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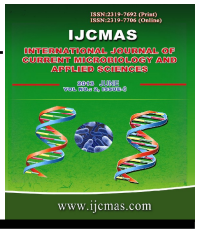

Original Research Article

Protein Profile Analysis of *Escherichia coli* O157:H7 from Human and Animals Origin

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Keywords

E. coli O157:H7; SDS-PAGE; human; animals; zoonotic.

A B S T R A C T

Healthy animals especially of the type of ruminants (cattle, sheep, and goats) and pigs are commonly carrying a large number of STEC and *Escherichia coli* O157:H7 in their feces, so they were considered as a main reservoir of the agent. The transmission of this agent to human commonly by consumption of less cooking meat, unpasteurized dairy product, water contaminated by feces, as well as person to person transmission has also been reported. Based on the importance of this agent to human healthy especially to the epidemiological aspect in order to know the pathogenesis of the agent, the genetic variation study of isolates originated from animals and human needs to be done. The study began by the cultivation of 20 *E. coli* O157:H7 isolates originated from animals and human, followed by analysis of the chemical characterization using SDS-PAGE (*Sodium Dodecyl Sulphonate Polyacrylamide Gel Electrophoresis*) method. The data from SDS-PAGE were analyzed using *simple matching coefficient* (Ssm) and *algorithm unweighted pair group method using arithmetic averages* (UPGMA). The results show that the protein profile of most *E. coli* O157:H7 local isolates are known to have similarity value more than 70%. These results prove that the isolates of animal origin can be found in humans or nature zoonotic.

Introduction

Healthy animals, especially certain types of ruminants (cows, goats, sheep) and pigs are known to contain large amounts of STEC and *E. coli* O157: H7 in their feces, so it was reported that types of animals are known as a natural reservoir of *E. coli* O157: H7 (Blanco *et al.*, 2004; Rey *et al.*, 2006). Transmission of this bacteria strain to humans occurs mainly through consumption of meat less cooked,

unpasteurized dairy products, water contaminated feces, and person-to-person transmission has also been reported (Karmali, 1989; Rey *et al.,* 2006).

Epidemiological studies of *E. coli* O157: H7 zoonotic agents from local isolates originated from animals and human through *analysis of Random Amplification of Polymorphic DNA* (RAPD) has been reported previously by Suardana *et al.,* (2011). The results obtained showed genomic DNA from isolates of animal and human origin are known to have the lowest similarity of 75.1% and as high as 96.6% when compared with control isolates ATCC 43894. On the other hand, besides the characterization and identification of microbial using molecular techniques (Kanso and Patel, 2003; Sembiring and Goodfellow, 2010), it is also known the characterization of microbial using chemical character (Schroll *et al.,* 2001).

Generally, the character of the chemical analysis based on comparison of the protein profiles, can be used for rapid microbial identification (Berber, 2004). Cellular protein profiles using SDS-PAGE (*Sodium dodecyl sulphonate polyacrylamide gel electrophoresis*) is one of the chemical character method that is widely used to strengthen of the identification, because it is through this method can be generated complex, specific, stable and reproducible of protein banding pattern so that it can be used to interpret and compare the tested strains with reference strains (Ghazi *et al.,* 2009).

Noting the lack of molecular studies in particular the analysis of chemical characterization of *E. coli* O157: H7 zoonotic agents for the purpose of exposing the pathogenesis of zoonotic agents from animals to humans as well as considering the need to confirm the results of previous studies by other characters approach, so that the study of genetic variation among isolates of *E. coli* O157: H7 derived from animal and human through analysis of chemical characterization by SDS-PAGE is interesting to be uncovered.

Materials and Methods

Bacterial strains of *Escherichia coli* O157: H7

Twenty isolates of *Escherichia coli* O157:H7 were used in this study composed of 11 clinical isolates originated from human fecal with renal failure symptoms i.e. KL-52(7), KL-87(7), KL-30(4), KL-45(1), KL-48(2), KL-85(1), KL-83(5), KL-24(5), KL-68(1), KL-106(3), and KL-55(6), 2 non-clinical isolates originated from healthy human fecal i.e. M-14(4), and M-17(1), 2 isolates originated from chicken fecal i.e. MK-35, and MK-19/8(4), 2 isolates originated from beef i.e. DS-21(4), and DS-16(2), 2 isolates originated from cattle fecal i.e. SM-25(1), and SM-7(1), and 1 control isolate ATCC 43894.

