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Confirmation of Aerogenic Strains of *Shigella boydii* 13 and Further Study of *Shigella* Serotypes by DNA Relatedness

DON J. BRENNER,[1]* ARNOLD G. STEIGERWALT,[1] H. GAIL WATHEN,[1] R. J. GROSS,[2] AND B. ROWE[2]

Enteric Laboratory Section, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333,[1] and Salmonella and Shigella Reference Laboratory, Central Public Health Laboratory, London NW9 5HT, England[2]

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Shigella boydii 13 strains are separable from other *Shigella* and *Escherichia coli* strains on the basis of DNA relatedness. From this observation, it was possible to confirm the existence of aerogenic *S. boydii* 13 strains. DNA relatedness studies also showed that strains of *E. coli* and strains representing all other serotypes of *Shigella*, including provisional strains, belong to the same genetic species.

Typical shigellae are nonmotile, lactose negative, and anaerogenic (they do not produce visible gas from the fermentation of glucose and other carbohydrates). These characteristics serve to separate shigellae from typical strains of *Escherichia coli* which are positive for these characters. Some *E. coli* biogroups are, however, nonmotile, lactose negative, and anaerogenic. Almost all *Shigella* O antigens are identical to or have reciprocal cross-reactions with one or more *E. coli* O antigens (7). Certain *E. coli* strains cause invasive diarrheal disease that is indistinguishable from shigellosis. Finally, shigellae and *E. coli* belong to the same genetic species (2, 3). Therefore, it is sometimes difficult, if not impossible, to identify atypical strains of the *E. coli-Shigella* group. Additional difficulties arise if a *Shigella*-like strain is aerogenic (produces gas from the fermentation of glucose and other carbohydrates).

Two biotypes of *Shigella flexneri* 6 (Manchester and Newcastle) are known to produce gas from glucose (5, 6). A strain of *Shigella dysenteriae* 3 was reported as aerogenic (10). Strains of *Shigella boydii* 14 (11) were thought to be aerogenic, but in subsequent studies (see reference 9) they were purported to be atypical *E. coli* strains. The most recent observation of aerogenic shigellae was in two strains of *S. boydii* 13 (9). *S. boydii* 13 is the only known group within the *E. coli-Shigella* complex that can be identified with certainty by DNA relatedness (2). The primary purpose of this study was to determine whether these aerogenic *S. boydii* 13 strains could be confirmed by DNA relatedness. A second objective was to further assess DNA relatedness among shigellae to determine whether any other serotype can be identified by this approach.

MATERIALS AND METHODS

Organisms. The *S. boydii* 13 and *E. coli* O28 strains used in this study are listed in Table 1. All other *Shigella* and *E. coli* strains used in DNA hybridization studies were sent to the Enteric Section, Centers for Disease Control, Atlanta, Ga., for identification between 1950 and 1975.

Media and biochemical tests. Cultures were stored on blood agar base deeps and were grown on brain heart infusion broth. The medium used to label cells with $^{32}PO_4$ has been described (1). Unlabeled DNA was prepared from cells grown to stationary phase in 1.5 liters of brain heart infusion broth on a dry air rotary shaker at 37°C. Media and reaction conditions for biochemical tests have been described recently (7, 8).

DNA preparation and DNA relatedness. The methods used to prepare DNA and to determine DNA relatedness have been described previously (4). All DNA reassociation reactions were done at least twice. Binding of homologous DNA to hydroxyapatite was between 65 and 90% before normalization. Control reactions containing only labeled DNA showed less than 3% binding to hydroxyapatite. The control values were subtracted from heterologous reaction values before normalization.

RESULTS AND DISCUSSION

DNA relatedness within *S. boydii* 13 strains and of *S. boydii* 13 to other shigellae and *E. coli* is shown in Table 2. Relatedness within *S. boydii* 13 strains was more than 90% when DNA reassociation was done at either an optimal (60°C) or a stringent (75°C) incubation temperature. The percent divergence (*D*) in these intraserotype reactions was 0.5 or less. The aerogenic *S. boydii* 13 strains (603-73 and 3555-77) could not be distinguished from anaerogenic *S. boydii* 13 strains on the basis of DNA relatedness. As shown previously (2), *S. boydii* 13 was easily separable from other *S. boydii* serotypes, other

