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Subtilase Cytotoxin-Encoding *subAB* Operon Found Exclusively among Shiga Toxin-Producing *Escherichia coli* Strains[▽]

Kinue Irino,[1]* Mônica A. Midolli Vieira,[2] Tânia A. Tardelli Gomes,[2] Beatriz E. Cabilio Guth,[2] Zita V. Furtado Naves,[1] Murilo Gomes Oliveira,[3] Luis Fernando dos Santos,[2] Mirian Guirro,[2] Claudio D. Timm,[4] Caroline P. Pigatto,[5] Sonia M. S. S. Farah,[6] and Tânia M. I. Vaz[1]

Department of Bacteriology, Instituto Adolfo Lutz, Av. Dr Arnaldo, 351/355, 9 andar, CEP 01246-902 São Paulo, SP, Brazil[1]; Department of Microbiology, Immunology and Parasitology, Universidade Federal de Sao Paulo, Sao Paulo, SP, Brazil[2]; Department of Clinical Analysis, Universidade Federal de Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil[3]; Department of Preventive Veterinary Medicine, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil[4]; Department of Veterinary Medicine, Universidade Federal do Parana, Curitiba, Parana, Brazil[5]; and Department of Bacteriology, Laboratorio Central de Saude Publica, Curitiba, Parana, Brazil[6]

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The presence of *subAB* was investigated for 3,453 *Escherichia coli* strains of various pathogenic categories. The occurrence of other virulence genes in *subAB*-positive strains was investigated. The *subAB* operon was detected among some Shiga toxin-producing *E. coli* (STEC) serotypes devoid of *eae* and carrying *ehx*A. Most *subAB*-positive strains also harbored stx_2, *iha*, *saa*, and $lpfA_{O113}$.

Subtilase cytotoxin, a new member of the AB_5 toxin family, was identified for the first time in 2004 in a virulent O113:H21 Shiga toxin-producing *Escherichia coli* (STEC) strain that caused an outbreak of hemolytic-uremic syndrome in South Australia (16, 18). The presence of *subAB* genes was further detected in other STEC strains belonging to different serotypes (19). Subsequently, *subAB* genes were identified among STEC strains isolated in other countries (3, 8, 9, 14, 25).

To evaluate how widely distributed the *subAB* operon is, we studied a large collection of STEC serotypes from nonhuman sources and *E. coli* strains of different pathogenic categories associated with human infections. The *subAB*-positive strains were further characterized regarding the presence of other virulence genes.

A total of 2,255 *E. coli* strains isolated from humans and belonging to enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), extraintestinal pathogenic *E. coli* (ExPEC), and *E. coli* strains not belonging to the diarrheagenic categories described so far were randomly selected. STEC strains isolated in Brazil from humans have previously been tested for the presence of *subAB* by our group (3). The 1,198 STEC strains from nonhuman sources were isolated from dairy cattle, beef cattle, buffaloes, and goats. Overall, 109 different STEC serotypes were tested. An STEC strain of serotype O113:H21 (3) was used as a reference strain for *subAB*, *cdt-V*, and $lpfA_{O113}$, and *E. coli* strain DH5α was used as a negative control.

The strains were screened for the presence of the *subAB* operon (encoding subtilase cytotoxin) using colony hybridization assays (21). The 1,823-bp *subAB*-specific DNA probe was derived from the STEC serotype O113:H21 (3) strain by PCR as previously described (19). Hybridization assays were performed under stringent conditions, and the probe was labeled with [α-^{32}P]dCTP (Amersham), using the Ready-To-Go DNA labeling kit (Amersham). All strains which yielded a positive or weak signal in hybridization assays with the *subAB* probe were retested by PCR (18, 19), and only those confirmed by PCR were considered to be carrying this sequence.

The genetic profiles of the *subAB*-positive strains were determined using our previously reported data for the same strains (6, 7, 12, 13, 20, 24) regarding the presence of the *ehx*A, *eae*, stx_1, stx_2, and adhesin-encoding genes (1, 10, 11, 15, 17, 22, 23).

A total of 130 STEC strains carrying the *subAB* operon, representative of each serotype and isolated from different animals, were analyzed by PCR for the presence of genes encoding Lpf_{O113} and cytolethal distending toxin (Cdt-V) (2, 5).

Expression of the SubAB and Cdt-V toxins was investigated using Chinese hamster ovary cells according to the methods of Paton et al. (18) and Bielaszewska et al. (2), respectively. Cells were exposed to filter-sterilized bacterial culture supernatants and observed daily for a period of 7 days. To confirm the loss of viability or morphological changes, trypan blue, violet crystal, and/or 4′,6-diamidino-2-phenylindole (DAPI) staining were performed. Control strains were included in all assays.

As shown in Table 1, the *subAB* operon was detected exclusively among STEC strains and corresponded to 25.5% (306/1,198) of the STEC collection. The presence of *subAB* was identified in 44.2% (141/319), 27.1% (29/107), and 23.8% (129/542) of STEC strains isolated from dairy cattle, buffaloes, and beef cattle, respectively. Only 3% (7/230) of STEC strains

* Corresponding author. Mailing address: Department of Bacteriology, Instituto Adolfo Lutz. Avenida Dr Arnaldo, 351, 9 andar, CEP 01246-902 São Paulo, Brazil. Phone: 55-11-3068-2896. Fax: 55-11-3085-3505. E-mail: kirino@ial.sp.gov.br or ikinue@hotmail.com.