Cultivation of *Escherichia coli* O157: H7 isolates

Cultivation of the 20 of *Escherichia coli* O157: H7 isolates was done by taking from stock isolates (stored in 30% glycerol with a storage temperature of -20°C) to subsequent cultivating of the isolates on lactose broth medium (LB) at 37°C for overnight. Isolates were re-confirmed using *E. coli* O157 latex agglutination test (Oxoid, DR120M), by reacting of isolates with latex reagent (1 drop isolates added 1 drop of latex reagent). Positive results are

characterized by the formation of precipitation, according to the available positive control (Anonymous, 2010). Confirmation was also made to the H7 flagella that was initially by grown of the isolates on brain hearth infusion broth (BHI) medium. Isolates have been grown subsequently inactivated using 40% formalin with reacting of 0.3 parts of isolates in 100 parts of formaldehyde BHI. These results were referred as the antigen. This antigen was tested with H7 antiserum (BD DifcoTM E.coli H7 antiserum, Cat.No. 221 591) which has been diluted with a ratio of 1:500. Tests carried out by reacting of 50 ml antigen with 50 ml antiserum in the plate and incubated at temperature 50°C in water bath for 1 h. Positive result is characterized by the formation of precipitation on the plate base (Anonymous, 2009).

Chemical Characterization

Isolates of *E. coli* O157: H7 fecal origin of animals and humans as well as control isolates ATCC 43894 were grown in lactose broth medium / LB (CM0137B) and incubated aerobically in a rotary incubator shaker at a speed of 125 rpm, 30^{o}C overnight. Suspension of each isolate centrifuged at 3000 rpm, 4°C for 10 minutes. Cell pellet was washed with 0.1 M PBS buffer (phosphate buffered saline) pH 7.0 and then centrifuged again with the temperature, time and the same speed. Washing is done 3 times. Pellet re-suspended with 0.5-1 ml PBS solution (depending on the volume of pellets earned) and then split using sonicator. Sonication was performed for 30 seconds repeated 6 times with a grace period of 5 seconds. Amplitude repetition duty cycle used was 0.7. Pellet that has been smashed centrifuged at 13,000 rpm for 10 min, and then the supernatant was taken and used as protein samples.

Protein content of the samples was measured by the method of Bradford (Bradford, 1976) using the Bio-rad protein assay. A total of 50 mL sample of protein (supernatant) was added 5X loading buffer (12.5 mL) and then incubated at 100°C for 2 minutes and quickly stored at 0°C for 10 minutes. Concentration of the protein samples synchronized with dilution. Soluble protein profiles visualized with *Sodium Dodecyl Sulphonate Polyacrylamide Gel Electrophoresis* (SDS PAGE). Gel used is discontinuous gel consisting of 5% stacking gel and 12.5% resolving gel. Concentration of protein samples is loaded into the wells and synchronized into 35 ug / mL. Marker proteins were products of Magic Mark XP Western Protein Standard 20-220 kDa (Invitrogen LC5602). Gel stained with coomassie brilliant blue and then distained with a solution of a mixture of methanol and glacial acetic acid and distilled water in the ratio 5:1:4, until the protein bands clearly visible.

Each molecular weight of protein in the gel for each isolate was determined by measuring the molecular mobility of proteins in polyacrylamide gels based on standard protein molecular weight. Protein marker is electrophoresed along with the protein of each isolate and then mobility (retention factor / Rf) is calculated using the formula:

$$Rf = \frac{\text{Distance movement protein bands from the early}}{\text{Distance movement of tracer from the initial color}}$$

Protein molecular weight of each sample is measured by measuring the linear regression equation of the relationship between the retention factor (Rf) as the X-

axis and a molecular weight logarithm as Y-axis

Chemical Characterization Data Analysis

Phenotypic characters obtained from SDS PAGE are calculated by the formula Rf value converted to 1 (positive) and 0 (negative). The data are presented in a matrix form n x t (n = number of isolates analyzed, t = phenotypic traits). Further data preparated on the PFE (programmers file editor). Similarity value determined by the method of *simple matching coefficient* (SSM) and grouped with the *unweighted pair group method algorithm using arithmetic averages* (UPGMA) in *multy variate statistical program package* (MVSP) 3.1 (Kovach 1988 *in* Sembiring, 2002).

