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Distribution of the O-Genotypes of *Escherichia albertii* Isolated from Humans and Environmental Water in Akita Prefecture, Japan

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Serological typing is useful for epidemiological studies of various pathogens. In the case of enterohemorrhagic *Escherichia coli*, a majority of the reported outbreaks have been attributed to several serogroups (i.e., O26, O111, and O157). In Japan, there have been an increasing number of reports on the isolation of *E. albertii* during human gastroenteritis outbreaks, a newly recognized enteric pathogen that is closely related to *E. coli* (1). *E. albertii* is subclinically carried by wild and domestic birds (2), and has been isolated from various animals (3). Furthermore, *E. albertii* has been widely detected in the environmental water of the Akita prefecture, Japan (4). A comprehensive scheme for the serological typing of *E. albertii* strains did not exist until recently, when Ooka et al. (5) developed the O-antigen classification method called EAO-genotyping. Therefore, little is known about the prevalence of different *E. albertii* serotypes. The aim of this study was to determine the distribution of O-genotypes of *E. albertii* strains derived from humans and environmental water of the Akita prefecture.

Forty-four *E. albertii* strains were examined in this study, of which 29 were of human origin and 15 were of environmental water origin. All strains were collected from the Akita prefecture, Japan (Table 1). Human strains were collected from distinct cases. One human strain was isolated in 2019, one environmental strain was re-identified from a stock strain that was originally classified as *E. coli*, and another environmental strain was isolated in 2019 from sewage effluents. Other strains were described previously (4,6,7).

All *E. albertii* strains were subjected to PCR

analysis for EAO-genotyping (5). During the EAO-genotyping, 40 primer pairs were used to detect each O-genotype and 2 primer pairs were used as internal controls. PCR was performed using KOD-Multi and Epi-DNA polymerase (Toyobo, Osaka, Japan) and under the following conditions; 94 °C for 2 min, 25 cycles of 94 °C for 10 sec, 60 °C for 30 sec, and 68 °C for 30 sec. The O-genotypes were identified on 2% agarose gels based on the size of the PCR amplicons, and in comparison to a 100 bp DNA ladder (Takara Bio Inc., Shiga, Japan). Cross-reactivity with the antisera against *E. coli* O-antigens was confirmed by the slide agglutination method, and using the *E. coli* antisera set 1 (Denka Seiken, Tokyo, Japan) (5).

Biochemical properties were determined with the help of the API 20 E system (bioMérieux, Tokyo, Japan), triple sugar iron agar (Eiken Chemical, Tokyo, Japan), lysine indole motility medium (Nissui, Tokyo, Japan), and the oxidation-fermentation medium (Eiken Chemical), as described previously (4,6).

The detection of *E. coli* attaching and effacing (*eae*), cytolethal distending toxin (*cdt*), and Shiga toxin (*stx*) genes was performed as described previously (4,6). Moreover, *cdt* genes were typed by PCR analyses (6).

The results showed that 38 *E. albertii* strains were shared by 19 O-genotypes while the remaining 6 strains were not typable (Table 1), however, the result for EC15062 has been described previously (5). Among these, 24 strains were agglutinated using antisera against *E. coli* O-antigens (Table 1). The most dominant O-genotype in human strains was EAOg18 (24.1%) (Fig. 1). Five O-genotypes, namely EAOg6, EAOg12, EAOg18, EAOg25, and EAOg32, were detected in both human and environmental strains (Fig. 1).

The results of biochemical tests are shown in Table 2. All *E. albertii* strains were non-motile, as per the conventional biochemical method, and produced indole and lysine decarboxylase. Thus, these strains could not be assigned to established biogroups, however, most *E. albertii* isolates collected in Japan were positive for both indole and lysine decarboxylase production and were assigned to biogroup 3 (8). Furthermore, these

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Table 1. Information of 44 *Escherichia albertii* strains analyzed in this study

Strain No.	Source	Sampling point ¹⁾		Isolation year and month	Results of EAO-genotyping ²⁾	Cross-reaction with antisera of <i>E. coli</i> O-antigens ³⁾	Reference
EC191	Human feces			Aug, 1995	8	O128/O55 ^w	(7)
EC193				Aug, 1995	5	-	(7)
EC264				Jul, 1996	UT	-	(7)
EC312				Aug, 1996	18	O115	(7)
EC596				Apr, 1997	8	O128/O55 ^w	(7)
EC800				Aug, 1997	15	O119	(7)
EC839				Sep, 1997	8	O128/O55 ^w	(7)
EC862				Oct, 1997	25	-	(7)
EC875				Oct, 1997	6	-	(7)
EC1498				Jul, 1998	27	-	(7)
EC1528				Jul, 1998	1	-	(7)
EC2478				Jul, 1999	UT	O103 ^w	(7)
EC2873				Sep, 1999	12	O63	(7)
EC3329				Feb, 2000	7	-	(7)
EC3763				Jul, 2000	32	-	(7)
EC4993				May, 2001	UT	O167	(7)
EC5340				Aug, 2001	18	O115	(7)
EC5533				Sep, 2001	18	O115	(7)
EC6953				Aug, 2002	8	O128/O55 ^w	(7)
EC6954				Aug, 2002	18	O115	(7)
EC9933				Jul, 2005	18	O115	(7)
EC12830				Aug, 2008	18	O115	(7)
EC13473				Aug, 2009	13	O103 ^w	(7)
EC14101				Jul, 2010	8	O128/O55 ^w	(7)
EC15062				Nov, 2011	4	O127a ^w	(6)
EC16692				Aug, 2016	1	-	(7)
EC16860				Jul, 2017	3	-	(7)
EC16882				Aug, 2017	18	O115	(7)
EC17412				Aug, 2019	8	O128/O55 ^w	this study
EC5150	Environmental water	River	E	Jul, 2001	32	-	this study
EC17073		River	B	May, 2018	33	-	(4)
EC17120		River	E	Jul, 2018	16	-	(4)
EC17263		River	E	Sep, 2018	6	-	(4)
EC17264		River	E	Sep, 2018	UT	-	(4)
EC17265		River	E	Sep, 2018	12	O63	(4)
EC17292		Moat		Nov, 2018	UT	-	(4)
EC17341		River	H	Nov, 2018	25	-	(4)
EC17393		River	A	May, 2019	33	-	(4)
EC17394		River	A	May, 2019	30	O124 ^w	(4)
EC17395		River	A	May, 2019	UT	-	(4)
EC17396		River	M	Jun, 2019	33	-	(4)
EC17397		River	N	Jun, 2019	18	O115	(4)
EC17398		River	N	Jun, 2019	11	O161	(4)
EC17413		Sewage effluent		Aug, 2019	29	O8	this study