TABLE 1. Sources of *S. boydii* 13 and *E. coli* O28 strains

Strain	Source	Gas from glucose
S. boydii		
C13	Standard strain; unknown	−
616	Unknown	−
2406-51	Stool, premature baby, Mexico	−
1201-54	Stool, child, Egypt; received from National Naval Medical Center, Bethesda, Md.	−
2045-54 (E. 7191, 3553-77)	Stool, child, Egypt; received from National Naval Medical Center	−
1610-55	Monkey, Florida	−
3552-77 (Boyd M425, E. 7192)	Original *S. boydii* 13 strain	−
3554-77 (E. 7142, DRL 341/59)	Bulgaria	−
3555-77 (E. 411/73, BR 1639)	Diarrheal stool, Belgian returning from India; E. Van Oye and B. Rowe	+
603-73 (E. 2054/71)	Stool, adult female, from Nigeria, isolated in British Isles	+
E. coli		
3556-77 (E. 2915/74)	British Isles	−
3557-77 (E. 4908/76)	Czechoslovakia	−
O28	Stock strain	+

TABLE 2. DNA relatedness of *S. boydii* 13 strains to one another and to other shigellae and *E. coli* strains

Source of unlabeled DNA	Source of labeled DNA				
	S. boydii 13 strain 1610-55			*E. coli* K-12	
	RBR[a] (60°C)	*D*[b]	RBR (75°C)	RBR (60°C)	RBR (75°C)
S. boydii 13					
1610-55	100	0.0	100	64	56
2045-54	94	0.5	97	68	
2406-51	94	0.5	99		
603-73				59	
3552-77	91	0.5	93	71	61
1201-54	88	0.5	90	67	60
3554-77	92	0.5	90	71	62
3555-77	89	0.0	86	61	57
616	92	0.0	92	68	60
Standard strain	92	0.5	98		
E. coli					
O28 (reference strain)	71				
O28 3556-77	66	6.0			85
O28 3557-77	72	5.0	67		79
K-12	68	6.5		100	100
D02	69		65	90	84
075	74	5.5	60		
09	60	10.0	26		
DK 73	72	5.5	64		
025	72	8.5	51		
S. boydii 10	69	6.5	66		
S. boydii 7	68	6.5	58		
S. flexneri 2 24570	69	5.0	63	86	87
S. flexneri 6	67	5.5	56		
S. sonnei	62	4.5	55		
S. dysenteriae 3	71	4.5	62	80	83

[a] RBR (relative binding ratio) = percent (heterologous DNA bound to hydroxyapatite)/(homologous DNA bound to hydroxyapatite) × 100.

[b] *D*, Percent divergence. *D* is calculated on the assumption that a 1°C decrease in the thermal stability of a heterologous DNA duplex compared with that of a homologous DNA duplex is caused by each 1% of unpaired bases within the duplex. *D* values are given to the nearest 0.5%.

TABLE 3. DNA relatedness among shigellae

Source of unlabeled DNA[a]	Source of labeled DNA					
	S. flexneri 24570 (2a)			*S. sonnei* 9020-75		
	RBR[b] (60°C)	*D*[b]	RBR (75°C)	RBR (60°C)	*D*	RBR (75°C)
S. dysenteriae						
(1)	78			74		
(2)	78			82		
(3)	80			79	1.0	73
5031-72 (4)	85	0.0	75	77		
785-59 (5)	80	0.0	83	76	0.5	74
852-59 (6)	83			76		
4788-55 (7)	83			78	1.0	71
2116-52 (8)	79			77		
4798-59 (9)	77			76	0.5	74
(10)	86			81	2.0	73
S. flexneri						
2746-71 (1a)	88	0.0	89	73	0.5	69
2702-71 (1a)	89	0.0	93			
4343-70 (1b)	87	0.0	85			
24570 (2a)	100	0.0	100	84	1.0	72
2850-71 (3a)	92	0.0	93	79	0.5	79
5091-70 (3a)	85	0.5	91			
4279-70 (3b)	85	0.5	83	76		
2243-71 (4a)	89	0.0	86			
688-71 (4a)	85	0.0	87	77		
5205-67 (4a)	86	0.0	92			
2196-69 (4a)	90	0.0	96			
5836-70 (4a)	85	0.0	88			
4643-64 (4b)	92	0.0	93	79		
2794-71 (5)	91	0.0	91			
5094-64 (5)	91	0.0	94	77		
109-65 (5)	87	0.0	92			
Aerogenic (6)	84	0.0	89			
S. boydii						
(1)	82			79		
6192-72 (2)	87			80		
1052-50 (3)	79			77		
3175-67 (4)	88			79	0.0	77
1751-70 (5)	94			84		
3467-56 (6)	83	0.0	89	81	0.0	81
(7)	83	2.0	82	87	1.5	74
(8)	88			79		
(9)	91			84		
(10)	86			81		
3962-53 (11)	87		83	78	0.5	77
6081-72 (12)	87			81		
(13)	63	7.0	42	67	8.0	41
2770-51 (14)	83			81	0.5	77
703-65 (15)	80	0.0	76	80	0.0	74
S. sonnei						
9020-75	87	0.5	80	100	0.0	100
484-74	82			91	0.0	90
2076-68				91	0.5	88
242-75				91	0.0	85
1185-75				90	0.0	90
1120-66				91	0.0	91
91-75				93	0.0	92
4560-74				86	0.0	87
1264-71				93	0.0	90
4822-66				90	0.0	88
4498-74				90	0.0	93
4446-74				93	0.0	84

