EX VIVO ADHERENCE TO MURINE ILEAL, BIOFILM FORMATION ABILITY AND PRESENCE OF ADHERENCE-ASSOCIATED OF HUMAN AND ANIMAL DIARRHEAGENIC *ESCHERICHIA COLI*

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Abstract. Diarrheagenic *Escherichia coli* (DEC) are important bacteria causing gastrointestinal infection, which can lead to severe forms of illnesses. This study focused on DEC adherent capabilities using murine intestinal tissue as a model. *Ex vivo* adherence results showed that enteroaggregative *E. coli* (EAEC) strain PSU280 exhibited the highest level of adherence, followed by strains from ETEC category. Scanning electron micrographs displayed tight binding and putative bacterial curli fibers, including putative fimbrial structures. The presence of putative curli fibers was confirmed by the presence of *csgA*, a curli major structural subunit gene. Five and 3 of 15 DEC possessed *lpf* (encoding long polar fimbriae) and *agn43* (encoding antigen43), respectively. Comparable biofilm formation efficiency but variable levels autoaggregation were observed among the DEC strains. In addition, yeast agglutination could be visualized in 11/15 strains. This study demonstrates the adherent ability of DEC strains isolated in southern Thailand as well as a number of crucial adherence-associated genes, information of importance to the understanding of DEC pathogenesis in this region of the country.

Keywords: *Escherichia coli*, adherence, antigen43, biofilm, curli, long polar fimbriae, murine intestine

INTRODUCTION

Diarrheal diseases are of major public health concern, accounting for 11% of children's deaths worldwide, which make diarrhea the second leading cause of mortality in children lower than 5 years of age (CDC, 2013). An updated report of global child mortality shows that of 7.6 million children deaths (age 1-59 months) in 2010, diarrhea is responsible for 9.9% (Liu *et al*, 2012), and the situation in Southeast Asian countries is similar (Liu *et al*, 2012).

One of the important causes of diarrhea in children, especially in developing countries, results from diarrheagenic *Escherichia coli* (DEC). DEC is classified into 6 distinct pathotypes, namely, enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), and

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diffusely adherent *E. coli* (DAEC) (Nataro and Kaper, 1998). These types of DEC differ in their virulence genes, which give rise to different mechanisms of pathogenesis, including degrees of outcome to the infected person. Routes of transmissions of DEC are mainly via food and water. In Thailand, although DEC can be isolated from food sources (Sukhumungoon *et al*, 2011; Phetkhajorn *et al*, 2014; Sirikaew *et al*, 2015) but infections caused by DEC have rarely been reported in the past three decades. This information is even more seldom in the southern part of Thailand.

Adherence of DEC to the host tissues is the most crucial step in pathogenesis (Torres *et al*, 2004) because without adherence, subsequent attack of host by other bacterial virulence factors cannot take place. One example in EPEC is the use of bundle-forming pili to cause a localized-adherent (LA) pattern on epithelial cells, causing effacement of microvilli (Clarke *et al*, 2003). In addition, tight binding between translocated intimin receptor (Tir) and intimin protein encoded by *eae* of EHEC has reported to occur at the early course of disease (Nataro and Kaper, 1998). However, the adherent ability of DEC strains from southern Thailand has not been fully documented. Thus, this study investigated the early pathogenesis capability of DEC isolated from human and animal sources using an *ex vivo* murine intestinal tissue model. Adherence-associated factors, biofilm formation ability and involved genes detection were characterized.

MATERIALS AND METHODS

Bacterial strains

Fifteen DEC isolated from both humans and animal sources between 2012 and 2014, reference DEC strains from Bangladesh and United States, and a commensal *E. coli* isolate from a healthy man (all stored at -80ºC) used in the study were listed in Table 1.

Murine intestinal tissue preparation and adherence assay

Murine intestinal tissue was prepared as previously described with slight modifications (Yamamoto *et al*, 1991). In short, a 12 week-old Wistar rat's ileal was obtained from a carcass and immediately washed in phosphate buffered saline, pH 7.2 (PBS). Slices of intestinal tissue (0.5 cm^2) were immediately used for the adherence assay.

Bacteria were cultured on colonization factor antigen (CFA) agar [1% casamino acid (Difco, Detroit, MI), 0.15% yeast extract (Difco), 0.005% $MgSO_4$, 0.0005% $MnCl_2$, and 2.0% agar (Difco)] for 18 hours. For each bacterial strain, an individual colony was inoculated into 3 ml of CFA broth and further cultured for 6 hours at 37ºC with shaking. Bacterial numbers were adjusted to 0.5 McFarland turbidity units (approximately 1.5×10^8 CFU/ml) with PBS, using a densitometer (Biosan, Riga, Latvia) in a total volume of 2 ml. Slices of rat intestinal tissues were immersed in bacterial suspension and statically incubated for 1 hour at 28ºC. Then, non-adherent bacteria were removed by washing with PBS and intestinal tissues were homogenized with PBS in sterile bags. A 10-fold serial dilution in PBS was performed. Adherent bacteria on the intestinal tissue were measured by surface plate counting on eosin methylene blue (EMB) agar (Becton, Dickinson, Sparks, MD). Each experiment was performed in triplicate.