¹⁾: cited from Takahashi, et al (4).²⁾: UT: untypable.³⁾: by the slide agglutination method using *E. coli* antisera set 1 (Denka Seiken). -: no agglutination; ^w: weak agglutination.

Table 2. Biochemical properties and the *cdt* types of each O-genotypes in *Escherichia albertii* strains

		Biochemical properties, %										
		Indole	Arginine dihydrolase	Acid from:								
		Lysine decarboxylase	H ₂ S									
		Ornithine decarboxylase	Motility ³⁾ (35°C)									
O-genotype	Number of strains	D-glucose, acid	Acid from: Amygdalin									Detection of the <i>cdt</i> type (%)
		D-glucose, gas	Inositol	ONPG ⁴⁾	L-arabinose	D-sorbitol	Sucrose					
		Acid from: D-mannitol	Lactose									
			Melibiose									
			L-rhamnose									
			D-xylose									
EA0g1	<i>n</i> = 2			100	100	0	0	0	0	0	II/V (100)	
EA0g3	<i>n</i> = 1			0	100	100	0	100	0	0	II/V (100)	
EA0g4 ¹⁾	<i>n</i> = 1			0	0	0	0	0	0	0	III/V (100)	
EA0g5	<i>n</i> = 1			100	100	0	0	0	0	0	III/V (100)	
EA0g6 ²⁾	<i>n</i> = 2			100	50	0	0	0	0	0	I/II/V (50), II/V (50)	
EA0g7	<i>n</i> = 1			100	100	0	0	0	0	0	III/V (100)	
EA0g8	<i>n</i> = 6			83.3	100	100	0	100	0	0	I/II/V (33.3), II/V (66.7)	
EA0g11	<i>n</i> = 1			0	100	100	0	100	0	0	III/V (100)	
EA0g12 ²⁾	<i>n</i> = 2			50	100	100	0	100	0	0	I/II/V (50), II/V (50)	
EA0g13	<i>n</i> = 1	100	0	0	0	100	100	100	100	0	II/V (100)	
EA0g15	<i>n</i> = 1			0	100	100	0	100	0	0	I/II/V (100)	
EA0g16 ²⁾	<i>n</i> = 1			100	100	100	0	100	0	0	II/V (100)	
EA0g18	<i>n</i> = 8			75	100	100	0	100	62.5	0	I/II/V (25), II/V (25), II/IV/V (50)	
EA0g25 ²⁾	<i>n</i> = 2			50	100	50	50	50	50	50	I/III/V (50), III/V (50)	
EA0g27	<i>n</i> = 1			100	100	0	0	0	0	0	II/V (100)	
EA0g29	<i>n</i> = 1			100	100	100	0	100	0	0	II/V (100)	
EA0g30 ²⁾	<i>n</i> = 1			100	100	100	0	100	0	0	II/V (100)	
EA0g32	<i>n</i> = 2			100	100	100	0	100	0	0	II/V (100)	
EA0g33 ²⁾	<i>n</i> = 3			0	100	100	100	100	100	100	I/II/V (100)	

¹⁾: cited from Konno, et al (6).

²⁾: results from strains of environmental water origin were cited from Takahashi, et al (4).

³⁾: on the lysine indole motility medium.

⁴⁾: ONPG; ortho-nitrophenyl-beta-galactoside.

8. Murakami K, Maeda-Mitani E, Kimura H, et al. Non-biogroup 1 or 2 strains of the emerging zoonotic pathogen *Escherichia albertii*, their proposed assignment to biogroup 3, and their commonly detected characteristics. *Front Microbiol.* 2019;10:1543.
9. Murakami K, Etoh Y, Tanaka E, et al. Shiga toxin 2f-producing

- Escherichia albertii* from a symptomatic human. *Jpn J Infect Dis.* 2014;67:204-8.
10. Kashio H, Konno T, Takahashi S, et al. Identification of multiple *Escherichia albertii* O-genotypes as etiological agents in a foodborne disease outbreak. *Jpn J Food Microbiol.* 2020;37:183-7. Japanese.