Results and Discussion

Chemical character of the *E. coli* O157: H7 isolates based on protein profiles using SDS-PAGE (*Sodium Dodecyl Sulphonate Polyacrylamide Gel Electrophoresis*) as shown in Figure 1a, 1b, and 1c with graphs representative as shown in Figure 2a, 2b, and 2c.

The results of protein profiles of *E. coli* O157: H7 isolates as shown in Figure 1a, 1b and 1c were clarified with representative graph such as Figure 2a, 2b and 2c. The data show some variation among isolates when compared with control isolates. SDS-PAGE analysis of the 20 isolates after analysis based on the value of retardation of the banding pattern found the variation in protein bands. The results of protein profiles are appropriated with the results previously by Kesava Naidu *et al.,* (2011) who found the variation of protein bands of the STEC isolates originated from human fecal, beef, and cattle feces ranging from a low of <20 kDa to the highest> 97.4 kDa.

Variations shown each isolate is a characteristic that distinguish between one isolate with the other isolates. Berber (2004) states that the chemical character based on comparative analysis of protein profiles can be used for rapid microbial identification. The same opinion was also agreed by Ghazi *et al.,* (2009) which states that the cellular protein profile using SDS-PAGE (*Sodium Dodecyl Sulphonate Polyacrylamide Gel Electrophoresis*) is one of the chemical character that is widely used to strengthen the identification, because by using SDS-PAGE it can be generated a complex, specific, stable and reproducible protein banding pattern, so that it can be used to interpret and compare the tested strains with reference strains. Other researchers Vijaya Bhaskar Reddy *et al.,* (2012) also stated that the protein profiles by SDS-PAGE is a molecular technique that can be trusted and can be repeated with good separation power which has been widely used by researchers to determine the various microorganisms for epidemiological purposes.

The variation in protein profiles among isolates were then used as a guide to assess the presence of polymorphisms or to assess the similarity between each isolate. Reliability of SDS-PAGE method in order to differentiate between the genus, species and strain of bacteria has been previously demonstrated by Walia *et al.,* (1988) who combining SDS-PAGE dendogram obtained from the numerical analysis that were originated from variety of protein banding pattern of

Figure.1a Protein profiles of SDS-PAGE produced by *E. coli* O157: H7 isolates. Line 1 positive control: ATCC 43894; line 2: KL-52 (7), line 3: KL-87 (7), line 4: KL-30 (4), line 5: KL-45 (1), line 6: KL-48 (2) and line 7: KL-85 (1)

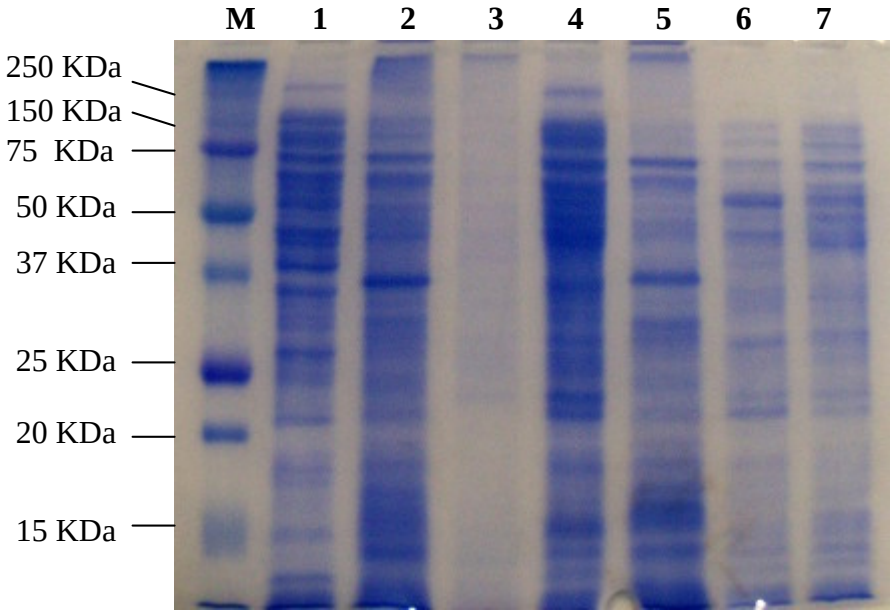


Figure.1b Protein profiles of SDS-PAGE produced by *E. coli* O157:H7 isolates. Line 8: KL-83(5), line 9: KL-24(5), line 10: KL-68(1), line 11: KL-106(3), line 12: KL-55(6), line 13: MK-35 and line 14: MK-19(8)/4.