Scanning electron microscopy (SEM)

The preparation of murine intestinal tissues and bacteria were performed as described above. Intestines were immersed

Table 1
Diarrheagenic *E. coli* strains used in the study.

Pathotype	Strain	Year of isolation	Origin	Serotype	Virulence genes (RPLA titer for Stx)	Reference
EHEC	PSU2	2012	Beef, Thailand	O157	stx_2 (≤ 4), *eae*	Sukhumungoon *et al*, 2013
	PSU54	2012	Beef, Thailand	O157	stx_2 (< 2), *eae*	Sukhumungoon *et al*, 2013
	EDL933	1982	Human, USA	O157	stx_1 (32), stx_2 (4,096)[a]	Riley *et al*, 1983
STEC	PSU1	2012	Beef, Thailand	O157	stx_1 (128), stx_2 (16)	Sukhumungoon *et al*, 2013
	PSU17	2012	Beef, Thailand	O8	stx_2 (16)	Sukhumungoon *et al*, 2013
	PSU70	2013	Beef, Thailand	O157	stx_2 (< 2)	Sukhumungoon *et al*, 2013
EPEC	PE-27	n.d.	n.d.	O111	*bfp*, *eae*	Reid *et al*, 1999
ETEC	KETE	Unknown	Human, USA	O6	*est*, *elt*	Stacy-Phipps *et al*, 1995
	H10407	1971-1973	Human, Bangladesh	O78	*elt*	Evans Jr and Evans, 1973
	PSU192	2014	Human, Thailand	O169	*est*, *astA*	Sirikaew *et al*, 2014
EAEC	PSU263	2013	Human, Thailand	O127a	*aggR*, *aggA*, *astA*	Sukkua *et al*, 2015
	PSU280	2013	Human, Thailand	O44	*aggR*, *aggA*, *aafA*, *pet*, *astA*	Sukkua *et al*, 2015
	PSU294	2013	Human, Thailand	O44	*aggR*, *aggA*, *aafA*, *pet*	Sukkua *et al*, 2015
O157	PSU120	2014	Human, Thailand	O157	*escV*	Themphachana *et al*, 2014
	PSU132	2014	Beef, Thailand	O157	-	This study
Commensal	P4	2014	Healthy human, Thailand	OUT	n.d.	This study (stock culture)

[a]Stx titer was measured in culture supernatant (Koitabashi *et al*, 2006); n.d., no data; OUT, O-untypeable.

in bacterial suspension for 1 hour, washed with PBS and further processed as the protocols described by Sukkua *et al* (2015) with slight modifications. In short, each sample was treated with 2.5% glutaraldehyde in cacodylate buffer for 90 minutes, washed twice with cacodylate buffer and dehydrated using a series of analytical-grade ethanol. Then, samples were fixed with osmium tetroxide prior to coating with gold and examined by SEM (Quanta, Hillsboro, OR) at magnification ranging from 6,000× to 90,000×.

Biofilm formation of DEC

Screening of biofilm formation was performed as previously described with slight modifications (Wakimoto *et al*, 2004). In short, an individual colony was cultured in 3 ml of Luria Bertani (LB) broth (Difco) supplemented with 0.45% glucose for 18 hours. Adjustment of bacterial number to 0.5 McFarland turbidity units was performed as described above. A 200 µl aliquot of bacterial cells were seeded into a 96-well flat-bottom polystyrene microtiter plate (NEST Biotech, Shanghai, China), incubated at 37ºC for 18 hours under static condition. Planktonic cells in LB broth were discarded and wells were washed twice with distilled water and air-dried. Attached biofilm was stained with 200 µl of 0.5% (w/v) crystal violet for 5 minutes, then rinsed twice and air-dried. Cell-bound crystal violet was released from bacterial cells by the addition of 200 µl of 95% ethanol. Absorbance at 570 nm was measured using a microplate reader (Biotek, Winooski, VT). Each experiment was performed in triplicate.

Autoaggregation assay

Autoaggregation was performed as previously described with slight modifications (Schembri *et al*, 2001). In brief, an 18-hour bacterial culture in LB broth was adjusted to 1.5×10^8 CFU/ml and cells were allowed to settle for 4 hours without agitation. A 100-µl aliquot of cells at the well bottom was cultured for a further 18 hours in 5 ml of LB broth at 37ºC with shaking. This procedure was repeated for four times. The settled (autoaggregatated) cells were monitored by eye after the fourth culture.

Agglutination of yeast cells

Adherence of *E. coli* to the host tissues is often paralleled by the ability to agglutinate erythrocytes or yeast cells (Schembri *et al*, 2000). This assay was performed as described by Schembri *et al* (2001) with slight modifications. In brief, a 30-µl aliquot of an 18-hour culture in LB broth was mixed with 5% *Saccharomyces cerevisiae* cells in 0.85% NaCl solution (NSS) on glass slide. Agglutination was monitored by eye and time at which agglutination took place was recorded. Positive agglutination reaction was judged within 1 minute after mixing. A suspension of yeast cells in LB broth was used as negative control.

