of Aceh most of local farmers are raise local breed of cattle, which called aceh cattle. Many of them are still raise this cattle using traditional management farming. Although they provided the cattle with house, but the sanitation is below recommendation standard [17]. This condition may increase the risk of existence of bacteria of *E. coli* O157. However, very little information available, if any, on the isolation as well as molecular subtyping of *E. coli* O157 in aceh cattle.

2. Method

2.1. Sample Isolation

In this study, three *E. coli* O157 isolates obtained from rectal swabs of aceh cattle were used for molecular analysis [18].The codes of those isolates were MA-35, MA-44, and MA-58.

2.2. Molecular Analysis

2.2.1. DNA Extraction

Total DNA was extracted separately using the gDNA PrestoTM Bacteria Mini kit (Geneaid) with slight modification. Purified total DNA (50 µL, ~200 µg/mL) was eluted and used as the template for PCR assays as described in previous studies [19, 20].

2.2.2. Polimerase Chain Reaction (PCR) Amplification for Gene 16S rRNA

Gene 16SrRNA amplification was conducted using PCR tehnique. The amplification of this gene were using general primers of BacF and Unib. The BacF primer was used since it complement with sustainable regions in bacterial domain. Then, UniB primer was used concerned to it universal sustainability for 16SrRNA gene to *E. coli* [19, 20]. A further step for this amplification processes were also followed the method and technique in prvious studies [19, 20].

2.2.3. DNA Sequencing

Determination of DNA sequence was conducted using a commercial service by Macrogen *Inc.*, Korea. The determination processes was used a method of Dye Terminator (3'-dyelabelled dideoxynucleotide triphosphate). This method is comprised of the steps of template preparation and sequencing reaction. Purification of PCR product and electrophoresis were used scanning fluorescence. The primers sequence of *rfb, fliC, and stx2* were used for multiplex-PCR analysis as shown in Table 1 [13, 21].

Table 1. The primers used for amplification of *rfb, fliC,* and *stx2* genes by PCR.

Gene	Target	Oligonucleotide sequence	Amplicon product (bp)
rfbF	O157	5'TCTCAGTGGGCGTTCTTATG-3'	339
rfbR		5'TACCCCCTCAACTGCTAATA-3'	
fliCF	H7	5'CCCCCTGGACGAAGACTGAC-3'	401
fliCR		5' ACCGCTGGCAACAAAGGATA-3'	
stx2F	Shiga-like toxin 2	5' GCGGTTTTATTTGCATTAGC-3'	115
stx2R		5' TCCCGTCAACCTTCACTGTA-3'	

2.3. Homology Analysis of DNA Sequence

The homology of sequence results of gene 16S rRNA were compared with available nucleotide in the GenBank database using the Basic Local Alignment Search Tool (BLAST) program from National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) [22]. At 100 DNA sequence with high percentage of homology was downloaded through that website. The downloaded files were saved in FASTA file for an easy editing purpose. The sequence results of BLAST will have different size and position. An approach of clustal W at MEGA 7.1 was used for the purpose of obtaining a same size and position with DNA samples [23].

2.4. Data Analysis

The results of this study were analysed descriptively for the molecular subtyping and homology of DNA sequence of *E. coli* O157.

3. Results and Discussions

3.1. Molecular subtyping of E. coli O157:H7 genes

The results of amplification of gene 16S rRNA was very clear as shown in Figure 1. It was indicated the presence of tape at a marker of 1500 bp with only one type of fragment of molecule of gene 16S rRNA. The amplification of gene 16S rRNA was using a general type of primer. One of this primer of BacF was complement with conserved area of bacteria domain. Then, another type of primer of UniB was based on universal conserved area of gene 16S rRNA to E.coli. The size of this primer band was at 1500 bp, which could amplify all the area of 16S rRNA for the whole bacteria. So, it can be concluded that the amplification process of fragment of gene 16S rRNA was successful and pure. Then, the process can be continued to the sequencing stage.

Figure 1. Result of amplification of gene 16S rRNA using primers of BacF and UniB.