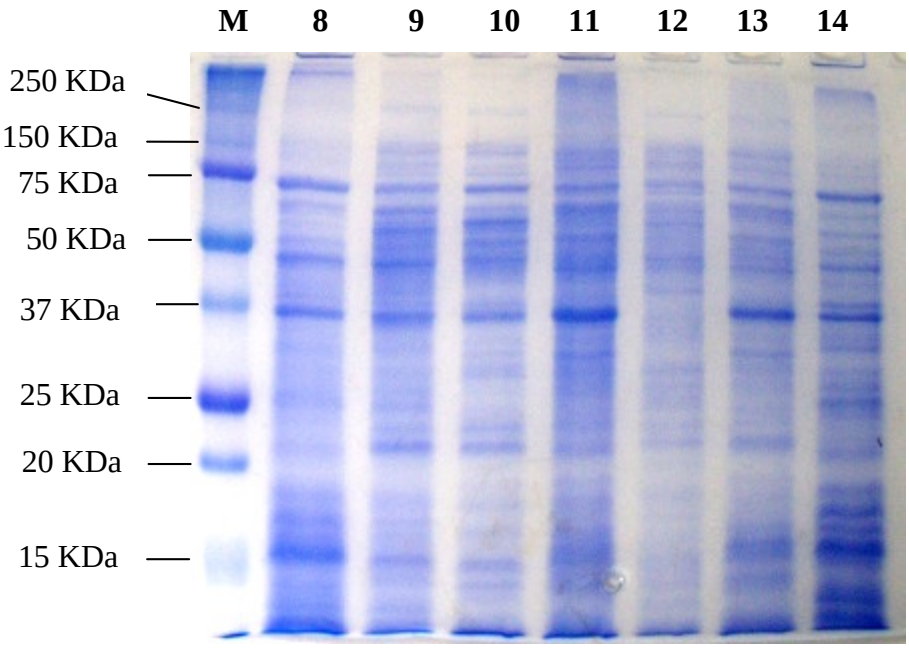


Figure. 1c Protein profile of SDS-PAGE produced by *E. coli* O157:H7 isolates. Line 15: M-14(4), line 16: M-17(1), line 17: DS-21(4), line 18: DS-16(2), line 19: SM-25(1) and line 20: SM-7(1).

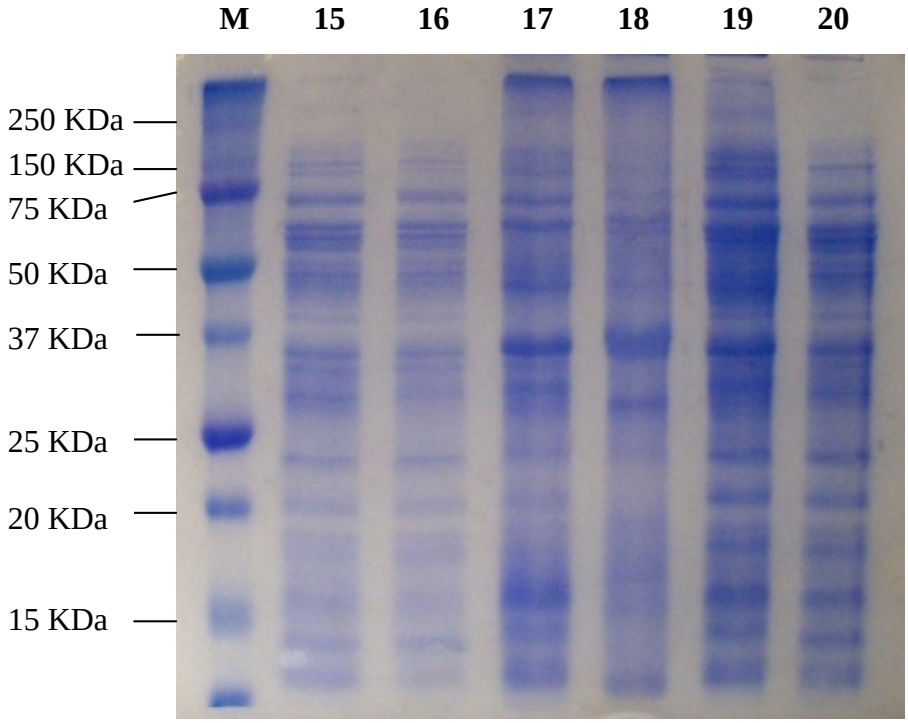


Figure. 2a Representative graph of protein profile of SDS-PAGE produced *E. coli* O157:H7 isolates. Line 1 positive control: ATCC 43894; line 2: KL-52(7), line 3: KL-87(7), line 4: KL-30(4), line 5: KL-45(1), line 6: KL-48(2) and line 7: KL-85(1)