PCR-based assay of adherence-associated genes

PCR template was prepared by a boiling method as described by Pannuch *et al* (2014). In brief, an 18-hour bacterial culture in tryptic soy broth (TSB) (Becton, Dickinson, Sparks, MD) was boiled for 10 minutes, immersed on ice for 5 minutes, sedimented, and supernatant was diluted ten-fold in sterile deionized water. DNA solution was kept at -20ºC until used. PCR was conducted in a 25-µl mixture consisting of 0.4 µM each primer pair (listed in Table 2), 0.1 mM dNTPs, 1X Go*Taq* DNA polymerase buffer (Promega, Medison, WI), 0.5 U Go*Taq* Flexi DNA polymerase and 2 µl of DNA template. Thermocycling (conducted in T100™ Thermal Cycler, Bio-Rad) conditions were as follows: 95ºC for

Table 2
Oligonucleotide primers used for amplification of DEC adhesion genes in the study.

Gene	Virulence factor	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
papC	P fimbriae	pap3	GCAACAGCAACGCTGGTTGCATCAT	336	Yamamoto *et al*, 1995
		pap4	AGAGAGAGCCACTCTTATACGGACA		
afa	Afa adhesin	afa1	GCTGGGCAGCAAACTGATAACTCTC	750	Le Bouguénec *et al*, 1992
		afa2	CATCAAGCTGTTTGTTCGTCCGCCG		
sfaDE	S fimbriae	sfaDE-F	CTCCGGAGAACTGGGTGCATCTTAC	408	Le Bouguénec *et al*, 1992
		sfaDE-R	CGGAGGAGTAATTACAAACCTGGCA		
fimH	Type 1 fimbrial tip	fimH-F	TGCAGAACGGATAAGCCGTGG	508	Johnson and Stell, 2000
		fimH-R	GCAGTCACCTGCCCTCCGGTA		
lpf	Long polar fimbriae	*lpfA1*-F	GGTCGTTTTTGCCTTAACCGC	≈500	Torres *et al*, 2004
		lpfA1-R	AGGTTGAAATCGACCTGCGC		
agn43	Antigen43	1-Kpn	GAACCTGTCGGTACCGATGCCCTCCC	≈900	Danese *et al*, 2000
		2-Bam	CGGGATCCGTTGCCACTGTACCGGGCTTGACGACC		
csgA	Curli major structural subunit	csgA-Fw	TGGTAACAGCGCCACTCTTG	≈155	Lloyd *et al*, 2012
		csgA-Rv	GACGGTGGAATTAGATGCAGTC		

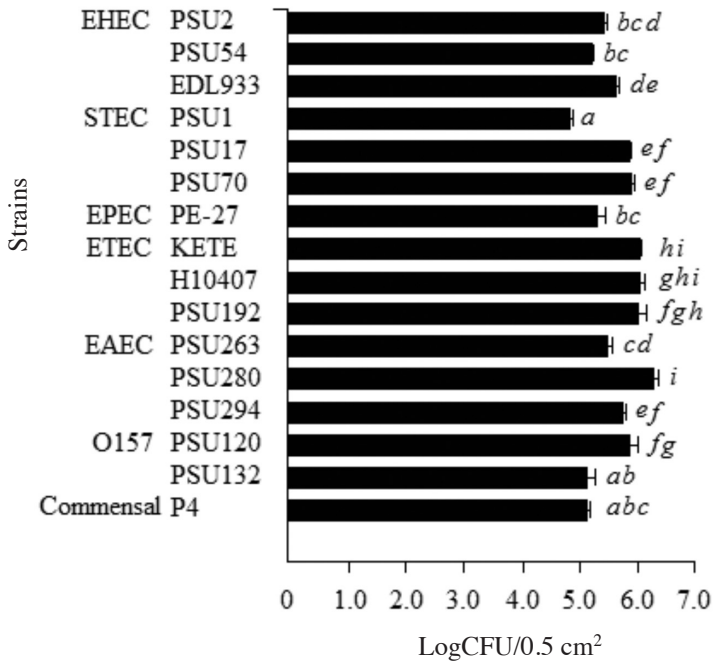


Fig 1–Adherence of diarrheagenic *E. coli* to rat intestinal tissues. A 0.5 cm^2 of 12 week-old Wistar rat's ileal was immersed in bacterial culture (0.5 McFarland turbidity unit) at 28ºC for 1 hour. Intestinal tissue was washed with PBS before measuring adherent bacteria by surface plate counting on EMB. Adherence is given as LogCFU/0.5 cm^2. Significant difference of adherent ability among DEC strains was analyzed by one-way ANOVA and showed 9 different groups which differed in adherent ability (*a* to *i*). Significant value is set at $p < 0.05$.