The results of multiplex-PCR analysis for DNA sequencing is shown in Figure 2. It was obtained that only the sample of MA-58 has genes for *E. coli* O157:H7. The figure showed the presence of gene *fli*C (401 bp), and *rfb* (339 bp). Then, the results of analysis was also indicated the presence of *stx*2 gene (115 bp), a virulence gene type in *E. coli* O157:H7. To the best of our knowledge, this is the first study conducted that confirmed the presence of *E. coli* O157:H7 using molecular analysis tehnique in the bacteria isolated from faecal swab sample of aceh cattle, which is one of bovine local genetic resources in Indonesia. This result has added the list of potential risk of local cattle in Indonesia as a source of infection. Formerly, a similar study has been conducted in bali cattle and obtained similar results [13, 24]. Both of those breed are categorised as beef cattle.

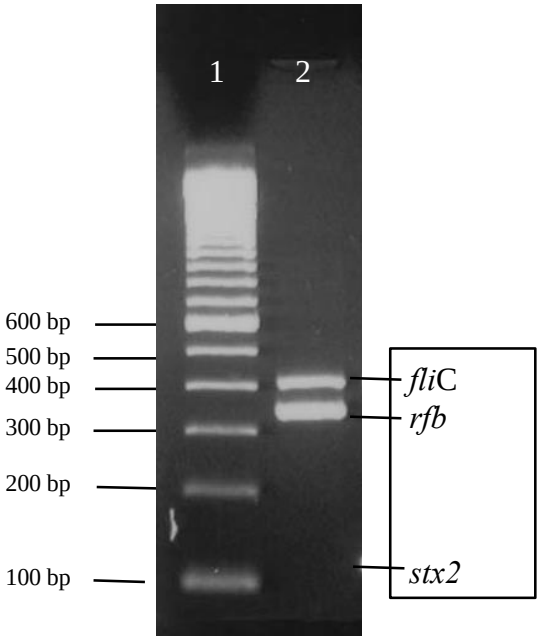

Figure 2. Determination of the presense of *E. coli* O157:H7 genes in sample obtained from faecal swab of aceh cattle by using the multiplex PCR technique with primers *fli*C (401 bp), *rfb* (339 bp), and *stx*2 (115 bp). 1: marker DNA 100 bp; 2. Sample isolate MA-58 (*E.coli* O157:H7).

The results of our study showed that the isolate *E. coli* O157:H7 of MA-58 was detected for the presence of *fli*C, rfb, and, Shiga-like toxin 2 (*stx*2) gene. This result is supported by some previous similar works for molecular subtyping of isolate obtained from cattle [13, 21, 25, 26, 27]. In contrast, the molecular analysis of *E. coli* O157:H7 bacteria isolated from faeces of bali cattle was detected for the presence of both *stx*1 and *stx*2 [13, 24]. In fact, the presence of one or two of those verocytotoxin genes in one isolate of *E. coli* O157:H7 is possible to happen [27, 28]. However, the findings for their appearance together in one isolate is very rare. Most molecular analysis for isolates of *E.coli* O157 or *E.coli* O157:H7 found that only produce *stx*2, but very rare for the presence of *stx*1 [4, 13, 24, 26, 29]. The presence of both of them is an indication of high pathogenicity of infection of *E. coli* O157:H7. The presence of *stx*1 in this strain of *E.coli* will cause hemorrhagic colitis in the infected individual. Then, if *stx*2 presence in the bacteria, it could result in hemolytic uremic syndrome in the infected animals or human. Therefore, a combination of those clinical signs will arise in infected individual when both verocytotoxin is presence in *E.coli* O157:H7 and could be fatal [6].

In addition to stx genes, several studies have been reported that some other virulence genes may be found in isolates of *E. coli* O157 or *E. coli* O157:H7 obtained from cattle. For example, numerous molecular analysis of those isolates have detected the presence of *efa*1 gene (also called *lif*A, for lymphocyte inhibitory factor), *eae*A, intimin, clyA (Cytolysin A), etpD (the Type II Secretion System (ETP) encoded gene), and enterohemolysin [4, 26, 30]. However, the finding of those genes is still very rare. Then, their essential to induce pathogenesis is still unclear [26, 31]. Perhaps, in the future molecular analysis subtyping of isolates of *E. coli* O157:H7 obtained from aceh cattle could be targeted for detection of the presence of those virulence genes.