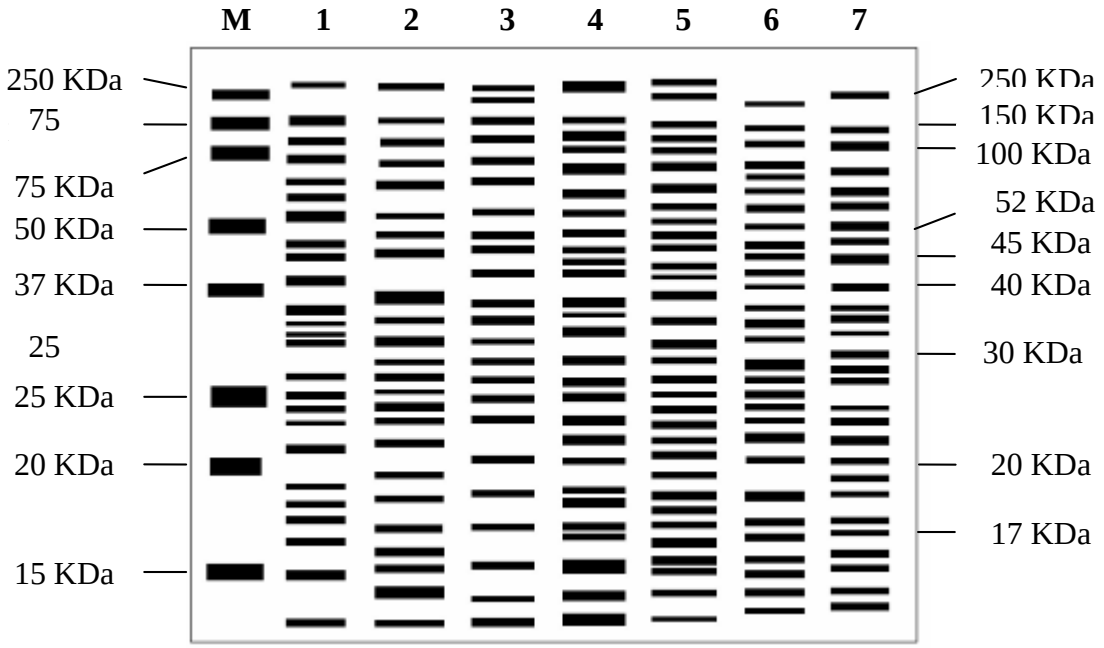


Figure.2b Representative graph of protein profile of SDS-PAGE produced *E. coli* O157:H7 isolates. Line 8: KL-83(5), line 9: KL-24(5), line 10: KL-68(1), line 11: KL-106(3), line 12: KL-55(6), line 13: MK-35 and line 14: MK-19(8)/4.