TABLE 3—*Continued*

Source of unlabeled DNA[a]	Source of labeled DNA					
	S. flexneri 24570 (2a)			*S. sonnei* 9020-75		
	RBR[b] (60°C)	*D*[b]	RBR (75°C)	RBR (60°C)	*D*	RBR (75°C)
Avirulent				94	0.0	96
Virulent				93	0.0	93
Provisional *Shigella*						
4358-60	86			84	1.5	74
4388-50	84			78		
2710-54	80	1.0	84	81		
1621-54	86			79		
2243-66	82			80		
4192-74	82			83	0.0	76
3615-53	83			81		
2367-71	71	2.5	69	72	1.0	64
2387-69	89			79		
1622-54	84			78		
3341-53	82	0.5	87	78	0.0	77
3047-61	88			82	0.5	77
5518-56				88	1.0	80
E. coli						
Aberrant 2000-53	74	1.0	73	79	0.5	78
Aberrant 5216-70	83	0.5	89	87	0.0	78
Aberrant 439-55	82	0.5	83	85	0.5	84
K-12	77	0.5	75	83	1.0	76
Salmonella typhimurium LT2	42	13.0	13	45	12.5	14
Proteus mirabilis PM-1	7		4	8	13.5	3

[a] Numbers in parentheses are serotypes.
[b] See footnote *a* of Table 2 for definitions of RBR and *D*.

Shigella species, and *E. coli* by DNA relatedness. It is significant that *S. boydii* 13 was clearly separable from a reference strain of *E. coli* O28. The O antigens from *S. boydii* 13 and *E. coli* 28 showed a reciprocal cross-reaction. The fact that *S. boydii* 13 is genetically different from *E. coli* O28 shows that, despite antigenic similarity, these organisms are not the same and that aerogenic strains 603-73 and 3555-77 are in fact *S. boydii* 13 and not *E. coli* O28.

Strains 3556-77 and 3557-77 are intermediate between *E. coli* O28 and *S. boydii* 13 on the basis of phenotype. They resemble *S. boydii* 13 more than *E. coli* O28 serologically (*S. boydii* 13 and *E. coli* O28 antigens cross reciprocally). They are ornithine negative, a characteristic that is variable in *E. coli* and usually positive in *S. boydii* 13. These strains ferment xylose and maltose, characteristics typical of *E. coli*, but not of *S. boydii* 13. The acetate reaction obtained with these two strains was positive at the Centers for Disease Control and negative at the Central Public Health Laboratory. *E. coli* strains are usually acetate positive, whereas *S. boydii* are uniformly acetate negative. DNAs from both strains are more closely related to a standard *E. coli* DNA than to DNA from an *S. boydii* 13 strain. The relatedness of these strains to *S. boydii* 13 DNA (66 to 71%; 5 to 6°C decrease in thermal stability) is similar to the general level of relatedness obtained between *E. coli* and *S. boydii* 13 (62 to 74%; 4.5 to 10°C decrease in thermal stability) and lower than that seen between *S. boydii* 13 strains (89 to 94%; 0.0 to 0.5°C decrease in thermal stability) (Table 2). It seems clear that these strains are not *S. boydii* 13. Further work is necessary before we can state definitively whether they are more like *E. coli* or a *Shigella* other than *S. boydii* 13.

As expected from previous work (2, 3), *E. coli*, including typical O28 strains, and shigellae, other than *S. boydii* 13, were not distinguishable on the basis of DNA relatedness to *E. coli* K-12 (Table 2). The *S. boydii* 13 strains, although substantially related to *E. coli*, were clearly separable from it. *S. boydii* 13 is therefore the second *Shigella* serotype in which the existence of aerogenic strains has been confirmed.

With the exception of *S. boydii* 13, all previously tested shigellae were 75% or more related (2). The previous work did not, however, attempt a comprehensive study of *Shigella* sero-

types. We have now systematically studied strains of all recognized *Shigella* serotypes, as well as representatives of provisional *Shigella* serotypes and of strains intermediate between *E. coli* and *Shigella*. The results obtained with labeled *S. flexneri* and *Shigella sonnei* DNAs are shown in Table 3. These data confirm and extend the earlier observation (2) that *S. boydii* 13 is the only group within *Shigella* that is easily separable from all others on the basis of DNA relatedness, and that all other shigellae, including the aerogenic biogroup of *S. flexneri* 6 and *E. coli*, are part of the same genetic species.

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