3.2. Homology of nucleotide sequence of E. coli O157:H7 genes

The results of analysis for homology of nucleotide sequence using BLAST program as shown in Table 2. The percentage of query coverage of nucleotide sequence of MA-58 was obtained at 100% aligned with the sequence in the GenBank database. Then, the results also showed that the E-value was at zero value and the percentage of maximum identity was at 100%. It has been suggested that 16S rRNA gene sequence data on an individual strain may represents a new species, when obtaining a score of

identity < 97%. However, when the identity score reaching above 97%, the meaning is remain unclear [32]. Although it is not a consensus, but based on the acceptance from most taxonomist that the species can be concluded homolog to the known species of bacteria when the percentage of identity is ≥99% [33]. Therefore, the results of BLAST obtained in this study were confirmed that the isolate of MA-58 has a homology of 100% with *E. coli* O157:H7 in the GenBank database.

The results obtained from analysis for homology of nucleotide sequence using BLAST program were showed that 16 different strains of *E. coli* O157:H7 in the Genbank database, which have 100 % query coverage, zero E-Value, and 100% identity of genes. 69% of those strains were have a complete genome sequence, while the rest of 31% were partial sequence. Overall, these results meant that nucleotide sequence of isolate of MA-58 is very homolog to the sequence in the GenBank database for *E. coli* O157:H7.

Table 2. The results of analysis for homology of nucleotide sequence *E. coli* O157:H7 strains using Genbank data with BLAST Program for Isolate *E. coli* O157:H7 of MA-58 obtained from faecal swab of aceh cattle. (Query Coverage: 100%; W-Value: 0; Maximum Identity: 100%)

Description	Accession Number
Article I. Escherichia coli O157:H7 pv15-279 DNA, **complete genome**	AP018488.1
Escherichia coli O157:H7 str. Sakai DNA, **complete genome**	BA000007.3
Escherichia coli O157:H7 strain 8368, **complete genome**	CP017444.1
Escherichia coli O157:H7 strain 3384, **complete genome**	CP017440.1
Escherichia coli O157:H7 strain JEONG-1266, **complete genome**	CP014314.1
Escherichia coli O157:H7 strain WS4202, **complete genome**	CP012802.1
Escherichia coli O157:H7 str. SS52, **complete genome**	CP010304.1
Escherichia coli O157:H7 str. EDL933, **complete genome**	CP008957.1
Escherichia coli O157:H7 str. SS17, **complete genome**	CP008805.1
Escherichia coli O157:H7 str. TW14359, **complete genome**	CP001368.1
Escherichia coli O157:H7 str. EC4115, **complete genome**	CP001164.1
Escherichia coli O157:H7 partial 16S rRNA gene, strain WAB1892	AM184233.1
Escherichia coli O157:H7 str. EDL933, **genome**	
Escherichia coli O157:H7 yaeD, rrsH, ileV, alaV, rrlH, rrfH, aspU, yafB genes for phosphatase, 16S rRNA, isoleucine tRNA 1B, alanine tRNA 1B, 23S rRNA, 5S rRNA, aspartate tRNA 1, aldose reductase, **complete and partial cds**	AB035926.1
Escherichia coli O157:H7 yrdA, rrsD, ileU, alaU, rrlD, rrfD, thrV, rrfF genes for transferase, 16S rRNA, isoleucine tRNA 1, 1340 alanine tRNA 1B, 23S rRNA, 5S rRNA, threonine tRNA 1, 5S rRNA, **partial cds**	AB035923.1
Escherichia coli O157:H7 yieP, rrsC, gltU, rrlC, rrfC, astP, trpT, yifA genes for hypothetical protein, 16S rRNA, glutamate tRNA 2, 23S rRNA, 5S rRNA, aspartate tRNA 1, tryptophan tRNA, hypothetical protein, **partial cds**	AB035922.1

4. Conclusion

In conclusion, the molecular analysis of the local isolate of bacteria of MA-58 obtained from aceh cattle confirmed that it is *E. coli* O157:H7 and detected with the presence of genes of *rfb*, *fliC*, and *stx2*. In addition, the bacteria is also confirmed homolog to the nucleotide sequence of 16 different strains of *E. coli* O157:H7 in the GenBank database.