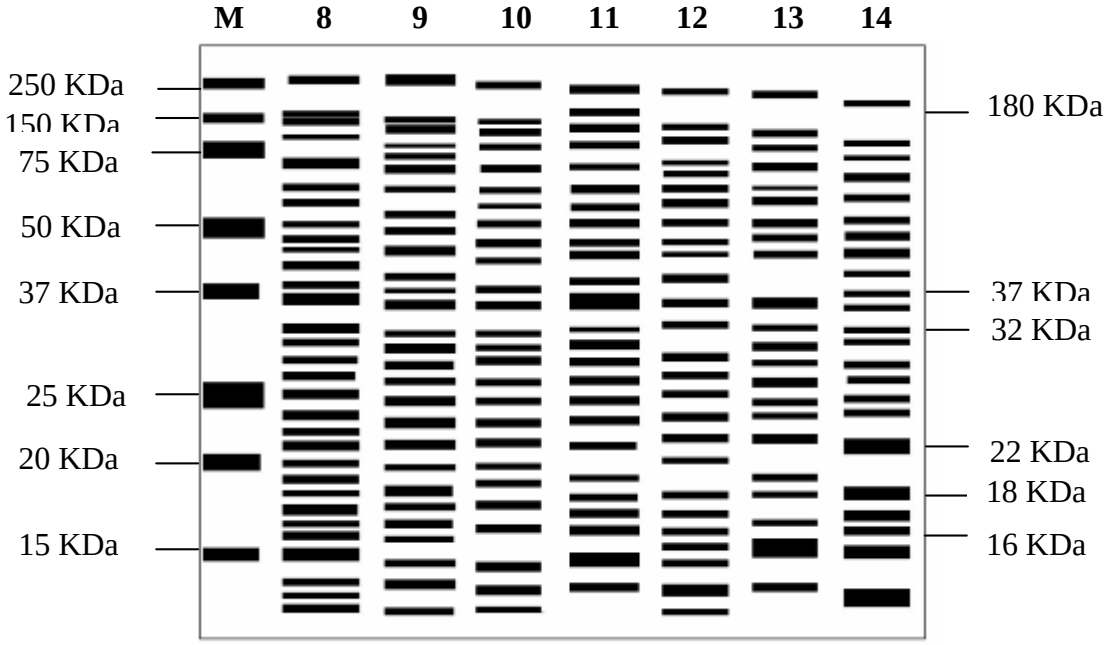


Figure .2c Representative graph of protein profile of SDS-PAGE produced *E. coli* O157:H7 isolates. Line 15: M-14(4), line 16: M-17(1), line 17: DS-21(4), line 18: DS-16(2), line 19: SM-25(1) and line 20: SM-7(1).

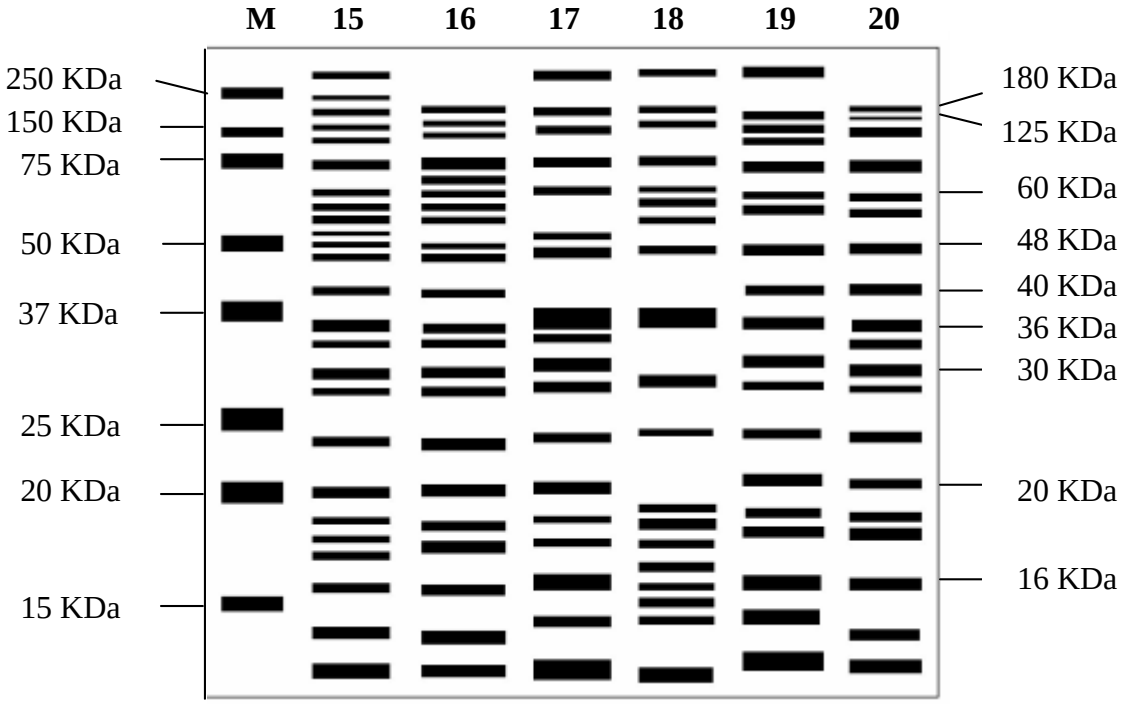
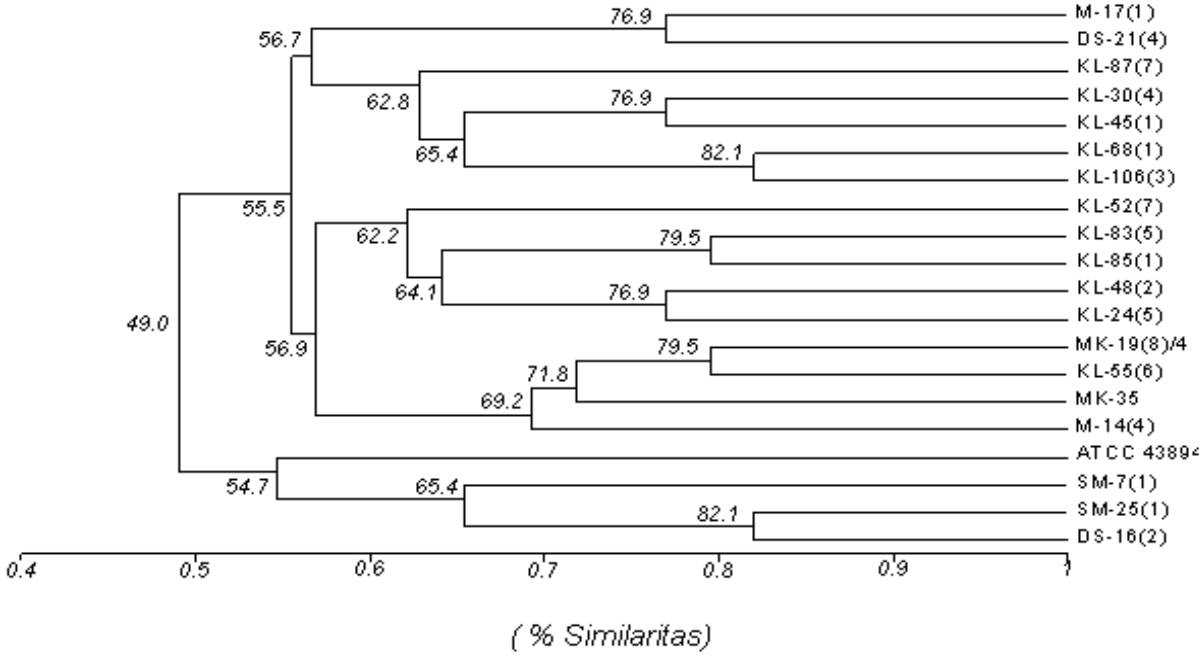