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TABLE 1. *E. coli* strains of different pathogenic categories searched for the *subAB* operon

E. coli category	Total no. of strains studied	No. (%) of strains positive for *subAB*
Enteropathogenic *E. coli* (EPEC)[a]	402	0
Enterotoxigenic *E. coli* (ETEC)[b]	264	0
Enteroinvasive *E. coli* (EIEC)[c]	266	0
Enteroaggregative *E. coli* (EAEC)	100	0
Extraintestinal *E. coli* (ExPEC)[d]	205	0
E. coli strains other than the diarrheagenic categories described so far	1,018	0
Shiga toxin-producing *E. coli* (STEC) from nonhuman sources[e]	1,198	306 (25.5)
Total	3,453	306

[a] EPEC, strains of serogroups O25, O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158.
[b] ETEC strains ST/LT or both toxin producers.
[c] EIEC strains of serogroups O28, O29, O112, O124, O121, O135, O136, O143, O144, O152, O159, O160, O164, O167, and O173.
[d] ExPEC *E. coli* strains isolated from blood, cerebrospinal fluid, and/or urine.
[e] STEC strains isolated from humans in Brazil have been previously tested by us (3).

isolated from goat carried *subAB*. Among the 109 different STEC serotypes tested, 21 carried the *subAB* operon. The presence of the *subAB* operon probably is associated with some STEC serotypes. In the present study, the rate of carriage of this sequence within each serotype ranged from 5.5 to 100% (Table 2). Among caprine STEC strains, only those belonging to O113:H21 carried the *subAB* operon. A total of 306 (306/1,198) STEC strains carrying *subAB* were detected. We have previously reported that among 49 human STEC strains isolated in Brazil, none carried the *subAB* sequence (3).

All strains carrying *subAB* were devoid of *eae*, and 59.8% (183/306) were associated with strains possessing the stx_2 gene alone, 39.9% (122/306) were carrying stx_1 plus stx_2, and only 0.3% (1/306) carried stx_1 alone, as previously reported (3, 8, 9, 14, 19). The most frequent adhesin-encoding genes among STEC strains carrying the *subAB* operon were $lpfA_{O113}$, *iha*, and *saa*, and all strains also carried *ehx*A.

Among the 130 selected STEC strains carrying the *subAB* operon, 98.5% (128/130) and 20% (26/130), respectively, harbored the $lpfA_{O113}$ and *cdt-V* sequences. In STEC strains carrying the *cdt-V* gene, 54% and 23% of the isolates, respectively, belonged to serotypes O116:H21 and O113:H21. Expression of the subtilase cytotoxin was detected in 40.7% (53/130) of the studied strains, while the *cdt-V* gene was expressed in 30.8% (8/26) of the strains. We observed that 24.5% (13/53) of the strains that expressed SubAB also harbored the *cdt-V* gene; however, none of the isolates coexpressed both cytotoxins. This result is in contrast with previous data in which coexpression of SubAB and Cdt-V in STEC isolates of serotype O113:H21 occurred (4). The expression of *subAB* genes in a collection of STEC strains belonging to several serotypes is reported here for the first time. The production of this toxin had been seen previously only in O113:H21 STEC (18).

To the best of our knowledge, the search for *subAB* in other *E. coli* categories has not been described before, and the present results showed that among *E. coli* strains, the *subAB* gene sequence was distributed only among some STEC serotypes.

TABLE 2. Distribution and frequencies of *subAB* operon-positive STEC serotypes isolated from dairy/beef cattle, buffalo, and goat

Serotype	No. of strains tested	No. (%) of *subAB*-positive strains
O39:H49[a]	11	9 (81.8)
O44:H25[b]	5	4 (80)
O59:H8[c]	1	1 (100)
O74:H28[a]	1	1 (100)
O74:H−[a]	3	3 (100)
O77:H18[b,c]	20	14 (70)
O79:H14[a,b]	35	34 (87.1)
O79:H28[c]	5	5 (100)
O79:H−[a]	8	8 (100)
O96:H19[a]	1	1 (100)
O96:H21[a]	5	5 (100)
O105:H18[b]	9	1 (11.1)
O113:H21[a,b,d]	52	36 (69.2)
O116:H21[a,b,c]	32	32 (100)
O141:H49[b,c]	18	18 (100)
O153:H25[b]	5	5 (100)
O163:H−[a]	1	1 (100)
O163:H19[a,b]	3	3 (100)
O174:H28[a]	1	1 (100)
O176:H18[a]	2	2 (100)
O178:H18[b]	4	2 (50)
O178:H19[a,b,c]	46	29 (63.0)
O179:H8[a,b]	9	3 (33.3)
ONT:H−[a]	18	1 (5.5)
ONT:H2[a]	21	2 (9.5)
ONT:H7[a,c]	53	6 (11.3)
ONT:H8[a,b]	38	7 (18.4)
ONT:H10[a]	4	3 (75)
ONT:H11[a]	4	2 (50)
ONT:H18[c]	14	4 (28.6)
ONT:H19[a,b]	59	34 (57.6)
ONT:H21[a,b,c,d]	64	8 (12.5)
ONT:H25[a]	4	2 (50)
ONT:H46[a,b]	16	15 (93.7)
ONT:H49[a]	4	3 (75)
OR:H19[a]	3	1 (33.3)
Total	579	306

[a] Isolated from beef cattle.
[b] Isolated from dairy cattle.
[c] Isolated from buffalo.
[d] Isolated from goat.

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