3 minutes; followed by 35 cycles of 94ºC for 1 minute, 55ºC for 1 minute (for *fimH*, *csgA*, and *lpf*), or 58ºC for 50 seconds (for *sfaDE*), or 60ºC for 40 seconds (for *papC* and *afa*), or 67ºC for 1 minute (for *agn43*), and 72ºC for 1 minute or 1.50 minutes for *agn43* and *lpf*; with a final step at 72ºC for 5 minutes. Amplicons were analyzed by 1.0% agarose gel-electrophoresis, stained with ethidium bromide and visualized using WSE-5200 Printgraph 2M gel imaging system (ATTO, Tokyo, Japan).

Statistical analysis

Data were analyzed using SPSS for Windows version 11.0 (SPSS, Chicago, IL). One-way ANOVA was used to determine significant difference in adherence among DEC strains and biofilm formation. Significance is set at $p < 0.05$.

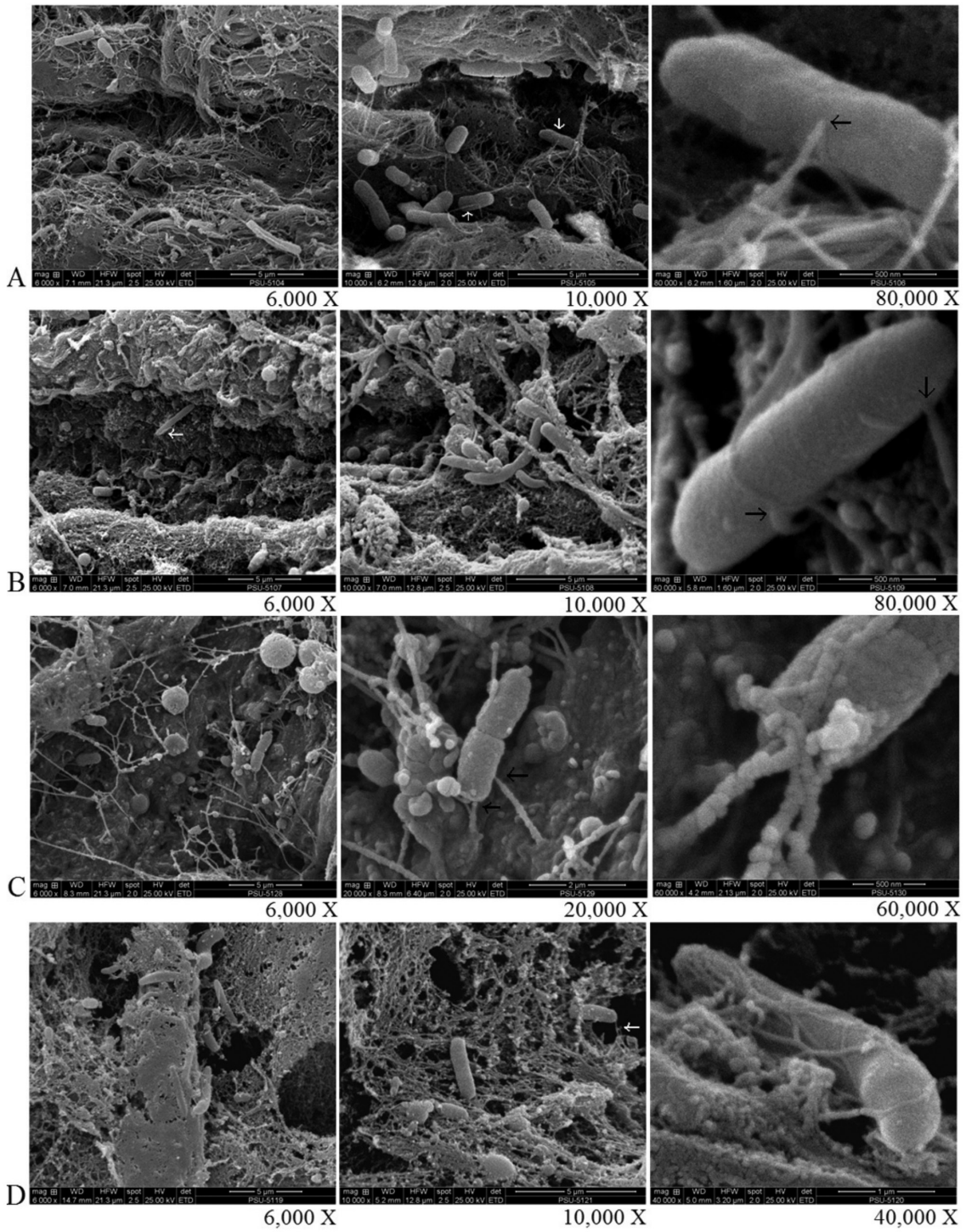
RESULTS

Adherence of DEC to murine intestinal tissue

Adherence to murine intestinal tissues suggests that a large portion of *E. coli* has pathological ability. The mean adherence values ranged from 4.93-6.07 log CFU/0.5 cm^2, with the highest level (6.07 logCFU/0.5 cm^2) observed for EAEC strain PSU280 ($p < 0.05$) (Fig 1). ETEC strain PSU192 isolated from a diarrheal patient in southern Thailand showed an adherence value as 5.80 logCFU/0.5 cm^2. Interestingly, a *stx*-negative *E. coli* O157 strain PSU120, also isolated from a diarrheal patient, was among the top five DEC strains that showed high levels of adherence. STEC strain PSU1 isolated from a beef sample demonstrated the lowest value (4.93 logCFU/0.5 cm^2) ($p < 0.05$) (Fig 1). A control commensal *E. coli* strain P4 exhibited a relatively low adherence value, comparable to STEC strain PSU1. Murine intestine alone did not give rise to any colonies on EMB, indicating that the normal microbiota left in the washed murine intestine samples used practically was non-existent.

SEM

All DEC pathotypes in the study


A
6,000 X
10,000 X
80,000 X
B
6,000 X
10,000 X
80,000 X
C
6,000 X
20,000 X
60,000 X
D
6,000 X
10,000 X
40,000 X

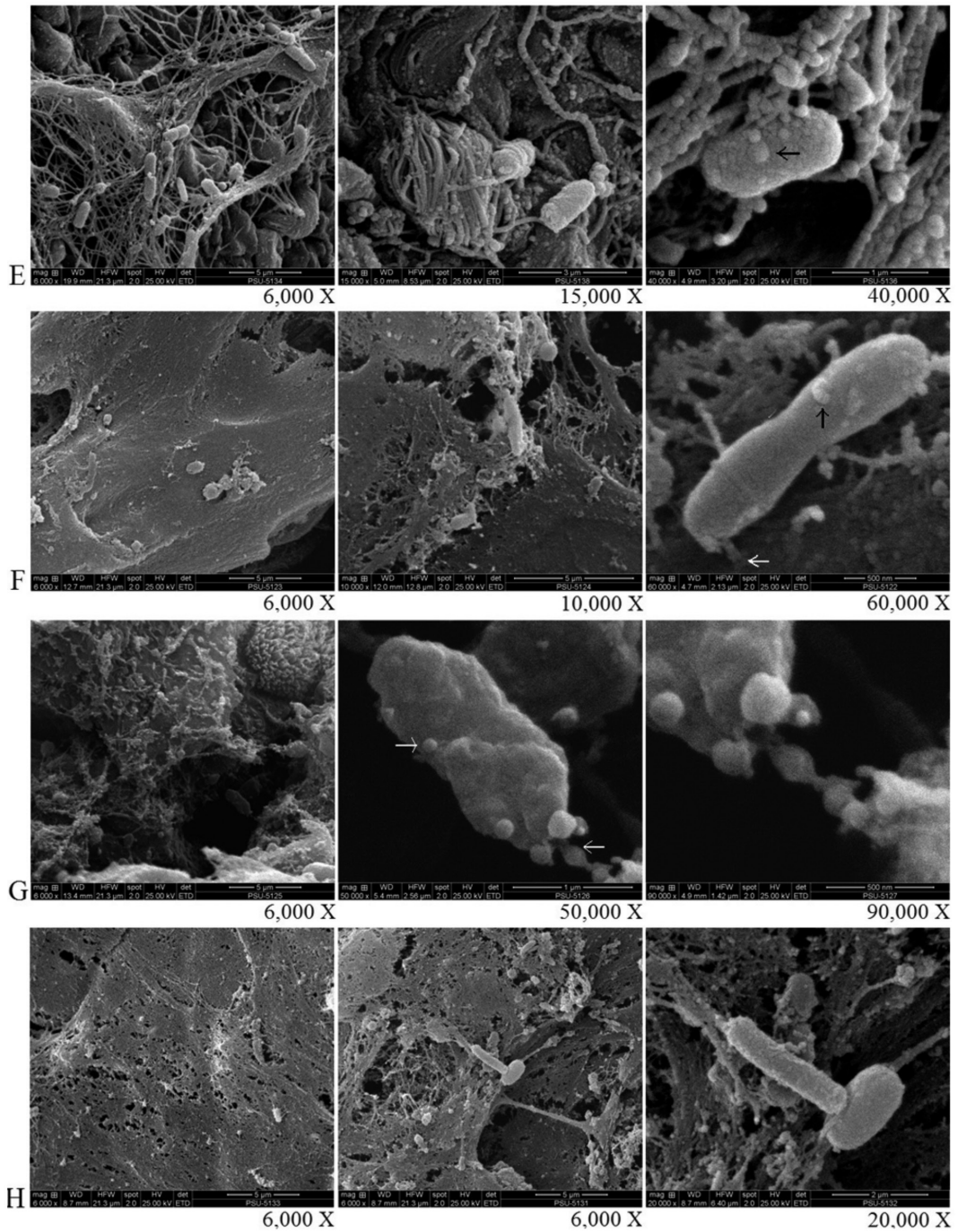


Fig 2–Scanning electron micrograph of representative strains from each DEC category adhered to murine intestinal tissues. A-H, EHEC strain PSU54, STEC strain PSU70, O157 strain PSU120, EPEC strain PE-27, ETEC strain KETE, ETEC strain PSU192, EAEC strain PSU280, and commensal *E. coli* strain P4, respectively. Black and white arrows in (A) and (D) are putative curli fibers. Black and white arrows in (B) and (C) indicate putative fimbriae, and black arrows in (E) and (F) and white arrows in (G) outer membrane vesicles.

