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Research Note

Modification of an Approved Medium for Fecal Coliform Detection in Seawater: A-1 Medium Minus Salicin

JOHN KAROLUS,[1]* MERCURIA CUMBO,[2] SUSAN BOEHLER,[3] AND LAURA SAVINA[4]

[1]*Connecticut State Department of Agriculture, Bureau of Aquaculture, 190 Rogers Avenue, P.O. Box 97, Milford, Connecticut 06460-0097;* [2]*Maine Department of Marine Resources, Lamoine, Maine 04605;* [3]*Massachusetts Division of Marine Fisheries, Pocasset, Massachusetts 02559; and* [4]*Massachusetts Division of Marine Fisheries, Gloucester, Massachusetts 01930, USA*

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ABSTRACT

Four hundred fifteen shellfish seawater samples from approved, conditionally approved, and restricted areas along the coastlines of Connecticut, Massachusetts, and Maine were tested in duplicate to compare results obtained with A-1 medium (AOAC official method no. 978.23) and those obtained with A-1 medium without salicin. Four laboratories used five sets of most probable number procedures to perform the analyses. No statistically significant differences between the two media were found with the *t* test, the *F* test, or the analysis of variance.

The A-1 medium (AOAC official method no. 978.23) is an acceptable bacteriological medium for the detection of fecal coliforms in seawater *(3)*. This medium, developed in 1972, contains the carbohydrate salicin, which was added because, its developers stated, it is readily used by *Escherichia* species *(2)*. Salicin is fermented by 44.5% of *Escherichia coli* strains, with fermentation being delayed by at least 2 days for 17% of these strains *(4)*. Since complete results are obtained in 24 h with A-1 medium, the strains with delayed reactions would probably not benefit from the presence of salicin. In addition, the fecal coliform group (composed primarily of *E. coli*) is defined by the American Public Health Association as facultative anaerobic, gram-negative, non–spore-forming rods that ferment lactose, with acid production and gas formation occurring within 24 h at 44.5°C *(1)*; no mention is made of salicin fermentation. However, the Food and Drug Administration (FDA) Shellfish Certified Laboratories are required to prepare A-1 medium from components instead of using commercially available dehydrated medium. Salicin is an expensive ingredient. Because of the cost of salicin and the questionability of its purpose in A-1 medium, a study was designed to compare results obtained with the AOAC formula A-1 medium and those obtained with the same medium without salicin to determine if salicin is necessary for the growth and gas production of fecal coliforms.

MATERIALS AND METHODS

Four FDA Shellfish Certified Laboratories participated in this study. These laboratories used rigorous FDA protocols for shellfish water testing. The initial tests started in June 1998 in the Connecticut laboratory. In November 1998, two laboratories in Massachusetts and one laboratory in Maine joined the project. A total of 415 samples were tested in duplicate at various times of year. Water samples used in this study were shellfish seawater samples routinely collected for each state's Shellfish Sanitation Program. Results generated in a laboratory by the AOAC A-1 method were incorporated in the shellfish water data of that laboratory's state. Therefore, every step of the study followed the strict guidelines of the National Shellfish Sanitation Program *(6)*. Water samples were collected according to approved protocols *(1)*. At least 150 ml was collected for each seawater sample. Samples were placed in an iced cooler to decrease their temperature to <10°C as quickly as possible. The samples were held below 10°C until they were tested. The laboratory analyses started within 30 h of sampling. Both types of media (with and without salicin) were prepared on the same day by the prescribed method *(3)*. The formula for single-strength A-1 medium is as follows: 5.0 g of lactose, 20.0 g of tryptose, 5.0 g of sodium chloride, 0.5 g of salicin, 1.0 ml of polyethylene glycol *p*-isoctyl-phenol ether (Triton X-100), and 1,000 ml of deionized H_2O. The medium was dispensed into culture tubes (each containing an inverted inner tube) and sterilized at 121°C for 10 min. The A-1 medium without salicin was prepared in the same manner, with the salicin being omitted from the formula. For each sample, both types of media were inoculated from the same collection bottle or dilution bottle. One medium was inoculated first, and then the second medium was inoculated. For the next sample, the order of inoculation was reversed.

