COMPARISON OF DIFFERENT ENRICHMENT METHODS FOR DETECTION OF *Listeria monocytogenes* FROM MILK SAMPLES*

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ABSTRACT

This study was conducted to compare the performance of different enrichment methods like the use of listeria enrichment broth (LEB), modified LEB (MLEB) and University of Vermount Medium (UVM broth) with four different concentrations of acriflavine and nalidixic acid for their ability to detect and recover L. monocytogenes from milk samples. Listeria monocytogenes (MTCC 1143) strain obtained from the Institute of Microbial Technology, Chandigarh was used in the study. The result of the present study shows that the LEB and UVMa are superior compared to other enrichment procedures used in the study followed by UVMb, MLEB, UVMc and UVMd. This study also reveals that there is no advantage in increasing the concentration of acriflavine and nalidixic acid in UVM for inhibiting the growth of contaminant bacteria.

INTRODUCTION

Listeria monocytogenes is recognized as a foodborne pathogen of major significance. This species is responsible for both sporadic and epidemic cases of listeriosis associated with a variety of foods, including meat products, raw vegetables, coleslaw, and dairy products. Currently there is a great deal of interest in culture media and methods for the selective recovery of *Listeria* spp. from food specimens, which stems from increasing human outbreaks of listeriosis (Vlaemynck *et al.,* 2000). These outbreaks emphasize the need for more effective detection and recovery methods for *Listeria monocytogenes*, especially from dairy products. The aim of this study was to compare the performance of different enrichment methods like listeria enrichment broth (LEB), modified LEB (MLEB) and University of Vermount Medium (UVM broth) with four different concentrations of acriflavine and nalidixic acid in their ability to detect and recover *L. monocytogenes* from milk samples.

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MATERIALS AND METHODS

Strain *Listeria monocytogenes* (MTCC 1143) strain obtained from the Institute of Microbial Technology, Chandigarh was used in the study.

Enrichment media LEB, MLEB and UVM broths (Himedia) were compared. The UVM broth with four different concentrations of acriflavin and nalidixic acid were subjected to the study. The concentrations used include UVMa (acriflavin 12mg/L and nalidixic acid 20mg/L), UVMb (acriflavin 18.5 mg/L and nalidixic acid 20 mg/L), UVMc (acriflavin 25mg/L and nalidixic acid 20mg/L) and UVMd (acriflavin 50 mg/L and nalidixic acid 40 mg/L). Thus, a total of six different enrichment techniques were tried.

Samples and inoculation Pasteurized milk samples screened and found negative for the

presence of *Listeria monocytogenes* were used to spike the culture. The *Listeria monocytogenes* concentration was adjusted to 1.5 x 10^3 organisms/ ml using Mac Farland standards. One milliliter of this was added to 9 ml of milk samples and was incubated at 30 °C for around 12 hrs. After this the enumeration of *Listeria monocytogenes* in this sample revealed 2.1 x 10^2 organisms/ ml of sample. One ml of these samples was then inoculated to 9 ml of all the six different types of enrichment broths and was incubated at 30 ° C for 24 hours. After this, the broth samples were serially diluted in normal saline and plated into PALCAM agar for the purpose of enumeration. The entire procedure was repeated thrice

RESULT

After the 12 hour incubation the milk sample had a total *Listeria monocytogenes* count of 2.1 x 10^2 organisms/ml. The counts obtained using different enrichment broths are given in the following table. One way ANOVA and Duncans Multiple Range Test (DMRT) were performed and it was found that no significant difference was observed between LEB and UVMa. These two broths were significantly different from the others.

Table 1. The *Listeria monocytogenes* Count obtained using different enrichment procedures.

Sl No.	Enrichment Broth	Yield In Palcam Agar Cfu/ml		
		1	2	3
1	LEB	$3.3x10^4$	$3.7 x10^4$	$3.5 x10^4$
2	MLEB	$2.5x10^4$	$2.3 x10^4$	$2.7 x10^4$
3	UVMa	$3.1x10^4$	$3 x10^4$	$3.4 x10^4$
4	UVMb	$2.8x10^4$	$3 x10^4$	$3x10^4$
5	UVMc	$2.6x10^2$	$2.7 x10^2$	$2.5 x10^2$
6	UVMd	$1.2x10^2$	$1.4 x10^2$	$1.6 x10^2$

DISCUSSION

The result of the present study shows that the LEB and UVMa are superior compared to other enrichment procedures used in the study followed by UVMb, MLEB, UVMc and UVMd. The ability of LEB and UVM in the recovery of *Listeria monocytogenes* has been reported in previous studies (Patel and Beuchat, 1995; Suh, J.H and Knabel, 2001). So, based on the results of this study, these two media can be suggested especially to recover organism from pasteurized milk, where there are competing thermoduric bacilli. As reported earlier by Salam *et al.* (2010), this study also reveals that there is no advantage in increasing the concentration of acriflavine and nalidixic acid in UVM for inhibiting the growth of contaminant bacteria.

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