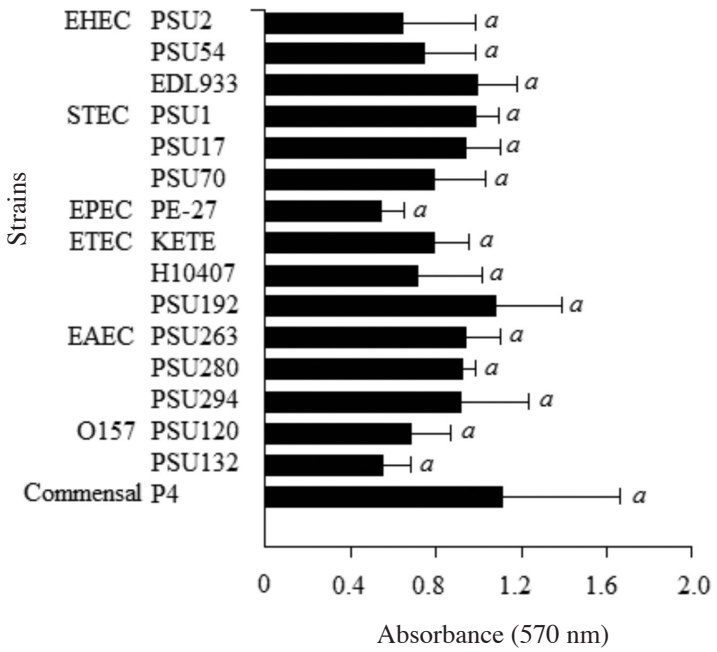


Fig 3–Biofilm formation of DEC strains. A bacterial culture (0.5 McFarland turbidity unit) was seeded into a 96-well flat-bottom polystyrene microtiter plate, incubated at 37°C for 18 hours statically. Biofilm was stained using crystal violet, washed, and biofilm level measured by the release of cell-bound crystal violet spectrophotometrically (570 nm). Statistical analysis was performed using one-way ANOVA. Lowercase letter (*a*) indicates insignificant difference among groups ($p > 0.05$).

exhibited cells ranging in length from approximately 1-2 μm. Upon adherence to murine intestine EHEC O157 strain PSU54 demonstrated putative thin curli fibers throughout the intestinal surface, and at a higher magnification a joint between curli and a bacterial cell (Fig 2A). The appendages of bacteria may not be composed of curli alone, and some might be fimbriae, which clearly could be seen in STEC O157 strain PSU70 (Fig 2B). Similar phenomenon of curli fiber formation was found in EPEC strain PE-27 (Fig 2D). ETEC and EAEC exhibited knobby outer membrane vesicles (OMVs) throughout the bacterial surface (Fig 2E, F and G). On the other hand, commensal *E. coli* strain P4 lacked curli fibers and fimbriae but had low amounts of small OMVs on the bacterial surface (Fig 2H).

Biofilm formation of DEC

The majority of DEC strains revealed comparable efficiency in biofilm formation, ranging from a mean value 0.508 (EPEC strain PE-27) to 1.027 (ETEC strain PSU192). ETEC strain PSU192 (O169), isolated from a diarrheal patient in Hat-Yai Hospital, showed the highest value for biofilm formation, but this value is not statistically different from other DEC strains (Fig 3).

Autoaggregation screening assay

Autoaggregation was observed macroscopically in all DEC strains, but at a low level (Table 3, Fig 4A). However, commensal *E. coli* strain P4 lacked autoaggregation production.

Agglutination of yeast cells

Of 15 DEC strains, 11 (73%) agglutinated yeast cells, 7 of which showing high levels of agglutination, with 5 exhibiting rapid agglutination (within 10 seconds) (Table 3). Observation of yeast agglutinates by Gram staining under bright field microscope revealed that DEC acted as a bridge between yeast cells, whereas commensal *E. coli* strain P4 did not exhibit this ability (Fig 4B).

