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Plating Medium for Differentiating *Salmonella arizonae* from Other Salmonellae

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A differential plating medium developed for isolation of *Salmonella arizonae* produces a uniform reaction for both lactose-negative and -positive *S. arizonae* and differentiates *S. arizonae* from other salmonellae.

Salmonella arizonae, capable of causing food-borne infections in man (3), can be easily overlooked on plating media used for detecting *Salmonella*. This is because 61.3% (2) of *S. arizonae* strains utilize lactose within 24 to 48 h, whereas almost all of the other *Salmonella* strains are incapable of using this carbon source. This large percentage of *S. arizonae* producing acid from lactose can be easily confused with lactose-fermenting microorganisms such as *Escherichia coli* and *Citrobacter* sp. Most plating media used routinely in *Salmonella* analysis use lactose utilization as an important differential characteristic. This property alone, however, is of limited value for the analysis of *S. arizonae*.

A plating medium was developed that produces a uniform reaction for both lactose-negative and positive *S. arizonae* strains and also differentiates *S. arizonae* from many other gram-negative organisms, including most other salmonellae. This medium was patterned after that used for Hektoen enteric agar (4). Salicin and lactose were deleted from the formulation, and malonate, dulcitol, and glucose were added. All other ingredients, including sucrose, were the same as in Hektoen enteric agar, except that the indicator phenol red was substituted for bromothynol blue and acid fuchsin. The formulation of *S. arizonae* agar is shown in Table 1.

The rationale for these modifications is based on differences in the ability of the two groups to utilize dulcitol, malonate, and lactose. The majority of *S. arizonae* utilize lactose and malonate but not dulcitol, whereas the majority of salmonellae use dulcitol but not lactose or malonate. The utilization of malonate by *S. arizonae* caused an alkaline reaction on the new plating medium, turning the indicator pinkish-red. Salmonellae produced acid from dulcitol, forming a yellow precipitate due to precipitation of the bile salts. Both *S. arizonae* and other salmonellae exhibited H_2S production on this medium. Some *Citrobacter* strains are also capable of H_2S production and incapable of dulcitol utilization. Such strains will be confused with *S. arizonae*. H_2S-positive *Proteus* can also be confused, since none use dulcitol. Therefore, sucrose was added to the plating medium to make it more selective for *S. arizonae*, since most strains do not use this disaccharide. The strains of *Citrobacter* and *Proteus* that utilize this sugar would give an acid reaction similar to that of salmonellae.

In preliminary studies, it was found that the addition of 0.1% glucose gave faster colonial growth, thus allowing better differentiation at 24 h. This small amount of glucose is rapidly utilized by both *S. arizonae* and other salmonellae without observable acid production.

The organisms used in evaluation of the medium were obtained from the culture collections of the Food and Drug Administration's Brooklyn laboratory and the National Center for Disease Control, Atlanta, Ga. The cultures were grown in brain heart infusion broth 24 h before testing. The medium was initially tested by streaking 24-h cultures of *S. arizonae*, salmonellae, and also strains of H_2S-positive *Citrobacter* and *Proteus* on plates, which were then incubated for 24 to 30 h at 35°C.

The appearance of typical colonies is described in Table 2. The medium readily distinguishes *S. arizonae* from other salmonellae. Some gram-negative organisms can be mistaken for *S. arizonae* on the new medium. Of particular concern are those organisms that produce H_2S and fail to use dulcitol or sucrose. Examples are certain strains of *Citrobacter*, *Proteus*, and *Pseudomonas putrefaciens*, which is the only H_2S-producing pseudomonad.

The ability of the medium to recover *S. arizonae* from foods and feeds was tested with samples inoculated with known strains of *S. arizonae* both as a pure culture and as mixed

cultures (combined with salmonellae or with salmonellae and *Citrobacter*). Twenty-five-gram samples of mushroom powder, oregano, pumpkin seeds, white pepper, fish meal, and flour were placed in 225 ml of lactose broth and inoculated with approximately 100 cells from an appropriate dilution of a 24-h brain heart infusion broth culture. The inoculated products were incubated and analyzed for *Salmonella* according to procedures of the Association of Official Analytical Chemists (AOAC) (1), except that the *S. arizonae* agar, instead of the prescribed plating media, was streaked. The isolates were confirmed as *S. arizonae* by AOAC biochemical and serological procedures. In every case, the inoculated strains of *S. arizonae* were easily recovered in the presence of other inoculated organisms and the natural flora in the product. Uninoculated controls were all negative for both *S. arizonae* and other salmonellae.

