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A Research Note

Productivity of Boiled and Autoclaved Violet Red Bile Agar

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ABSTRACT

Violet Red Bile (VRB) Agar which was boiled had significantly greater productivity than did autoclaved VRB Agar when a 4-h *Escherichia coli* culture was enumerated. There was no significant difference between the boiled and autoclaved medium when a 16-h culture was examined.

The 13th Edition of *Standard Methods for the Examination of Dairy Products* (SMEDP) states in Chapter 4 that Violet Red Bile (VRB) Agar may be used after boiling for 2 min. It further states that VRB . . . . "can be autoclaved without serious impairment of productivity" (*1*).

Since the philosophy of SMEDP editors has been to recommend only one method rather than alternative procedures, and since the statement regarding preparation of VRB either by boiling or autoclaving is apparently without documentation, this study was undertaken to determine the preferable method.

MATERIALS AND METHODS

Culture

Escherichia coli ATCC 25922, used throughout the experiment, was maintained on Brain Heart Infusion slants and transferred weekly. For use, 9 ml of Nutrient Broth (Difco) was inoculated from the slant and incubated at 35 C for 20 h.

A low form culture flask containing 1 liter of Nutrient Broth, adjusted to 35 C, was then inoculated with 1 ml of the 20-h seed culture and rotated on a mechanical shaker at 100 rpm. Incubator facilities were not adequate to allow incubation of the low form flask at a temperature other than 35 C. Samples were taken after 4 and 16 h of incubation (predetermined to represent mid-log and mid-stationary phases of growth).

A known pure culture was used for this study rather than a natural source, such as raw milk, to make the procedure entirely reproduceable.

Media

VRB and Standard Plate Count (SPC) agars were both manufactured by Difco. VRB was prepared according to manufacturer's instructions and sterilized either by boiling for 2 min, as described in Chapter 4, 13th Edition SMEDP (*1*), or by autoclaving at 121 C for 15 min. SPC Agar was prepared according to manufacturer's instructions and autoclaved at 121 C for 15 min. All media were prepared fresh the day of use.

Procedure

The 4- and 16-h cultures were diluted to a working level in phosphate buffered dilution water prepared according to 13th Edition of SMEDP. The culture was plated in replicates of 20 plates each for VRB-boiled, VRB-autoclaved,a nd SPC agars. Plates were incubated at 32 C for 18-24 h and enumerated using a Quebec Darkfield Colony Counter. The plating scheme and statistical analysis were done as described in sections 4.86 and 4.87 of the 13th Edition of SMEDP.

RESULTS

The procedure was done on two separate occasions, Trial 1 and Trial 2, and results are presented in Tables 1 and 2. For each trial, the 4-h culture exhibited significantly greater yields on the VRB-boiled than on the VRB-autoclaved medium. The SPC Agar was included to give some indication of culture yield on a non-selective

TABLE 1. *Comparison of yield of VRB-boiled, VRB-autoclaved, and SPC agars when tested with* E. coli *ATCO 25922*

	4-h Culture				16-h Culture		
	VRB agar				VRB agar		
	Boiled	Autoclaved	SPC agar		Boiled	Autoclaved	SPC agar
Trial 1							
# of replicates	20	20	20		20	20	20
$\bar{X}$ plate count ($\times 10^6$)	301.4	275.85	294.7	($\times 10^8$)	48.3	46.1	49.8
Range ($\times 10^6$)	268-334	239-323	270-331	($\times 10^8$)	37-59	34-61	39.65
$\sim$	15.53	22.32	19.74		5.71	7.06	7.27
Trial 2							
# of replicates	20	20	20		20	20	20
$\bar{X}$ plate count ($\times 10^6$)	303.4	270.9	309.6	($\times 10^8$)	49.9	47.2	58.9
Range ($\times 10^6$)	253-334	227-298	285-357	($\times 10^8$)	37-63	37-59	41-66
δ	18.3	16.9	16.3		7.0	6.6	6.6

TABLE 2. *t-Values for differences in productivity between VRB-boiled, VRB-autoclaved, and SPC agars*

	Trial 1		Trial 2	
	4-h Culture	16-h Culture	4-h Culture	16-h Culture
VRB-boiled versus VRB-autoclaved	4.194[a]	2.03	5.83	1.26
SPC versus VRB-boiled	1.192	0.72	1.13	4.21
SPC versus VRB-autoclaved	2.82	1.64	7.37	5.65

[a]A t-value greater than 2.70 is significant.

medium. The VRB-boiled and SPC counts were comparable for both trials for the 4-h culture, but the difference in productivity between SPC and VRB-autoclaved agars was significant, indicating that the VRB-autoclaved medium is significantly more inhibitory to a young culture than either VRB-boiled or SPC agars.

For the 16 h mid-stationary culture, no significant differences in productivity were noted between VRB-autoclaved and VRB-boiled agars in either trial. In one trial, the SPC count was significantly greater when compared to results obtained with VRB when prepared either way, but the two VRB results were comparable.

Consequently, it appears that autoclaved VRB is significantly inhibitory to young, actively growing cultures, whereas there seems to be no effect on a more mature culture. Therefore, it is recommended that VRB agar be boiled for 2 min rather than autoclaved before use.

REFERENCE

1. Hausler, W. J., Jr. (ed). 1972. Standard methods for the examination of dairy products, 13th ed. American Public Health Association, Washington, D.C.