Presence of adhesion genes

The presence of adherence-associated

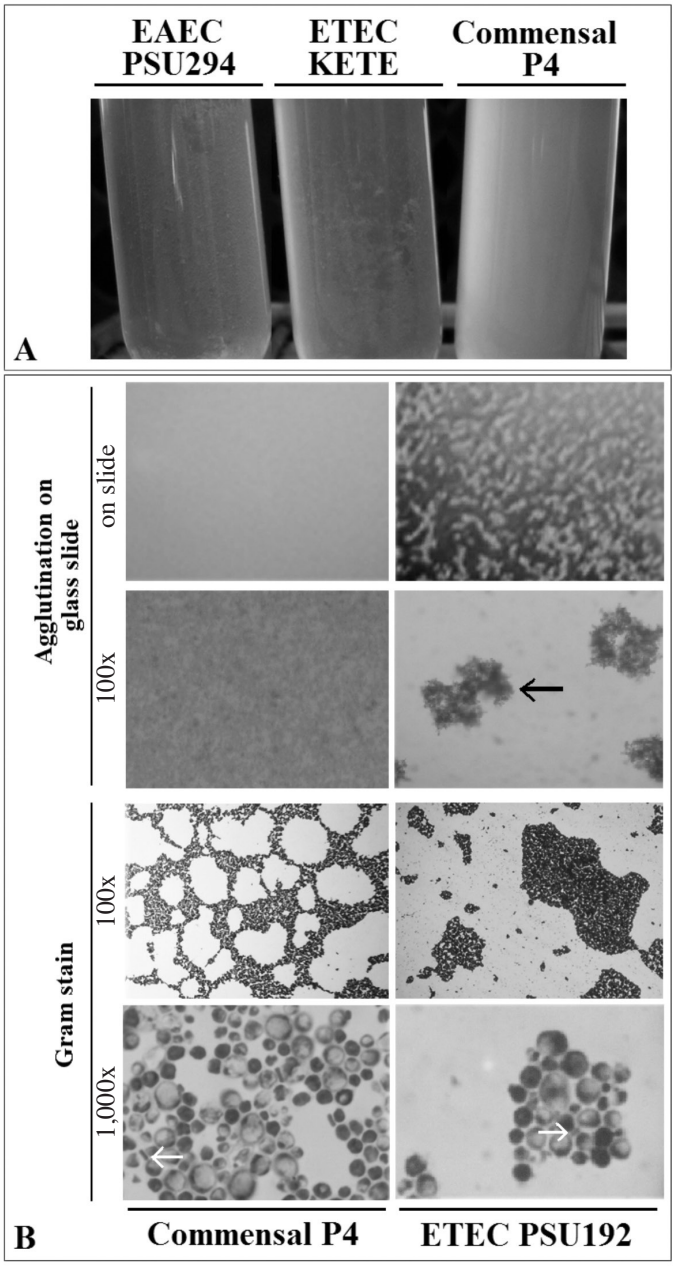


Fig 4–Autoaggregation (A) and agglutination of yeast cells (B). Autoaggregation was performed by allowing bacterial culture (0.5 McFarlarland turbidity unit) to settle for 4 hours. This procedure was repeated 4 times before autoaggregation ability was determined by eye at the fourth culture. For yeast cell agglutination, an 18-hour bacterial culture was mixed with 5% *Saccharomyces cerevisiae* in NSS on a glass slide. Agglutination was monitored by eye and confirmed microscopically under a bright field microscope (100× magnification; black arrow indicates a yeast agglutinate). In Gram stained images (100x and 1,000x magnification) white arrow (right panel) indicates yeast agglutination, whereas white arrow (left panel) indicates non-agglutination.

Table 3
Analysis of adherence-associated genes harbored by DEC strains.

Pathotype	Strain	Adherence-associated gene							Autoaggregation[a]	Agglutination of yeast cells[b]
		fimH	*agn43*	*csgA*	*papC*	*afa*	*sfaDE*	*lpf*		
EHEC	PSU2	+	-	+	-	-	-	+	+	-
	PSU54	+	-	+	-	-	-	+	+	+
	EDL933	+	-	+	-	-	-	+	+	-
STEC	PSU1	+	-	+	-	-	-	-	+	+
	PSU17	+	+	+	-	-	-	-	+	++*
	PSU70	+	-	+	-	-	-	-	++	++
EPEC	PE-27	+	-	+	-	-	-	-	+	-
ETEC	KETE	+	-	+	-	-	-	-	++	++
	H10407	+	-	+	-	-	-	-	+	-
	PSU192	+	-	+	-	-	-	+	+	++*
EAEC	PSU263	+	-	+	-	-	-	-	+	++*
	PSU280	-	+	+	-	-	-	-	++	+
	PSU294	-	+	+	-	-	-	-	++	+
O157	PSU120	+	-	+	-	-	-	-	++	++*
	PSU132	+	-	+	-	-	-	+	+	++*
Commensal	P4	-	-	-	+	-	-	-	-	-

[a]++, obvious autoaggregation; +, low autoaggregation; -, no autoaggregation. [b]++, high level of agglutination; +, low level of agglutination; -, no agglutination. *Agglutination occurs within 10 seconds.

genes can, in part, indicate bacterial adhesion structures. All DEC possessed *csgA*, 5/15 (33%) strains contained *lpf* and 3/15 (20%) *agn43* (also known as *flu* for flocculation) (Table 3).

