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FERMENTATION OF RAFFINOSE BY SHIGELLAE

R. W. Reavis and W. H. Ewing

International Shigella Center, Atlanta

Communicable Disease Center
Public Health Service, U. S. Department of
Health, Education, and Welfare
Atlanta, Georgia

The purpose of this paper is to report the results of studies on the fermentation of raffinose by relatively large numbers of cultures that belonged to the four Shigella subgroups and to the Escherichia coli group. The studies reported comprised a portion of an extensive reinvestigation of the biochemical reactions given by shigellae, including evaluations of the usefulness of several newer tests and methods in taxonomy. The results of the more comprehensive studies will be published elsewhere.

A total of 1263 Shigella cultures were tested in 0.5 per cent raffinose broth containing Andrade's indicator.¹ The strains employed were submitted to the laboratory during the last several years, but the majority were recently isolated. After inoculation the raffinose broth tubes were incubated at 37° C and observed daily for two weeks, after which they were examined at intervals of 3 days until a total of 30 days had elapsed.

The results of the tests are recorded in table 1. Cultures that belonged to the Shigella dysenteriae and Shigella boydii serotypes, including several provisional serotypes, did not

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¹A mimeographed outline of biochemical methods is available upon request.

ferment raffinose, whereas the majority of Shigella flexneri, and all of Shigella sonnei strains utilized this substrate. Of 646 S. flexneri cultures tested, approximately 59 per cent fermented raffinose (20 per cent within 1 day, 39 per cent delayed) and 41 per cent did not. Only cultures of the mannitol negative variety of S. flexneri 4a and S. flexneri 6 (all biotypes) consistently failed to produce acid from this test substance. These two types constituted about 27 per cent of the 646 S. flexneri cultures tested. Of the remaining 475 S. flexneri strains, approximately 81 per cent utilized raffinose (27 per cent within 1 day, 54 per cent slowly) and 19 per cent did not.

Several S. sonnei strains were studied to determine whether their fermentation of raffinose was mutative. The cultures were inoculated onto agar plates and after incubation, 15 to 20 colonies from each plate were subcultured to individual tubes of raffinose broth. The progeny of each of the isolated colonies from the S. sonnei cultures studied produced acid from raffinose in approximately the same length of time. For example, the progeny from 18 of 19 colonies from one S. sonnei culture fermented this substance after 7 days' incubation while the nineteenth tube became acid after 8 days' incubation. The results of these limited studies indicated that fermentation of raffinose by S. sonnei strains may not be mutative in character. However, many additional S. sonnei cultures should be examined before definite conclusions are drawn. Also, S. flexneri strains should be studied in this regard.

In addition to the above-mentioned Shigella cultures, 344 E. coli strains were tested for raffinose fermentation. Seventy-six of these were aerogenic, motile biotypes and 268 were of the anaerogenic nonmotile biotypes commonly referred to as members of the Alkalescens-Dispar (A-D) group. Of the total E. coli cultures studied 58, or 17 per cent, fermented raffinose. Twenty-eight per cent of the 76 aerogenic, motile E. coli types and 14 per cent of the A-D group strains produced acid from raffinose. It was of interest to note that cultures belonging to A-D O antigen groups 1 (107 strains) and 2 (89 strains) failed to ferment raffinose.

The Shigella group or genus is differentiated from other Enterobacteriaceae by means of their biochemical reactions

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and the group is divided into four subgroups or species by means of a combination of the biochemical and serological characteristics of the types contained in each subgroup. The results of the studies reported indicated that raffinose was of little value in distinguishing members of the Shigella group from anaerogenic, nonmotile E. coli cultures in general. However, the use of raffinose may be of assistance in the differentiation of A-D O1 and O2 strains from the majority of S. flexneri cultures, since strains belonging to these commonly occurring A-D O antigen groups did not utilize raffinose while the majority of S. flexneri cultures did so. Of more importance was the fact that within the Shigella group itself, raffinose was of considerable value in the biochemical differentiation of members of subgroups B (S. flexneri) and C (S. boydii), in the delineation or characterization of the four Shigella subgroups or species, and in the determination of the subgroup to which a culture may belong.

SUMMARY

Twelve hundred sixty-three cultures that belonged to the four Shigella subgroups or species and 344 Escherichia coli strains were tested for their ability to ferment raffinose. Members of subgroups A (Shigella dysenteriae) and C (Shigella boydii) did not utilize this substance whereas the majority of subgroup B (Shigella flexneri) and all of the subgroup D (Shigella sonnei) cultures did so. The majority of the E. coli strains tested failed to produce acid from raffinose.

The results indicated that the use of raffinose as a test substrate was of particular value in the biochemical differentiation of strains belonging to the S. flexneri and S. boydii subgroups and in the delineation or characterization of the four Shigella subgroups or species.

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Table I. Fermentation of Raffinose by Shigellae

Subgroup		No. Tested	Positive		Positive Delayed	Negative
			1 day			
A						
<u>S. dysenteriae</u>	1	42	0	0		42
	2	83	0	0		83
	3	30	0	0		30
	4	12	0	0		12
	5	11	0	0		11
	6	2	0	0		2
	7	7	0	0		7
	8	13	0	0		13
Serotype	58	4	0	0		4
Serotype	3873-50	2	0	0		2
Serotype	2050-52	3	0	0		3
Total		209	0	0		209
B						
<u>S. flexneri</u>	1a	26	4	21 (3 to 5 days)		1
	1b	51	2	47 (2 to 14, majority in 2 days)		2
	2a	99	4	73 (2 to 28, majority in 2 or 3 days)		22
	2b	24	0	21 (3 to 12, majority in 4 or 5 days)		3
	3	98	64	20 (2 to 19, majority in 2 to 5 days)		14
	4a (+)	75	44	4 (2 to 3 days)		27
	4a (-)	36	0	0		36
	4b	16	4	11 (2 to 8, majority in 2 days)		1
	5	35	2	26 (2 to 10, majority in 3 or 4 days)		7
	6*	141	0	0		141
	X var.	8	2	5 (3 to 5 days)		1
	Y var.	30	4	20 (4 to 25, majority in 2 days)		6
	XY var.	7	0	6 (4 to 25, majority in 4 days)		1
Total		646	130	254		262
C						
<u>S. boydii</u>	1	18	0	0		18
	2	32	0	0		32
	3	8	0	0		8
	4	66	0	0		66
	5	5	0	0		5
	6	4	0	0		4
	7	41	0	0		41
	8	10	0	0		10
	9	10	0	0		10
	10	17	0	0		17
	11	34	0	0		34
Serotype	123	4	0	0		4
Serotype	425	10	0	0		10
Serotype	703	7	0	0		7
Serotype	2770-51	7	0	0		7
Total		273	0	0		273
D						
<u>S. sonnei</u>	135	1	134 (2 to 10 days, majority in 5 days)			0

(+) Mannitol positive variety

(-) Mannitol negative variety

* Includes all known biotypes