The *S. arizonae* agar medium has been included as an additional plating medium along with bismuth sulfite, brilliant green, salmonella-shigella, and Hektoen enteric agars, which are normally used for *Salmonella* analysis in this laboratory. A comparison of colonial characteristics for salmonellae and *S. arizonae* is shown in Table 3. With the media used routinely, colonies are normally picked only if they conform to that described for salmonellae or lactose-negative *S. arizonae*. With bismuth sulfite agar, lactose-positive *S. arizonae* colonies could possibly be picked; however, they cannot be distinguished from other *Salmonella* and can be missed if high numbers of other *Salmonella* are also present. The new medium was formulated specifically for *S. arizonae*, and only colonies exhibiting alkaline (red) reactions with H_2S production should be picked. The other four media should be used for the isolation of other potential *Salmonella*.

A total of 94 food and feed samples were tested (Table 4). *S. arizonae* were detected in five samples using the new medium, whereas the routine agars detected *S. arizonae* from only two of the samples. The *S. arizonae* that were missed from the other three samples using the routine agars were all lactose positive. Of the two samples from which *S. arizonae* were detected on the routine agars, one isolate was lactose negative; the other was lactose positive and was picked only from bismuth sulfite plates.

TABLE 1. *Formulation of S. arizonae agar*[a]

Ingredient	Amt (g)
Proteose peptone (Difco)	12
Yeast extract	3
Bile salts no. 3 (Difco)	9
Sucrose	12
Malonic acid disodium salt	6
Dulcitol	20
Glucose	1
Sodium chloride	5
Sodium thiosulfate	5
Ferric ammonium citrate	1.5
Agar	14
Phenol red	0.04
Distilled water	1,000 ml

[a] Do not autoclave. Add ingredients, heat to boiling, adjust pH to 7.1 to 7.2 (pinkish-red color), and pour plates as desired.

TABLE 2. *Colonial appearance on S. arizonae agar at 24 h*

Group of organisms	Colonial appearance
S. arizonae (lactose + and lactose − strains)	Pinkish-red, black centered colonies, 2–3 mm in diameter. The medium surrounding the colony is pinkish-red.
Other salmonellae	Dark-centered, yellow colonies, surrounded by a yellow precipitate. Slow dulcitol-utilizing strains give weak acid reactions at 24 h and typical reactions at 30 h.
Citrobacter and *Proteus* (H_2S + strains)	Resembles salmonellae if dulcitol or sucrose is fermented. Resembles *S. arizonae* if neither substrate is utilized.

TABLE 3. *Comparison of colonial descriptions on various plating media*

Medium	Colonial appearance		
	Salmonellae	*S. arizonae* Lac$^-$	*S. arizonae* Lac$^+$
Brilliant green	Pinkish-red	Pinkish-red	Yellow-green
Salmonella-shigella	Uncolored, black centered	Uncolored, black centered	Red, black centered
Bismuth sulfite	Black	Black	Black
Hektoen enteric	Green, black centered	Green, black centered	Salmon, dark centered
S. arizonae	Yellow, dark centered	Red, black centered	Red, black centered

TABLE 4. *Results of Salmonella analysis of food and feed samples using S. arizonae agar along with routinely used plating media*

Product	Total tested	No. positive for *S. arizonae*[a]	
		S. arizonae agar	Routine agars
Frozen frog legs	40	4	2
Nut meats	14	1	0
Frozen foods	5	0	0
Animal feeds	9	0	0
Chocolate products	7	0	0
Spices	4	0	0
Noodle products	8	0	0
Soup mixes	7	0	0
Total	94	5	2

[a] Numbers refer to samples containing *S. arizonae* as confirmed biochemically and serologically by AOAC methods.

On the basis of the work reported here, *S. arizonae* agar proved superior to the four agars routinely used in a *Salmonella* analysis for the isolation of *S. arizonae* from foods and feeds. It should be emphasized that the new medium will not serve as a replacement for the media used routinely for isolation of *Salmonella*. It will, however, serve to differentiate *S. arizonae* from the other salmonellae in those cases where this information is desired.

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