Figure.3 Dendogram similarity of protein profile of SDS-PAGE produced *E. coli* O157:H7 isolates. Dendogram are determined by Simple Matching Coefficient (Ssm) and algorithm Unweighted Pair-Group Method using Arithmetic Average (UPGMA).



strain. Other researchers Celebi *et al.*, (2007) also using the same SDS-PAGE method for determining taxonomic and epidemiological studies of urophatogenic *E. coli*.

SDS-PAGE usually combined with dendogram derived from the numerical analysis of the whole cell protein patterns of the strains has been used extensively to study similarity or differences among genera, species, and even strains (Adwan and Adwan, 2004). Further studies to determine the level of similarity between each isolate in the form of dendogram similarity are presented in Figure 3, and the matrix of similarity values for each isolates are completely presented in Table 1.

Dendogram in Figure 3 shows a grouping of 20 isolates that were tested originated from different source into 13 clusters / clade with 19 branching points (nodes) that have similarity values range from 49 to 82.1%. Cluster 1 as the initial cluster was formed by isolates KL-68 (1) and KL-106 (3) clinical human fecal origin with a similarity value of 82.1%, as well as between isolates of cluster 2 formed by SM-25 (1) origin of cow feces and DS-16 (2) origin of beef with a similarity value of 82.1%.

Cluster 3 is formed by isolates KL-83 (5) and KL-85 (1) clinical human fecal origin with similarity value 79.5%. Cluster 4 was formed by isolates MK-19 (8) / 4 chicken fecal origin with isolates KL-55 (6) clinical human fecal origin with the same value of similarity with cluster 3 is 79.5%. This will continue until clusters 13 with similarity value its own.

Table.1 Similarity value of SDS-PAGE analysis among isolates of *E. coli* O157: H7 with other isolates. Similarity matrix derived from the value of *Simple Matching Coefficient* (Ssm) and *algorithm Unweighted Pair-Group Method using Arithmetic Average* (UPGMA).

