Lactose-Fermenting *Salmonella* from Dried Milk and Milk-Drying Plants

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A study of 552 salmonella cultures revealed that 86 (15.6%) of the cultures fermented lactose. These had been isolated from dried milk products and milk-drying plants. Acid and gas were produced in lactose broth. Solid media containing lactose as the key ingredient for the differential reaction were not satisfactory for recognizing salmonella colonies. No problem was encountered in selecting salmonella colonies when bismuth sulfite agar was used.

Salmonella are generally considered non-lactose-fermenting organisms. This is reflected in the definitions of the genus *Salmonella* in *Bergey's Manual of Determinative Bacteriology* (2) and by Edwards and Ewing (3). Kauffmann (12) gave a similar definition of the genus except that he included the *Arizonae*, which usually ferment lactose. In 1966 Ewing and Ball (5) reported that 3 (0.8%) of 371 unselected salmonella serotypes fermented lactose. A later report by Ewing (4) indicated that 3 (0.3%) of 787 cultures fermented lactose. Reports of lactose-positive *Salmonella* have been made by Twort (20), Kristensen (13), Kauffmann (10, 11), Seligmann and Saphra (19), Saphra and Seligmann (18), Falkow and Baron (7), McCoy (14), Gonzalez (8), Pickett and Agate (15), Poelma and Romero (16), Rokey and Mecca (17), and Hughtanen and Naghski (9). Some of these lactose-positive variants resulted from experimental processes. However, most were from natural sources.

Twort (20) and Kristensen (13) indicated that the environment of salmonella organisms may influence their ability to ferment sugars. The salmonella cultures received at the Veterinary Services Diagnostic Laboratory were isolated from dried milk and milk-drying plants. Such an environment afforded exposure to lactose in the milk. This report concerns a study of these cultures with emphasis on their ability to ferment lactose.

MATERIALS AND METHODS

Cultures. Cultures (552) were obtained from laboratories of the Agricultural Marketing Service of the U. S. Department of Agriculture. They were received over a period of approximately 3 years and were isolates from dried milk and milk-drying plants. Approximately 175 plants were sampled during a salmonella control program.

Media. Brilliant green sulfadiazine (BGS) agar was prepared by a standardized method (1). Bismuth sulfite (BS) agar, triple sugar iron (TSI) agar, and lysine iron agar were prepared by the directions of the manufacturer (Difco). Lactose broth, dulcitol broth, malonate broth, nutrient gelatin, and Jordan tartrate agar were prepared as described by Ewing and Davis (6).

Procedures. Plates of BGS and BS were inoculated with each culture. The plates were incubated at 37 C and colonial characteristics were recorded at 24 and 48 h. One colony from each plate was inoculated into lactose broth. When lactose fermentation was indicated on BGS plates (green colonies), one of the colonies was chosen to inoculate the lactose broth. The lactose broth cultures were observed periodically for 30 days before interpreting the test as negative. All cultures that gave no indication of lactose fermentation on the BGS plates were tested for β-D-galactosidase activity (ONPG test) by using the method described by Ewing and Davis (6). Lactose-positive cultures were further examined by inoculating TSI agar, lysine iron agar, tartrate agar, nutrient gelatin, malonate broth, and dulcitol broth. The TSI and lysine iron agar slants were inoculated by stabbing the butt and streaking the slant. Lead acetate strips were placed in the tops of the TSI agar tubes as an additional indicator of hydrogen sulfide formation. All cultures were serotyped by the procedures of Edwards and Ewing (3).

RESULTS

Eighty-six (15.6%) of the 552 cultures examined were positive in lactose fermentation tests. All lactose-positive cultures produced green colonies on BGS agar, black or brown colonies with sheen on BS agar, and fermented lactose broth within 48 h. β-D-Galactosidase

activity was not detected in any lactose-negative culture.

Reactions produced in TSI agar by the lactose-positive cultures, with one exception, were atypical for *Salmonella*. Seventy-two cultures produced acid throughout the medium with gas, and 13 cultures produced acid throughout the medium with no gas. Hydrogen sulfide production was indicated by blackening in the medium by 19 cultures and by blackening of the lead acetate strips by all 86 cultures.

Reactions in lysine iron agar were typical for *Salmonella*. All of the lactose-positive cultures gave a positive reaction to the test for lysine decarboxylase. Hydrogen sulfide production in this medium was not recorded.

All of the lactose-positive cultures fermented dulcitol with the production of acid and gas and produced acid in Jordan tartrate agar. None utilized sodium malonate and none liquefied gelatin.

Thirty-one serotypes were identified from the 552 cultures (Table 1). The lactose-fermenting cultures were limited to three serotypes. *S. anatum*, *S. tennessee*, and *S. newington*. These serotypes were among the five most common types identified in the study. Lactose-fermenting strains of each of these types were found in samples from each of the 3 years. However, the percentage of these types that fermented lactose decreased each year.

DISCUSSION

The results of this study were compatible with those reported by Twort (20) and Kristensen (13). The lactose-positive strains were serotypes that appeared to be indigenous to the milk plant environment and thus exposed continually to lactose. The three serotypes involved were among the five most commonly found and were isolated during each of the 3 years. It is possible that the ability of these strains to ferment lactose was influenced by their environment.

Although the percentage of the cultures fermenting lactose was very high compared to previous statistics, it is not wise to draw conclusions from this concerning their prevalence. These cultures were from a specialized environment which could have influenced the variation. Emphasis is given to the fact that one should continually be alert to variant forms, particularly when examining source materials containing lactose.

The observed annual drop in the percentage of lactose-positive variants is interesting. However, information was not sufficient to support any definite conclusions as to the reason for this

TABLE 1. *Results of lactose fermentation tests and serotyping of 552 salmonella isolates from dried milk and milk-drying plants*

Serotype	1968		1969		1970	
	+	−	+	−	+	−
Cubana		6		67		22
Anatum	14	8	18	18	12	24
Tennessee	11	3	19	7	2	38
Montevideo		6		19		28
Newington	5	4	2	17	3	7
Senftenberg		2		5		26
Bredeney				26		3
Oranienberg		3		6		1
New brunswick		1		15		3
Worthington				5		7
Kentucky				5		7
Schwarzengrund						12
Minnesota		1				8
Infantis				6		2
Binza		2		1		2
Give		1				3
Typhimurium				2		3
Eimsbuettel		1		2		
Derby				3		
Lexington		2				
Ohio		2				
Muenchen						2
Enteritidis				2		
Siegburg				1		1
Cerro						2
Java				1		
Orion				1		
Newport				1		
Taksony						1
Heidelberg						1
Drypool						1

drop. A possible explanation could be that chronic sources of contamination were detected and eliminated.

The observed lack of evidence of hydrogen sulfide production in TSI agar further complicates the process of identification of lactose-positive *Salmonella*. The use of lead acetate strips, although giving positive results, did not appear to be a completely satisfactory answer to the problem. Lead acetate is the more sensitive indicator of hydrogen sulfide production and, therefore, the results cannot be equated to those in TSI, which is the usual standard and is less sensitive.

The results of this study indicate that bismuth sulfite agar and lysine iron agar are

especially useful in the isolation and identification of lactose-positive *Salmonella*. Probably the most important factor is to include a differential plate medium that does not key on lactose fermentation.

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