Five sets of most-probable-number (MPN) procedures were carried out. The first set of procedures involved three tubes and three dilutions (10, 1, and 0.1 ml), with an MPN range of <3 to >1,100 fecal coliforms per 100 ml; the second set involved five tubes and three dilutions (1, 0.1, and 0.01 ml), with an MPN range of <1.8 to >16,000 fecal coliforms per 100 ml; the third set involved five tubes and three dilutions (5, 1, and 0.1 ml), with an MPN range of <3 to >2,400 fecal coliforms per 100 ml; the fourth set involved 12 tubes and a single dilution (1 ml), with an MPN range of <8.7 to >248 fecal coliforms per 100 ml; and the fifth set involved 12 tubes and a single dilution (5

* Author for correspondence. Tel: 203-874-0696; Fax: 203-783-9976; E-mail: dept.agric@snet.net.

TABLE 1. *Statistical comparison of A-1 medium and A-1 medium minus salicin for five sets of MPN tests*

MPN test criteria									
No. of dilutions	No. of tubes	Volume (ml)/tube	No. of samples	*t*	*t* test *P* value	*F*	*F* test *P* value	ANOVA[a]	ANOVA *P* value
1	12	5	77	−0.181	0.857	0.932	0.760	0.08	0.773
1	12	1	39	−0.380	0.705	0.904	0.753	0.14	0.705
3	5	1, 0.1, 0.01	54	−0.236	0.814	1.034	0.887	0.08	0.781
3	5	5, 1, 0.1	133	0.042	0.966	1.011	0.949	0.02	0.881
3	3	10, 1, 0.1	112	−0.408	0.684	1.019	0.922	0.02	0.656

[a] ANOVA, analysis of variance.

ml), with an MPN range of <1.8 to >50 fecal coliforms per 100 ml. Single-strength A-1 medium and A-1 medium without salicin were inoculated with 1 ml of seawater, 1½-strength media were inoculated with 5 ml, and double-strength A-1 medium was inoculated with 10 ml. Samples were incubated at 35 ± 0.5°C in an air incubator containing a circulating fan. After 3 ± 0.5 h, the samples were placed in a circulating water bath set at 44.5 ± 0.2°C. After 24 ± 2 h, the samples were observed and results were recorded. Any amount of gas production in an A-1 broth culture tube was considered a positive reaction indicating coliforms of fecal origin. MPN values were based on the dilutions used and the number of positive tubes. On the basis of these MPN values, results were derived from the appropriate tables *(1, 6)*. All numerical results were entered into an Excel worksheet and converted to logarithmic values. The data were analyzed with the Statgraphics plus 5 program (Manugistics, Rockville, Md.). The *t* test and the analysis of variance were used to compare the means between the two populations, and the *F* test was used to compare the variances. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The statistical results obtained in this study indicate that the two media were comparable in terms of means and variances; there were no statistically significant differences (Table 1). Thus, salicin was not required for the A-1 medium. Salicin is an expensive ingredient, and its elimination can save money for laboratories that use the A-1 medium. The sampling sites included approved, conditionally approved, and restricted areas and provided fecal coliforms at various concentrations. The sampling sites were statistically analyzed separately. The results of these analyses indicate that the concentration of fecal coliforms did not cause the results to differ significantly. Hunt and Springer *(5)* discussed the potential problem of geography-based differences between fecal coliform detection results obtained with A-1 medium and those obtained with EC medium, but these investigators concluded that there was good correlation between the two media despite different locations. In the present study, analyses covered the northeast section of the United States. We recommend that individual laboratories run their own comparison studies to address geographical concerns.

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