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References

[1] Dorn CR, Angrick EJ 1991 *J Clin Microbiol* **29** 1225–1231.
[2] Besser TE, Hancock DD, Pritchett LC, McRae EM, Rice DH, Tarr PI 1997 *J Infect Dis* **175** 726–729.
[3] Dunn JR, Keen JE, Vecchio RD, Wittum TE, Thompson RA 2004 *J Food Prot* **67** 2391–2396.
[4] To´th I, Schmidt H, Kardos G, Lancz Z, Creuzburg K, Damjanova I, Pa´szti J, Beutin L, Nagy B 2009 *Applied And Environmental Microbiology* **75** (19) 6282–6291.
[5] Gyles CL (2007) *J Anim Sci*. **85** E45–E62.
[6] Fraser ME, Fujinaga M, Cherney MM, Meltoncelsa AR, Twiddy EM, O'Brien OD, James NG 2004 *J.Biological Chemistry* **279** 27511-27517.
[7] Chapman PA, Siddons CA, Wright DJ, Norman P, Fox J, Crix E 1993 *Epidemiol Infect* **111** 439–447.
[8] Brusa V, Aliverti V, Aliverti F, Ortega, EE, de la Torre JH, Linares LH 2013 *Front Cell Infect Microbiol* **2** (171).
[9] Elder RO, Keen JE, Siragusa GR, Barkocy-Gallagher GA, Koohmaraie M, Laegreid WW 2000 *Proceedings of the National Academy of Sciences* **97** (7) 2999-3003.
[10] Nastasijevic I, Mitrovic R, Buncic S 2008 *Letters in Applied Microbiology* **46** (1) 126-131.
[11] Lin YL, Chou C, Pan T 2001 *J Microbiol Immunol Infect* **34** 17–24
[12] Fox JT, Renter DG, Sanderson MW, Nutsch AL, Shi X, Nagaraja TG 2008 *J Food Prot* **71** 1761–1767.
[13] Suardana, IW, Artama WT, Asmara W, Daryono BS 2010 *Jurnal Veteriner* **11** (4) 264-270.
[14] Islam MZ, Musekiwa A, Islam K, Ahmed S, Chowdhury S, Ahad A, Biswas PK 2014 *PLoS ONE* **9**(4) e93299.
[15] Sumiarto B 2004 *Jurnal Veteriner* **5** (3) 85-90.
[16] Suardana, IW, Utama IH, Wibowo MH 2014 *Jurnal Kedokteran Hewan* **8** (1) 1-5.
[17] Abdullah MAN, Noor RR, Hendiwirawan E 2008 *J Indon.Trop.Anim.Agric*. **33**(1) 1-10.
[18] Abrar M, Ferasyi TR, Amiruddin 2017 *Laporan Hibah Penelitian Profesor LPPM Universitas Syiah Kuala.*
[19] Balqis U, Hambal M, Admi M, Safika, Meutia N, Sugito, Dasrul, Abdullah MAN, Ferasyi TR, Lubis TM, Abrar M, Darmawi 2018 *Malaysian Journal of Microbiology* **14** (3) 229-235
[20] Sari WN, Safika, Darmawi, Fahrimal Y 2017 *Veterinary World* **10** 1515- 1520**.**
[21] Hu Y, Zhang Q, Meitzler JC 1999 *Journal of Applied Microbiology* **87** 867–876
[22] Ghasemia Y, Sara RA, Alireza E, Aboozar K, Maryam S, Talebniab N 2011 *Iranian Journal of Pharmaceutical Sciences* **7**(3) 175-180.
[23] Kumar S, Stecher G, Tamura K 2016 *Mol Biol Evol.* **33** 1870-1874.
[24] Lestari D, Pinatih KJP, Suardana IW 2015 *Indonesia Medicus Veterinus* **4** (4) 295-304.
[25] Shayegh J 2017 *Microbiology Research* **8** (1).
[26] Cadirci O, Siriken B, Inat G, Kevenk TO 2010 *Meat Sci* **84**(3) 553-6.
[27] Avery SM, Small A, Reid CA, Buncic S 2002 *Journal of Food Protection* **65** (7) 1172-1176.
[28] Foley SL, Simjee S, Meng J, White DG, McDermott PF, Zhao S 2004 *Journal of Food Protection* **67**(4) 651-657.
[29] Griffin PM, Tauxe RV 1991 *Epidemiologic Reviews* **13** 60–98.
[30] Nataro JP, Kaper JB 1988 *Clinical Microbiology Review* **11** 142–201.
[31] Blanco JE, Blanco M, Alonso MP, Mora A, Dahbi G, Coria MA, Blanco J 2004 *Journal of Clinical Microbiology* **42** 311–319.

[32] Janda JM, Abbot SL 2007 *J. Clin. Microbiol.* **5**(9) 2761-2764.
[33] Petti CA 2007 *Clin. Infect. Dis.* **44** 1108–1114**.**