DISCUSSION

EAEC has been shown to cause aggregative adherence pattern in cell cultures including abiotic surfaces (Dudley *et al*, 2006). EAEC strain PSU280 demonstrated the highest adherence values, which was not unexpected because this EAEC strain has previously been shown to have large numbers of fimbrial and crucial toxin genes (Sukkua *et al*, 2015). The adherence values of EAEC strains PSU263 and PSU294 were comparable to, but still lower than, those of PSU280. This may be attributed to their lower numbers of adhesin genes.

This study also demonstrated the relatively high levels of adherence to murine intestinal tissues by ETEC strains, including that of strain PSU192 isolated from a diarrheal patient. ETEC possesses at least 23 colonization factors (currently called coli surface antigens), which mediate the initial steps of ETEC pathogenesis (Gaastra and Svennerholm, 1996).

In the current study, SEM images of DEC cells after binding to murine intestinal tissues showed distinct appearance of adherence-associated factors such as putative fimbriae and curli fibers, except commensal *E. coli* P4. Curli structure was first discovered in late 1980s in *E. coli* that caused bovine mastitis (Barnhart and Chapman, 2006) and has been implicated in many pathological processes of *E. coli* and *Salmonella* spp, for example, and of cell adhesion, cell aggregation, biofilm formation, and cell invasion, as well as being a potent inducer for host inflammation (Prigent-Combaret *et al*, 2001; Cookson *et al*, 2002). It is worth noting that biofilm formation by DEC in this study might result, in part, from the presence of putative curli fibers.

Long polar fimbria plays a role in both *in vivo* and *in vitro* adherence by *E. coli* O157:H7 as well as other pathogenic *E. coli* strains (Jordan *et al*, 2004; Fitzhenry *et al*, 2006). In this study, all 3 EHEC strains possessed long polar fimbriae-coding gene, *lpf*. The possession of *lpf* together with *fimH* and *csgA* in these 3 strains, were led to the higher possibility of human intestinal colonization due to the *in vivo* colonization capabilities in other mammals, sheep, conventional pig, and gnotobiotic piglet, had been clearly shown (Jordan *et al*, 2004).

All ETEC and EAEC strains in the study exhibited OMVs (SEM images). OMV is formed at the outer membrane from blebbing of the periplasmic content (Beveridge, 1999) and OMV formation is increased during colonization of host tissues (Ellis and Kuehn, 2010). OMV displays the ability to act as a virulence factor-delivery system, for instance, Shiga toxins from STEC (Kolling and Matthews, 1999), heat-labile enterotoxin from ETEC (Horstman and Kuehn, 2000), and cytotoxic necrotizing factor-1 from UPEC (Kouokam *et al*, 2006). Thus, the presence of OMV in ETEC and EAEC strains in this study supports, at least in part, their virulent roles.

It was noted that the characteristics of tissue-bound *E. coli* cells were different from the SEM images of palletized bacterial cells grown in broth culture (Sukkua *et al*, 2015). It is thought that various types of adherent factors may be induced by intestinal tissue binding (An and Friedman, 1998). In addition, intestinal tissue may serve as a substratum for bacteria to

attach, preventing the destruction of appendages by shear forces.

FimH (encoded by *fimH*) is thought to confer the autoaggregation of DEC, resulting in the colonization of mammalian hosts (Schembri *et al*, 2001). In this study, 2/15 DEC strains (EAEC PSU280 and PSU294) did not possess *fimH*, but displayed autoaggregation at a high level. This may be explained by the presence of other autoaggregation-inducing factors, such as Agn43 which confer the Agn43-Agn43 interaction, visualized by settling of standing liquid culture (Schembri *et al*, 2004**)**. In addition, Agn43 is responsible for biofilm formation. Curli fibers also involved in intercellular fiber-associated precipitation, mediated by nucleator protein–mediator secreted from adjacent cells (Schembri *et al*, 2001). Fimbriae, AAF/I and AAF/II in EAEC are supposed to be the cause of autoaggregation in these two EAEC strains (Sukkua *et al*, 2015).

Among the DEC categories, attention should not be focused only on EAEC pathogenesis but EHEC and Shiga toxin-producing *E. coli* (STEC) also should be considered. In addition to bloody diarrhea, Shiga toxins produced from EHEC or STEC are able to bind to their specific globotriaosylceramide (Gb3) receptors causing severe complications to the brain (Fujii *et al*, 2008) and kidney (hemolytic uremic syndrome) (Trachtman *et al*, 2012), leading to high mortality rates. In this present study, STEC O8 strain PSU17 showed relatively high levels of adherence to the murine intestinal tissue. This strain can produce Stx2 at a titer of 16, which is considered as at the dangerous level and can end up with illnesses (Sukhumungoon *et al*, 2013).

In conclusion, regarding the high virulence background of DEC strains in Thailand, appending the clear evidence of additional uncovered virulence genes, including the striking adherence capabilities shown in this study, we conclude that these DEC strains are ferocious and when they reach their infectious doses, they are indeed threaten to human in this area. Biofilm produced by those DEC helps protect them from antibiotic attack, exacerbating the course of infection.

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