	ATCC 43894	KL-52(7)	KL-87(7)	KL-30(4)	KL-45(1)	KL-48(2)	KL-85(1)	KL-83(5)	KL-24(5)	KL-68(1)	KL-106(3)	KL-55(6)	MK-35	MK-19(8)/4	M-14(4)	M-17(1)	DS-21(4)	DS-16(2)	SM-25(1)	SM-7(1)
ATCC 43894	1.000																			
KL-52(7)	0.487	1.000																		
KL-87(7)	0.385	0.590	1.000																	
KL-30(4)	0.590	0.487	0.692	1.000																
KL-45(1)	0.462	0.564	0.615	0.769	1.000															
KL-48(2)	0.205	0.564	0.667	0.564	0.641	1.000														
KL-85(1)	0.462	0.667	0.513	0.513	0.641	0.641	1.000													
KL-83(5)	0.462	0.615	0.564	0.564	0.641	0.590	0.795	1.000												
KL-24(5)	0.333	0.641	0.538	0.487	0.513	0.769	0.718	0.615	1.000											
KL-68(1)	0.487	0.436	0.641	0.692	0.718	0.718	0.564	0.564	0.590	1.000										
KL-106(3)	0.513	0.410	0.564	0.615	0.590	0.590	0.487	0.538	0.564	0.821	1.000									
KL-55(6)	0.436	0.538	0.487	0.538	0.615	0.615	0.564	0.615	0.641	0.692	0.769	1.000								
MK-35	0.359	0.513	0.513	0.410	0.641	0.641	0.641	0.590	0.667	0.564	0.590	0.718	1.000							
MK-19(8)/4	0.436	0.487	0.436	0.436	0.615	0.564	0.462	0.410	0.538	0.641	0.667	0.795	0.718	1.000						
M-14(4)	0.410	0.462	0.513	0.462	0.538	0.641	0.590	0.538	0.667	0.615	0.641	0.718	0.692	0.667	1.000					
M-17(1)	0.487	0.436	0.487	0.538	0.564	0.462	0.615	0.615	0.487	0.590	0.513	0.487	0.564	0.436	0.462	1.000				
DS-21(4)	0.462	0.564	0.513	0.564	0.590	0.538	0.436	0.538	0.513	0.667	0.641	0.718	0.590	0.667	0.487	0.769	1.000			
DS-16(2)	0.538	0.333	0.385	0.385	0.462	0.462	0.410	0.410	0.436	0.590	0.615	0.538	0.615	0.641	0.615	0.487	0.513	1.000		
SM-25(1)	0.615	0.359	0.359	0.359	0.436	0.436	0.538	0.436	0.513	0.615	0.641	0.513	0.538	0.615	0.590	0.564	0.538	0.821	1.000	
SM-7(1)	0.487	0.436	0.333	0.487	0.564	0.564	0.615	0.513	0.692	0.538	0.513	0.487	0.564	0.487	0.564	0.590	0.513	0.641	0.667	1.000

Similarity value as a result of the SDS-PAGE analysis that lie between 49 to 82.1% is slightly different from the results of previous study using PCR-RAPD method which shows the similarity values between 75.1 to 96.6%. The difference of those as a result of a number of bands generated from each character are not same. This result is appropriate with the study has been done before by Maiti *et al.,* (2009) that evaluating of the PCR-RAPD method with protein profile analysis which showed no significant difference of the pattern of discrimination that were resulted between two methods.

The data in Table 1 as a continuation of the dendogram Figure 3 illustrates the similarity values between one isolate with others. The high similarity (> 70%) among clinical isolates of human origin (isolated from patients with renal failure) with the isolates of fecal cattle origin, beef, and chicken feces, indicating that the same isolates have been found among different host. This opinion is supported by Rosello-Mora and Amman (2001) which revealed that in general a prokaryotic organisms within species can be grouped into the same strain if it is known to have its genome similarity of 70% or greater.

The existence of high similarity between isolates of *E. coli* O157: H7 human origin with the main reservoir in animals, indicating the transfer of agent from animals to human has been occurred or the agent as a zoonotic. This indication is supported by the opinion of Nature and Caper (1998) which states that animals like cows, goats, sheep, pigs, cats, dogs and birds are the natural reservoir of *E. coli* O157: H7. The agent is usually transferred to humans through contaminated food or drink. This results are strengthen by the results of previously by Suardana *et al.,* (2011) who gave evidence the potential transmission of zoonotic agents *E. coli* O157: H7 from animal origin to human using PCR-RAPD method. In conclusion, results of the research showed that 1). Local isolates of *E. coli* O157: H7 originated from animals and humans have a similarity value ranges from 49 to 82.1%. 2) Cattle and chickens proved to be a main reservoir that can move the agent into humans.

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