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Comparison Study and Evaluation of Selective Enrichment Broth for Coliforms with Commercial Broth Media

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ABSTRACT

Background: Several bacteriological broths and solid media are available for growth of coliforms. Many are generalized and some of them works as selective. Now a days, in this category with development of chromogenic and flourogenic media, enumeration and detection have work become very easy. Despite the fact most of the countries are using approved methods like IS-5887 (Part-I) 1976 and IS-5401 Part-1 (2012) protocol for monitoring *E. coli* and coliforms in dairy products respectively. This is due to reliability of these media in giving results. Even though results obtained using these methods need confirmation as there are chances to have other organisms/contaminants in the broth or media which does not actually belong to coliforms groups. This leads to further testing to get presence of coliforms and *E. coli.*

Methods: The present investigation comprised comparison and evaluation of formulated selective coliforms broth (Gawai, 2020) with commercially available broths namely Lactose broth, Coliform broth, Tergitol-7 broth, MacConkey's broth, Violet red bile broth, Lauryl sulphate tryptose broth and Nutrient broth at specified time intervals.

Result: The growth pattern of *Escherichia coli* ATCC 25922, Coliforms cocktail and *Salmonella typhimurium* ATCC 14028 were studied in developed selective coliform broth along with seven commercial broths. It was observed that *E.coli* ATCC 25922 showed good growth in all the broth tested and highest growth was reported with Formulated broth *i.e.* 9.60 log cfu/ml and least growth was in MacConkey broth *i.e.* 8.60 log cfu/ml after 24 h of incubation. Coliform cocktail culture showed the best result in Formulated selective broth and Tergitol broth after 24 h of incubation *i.e.* 9.40 log cfu/ml while formulated coliforms broth showed the best reduction of *Salmonella typhi* ATCC 14028 in comparison with other tested broths.

Key words: Coliforms, Comparison study, *E.coli,* Growth patterns, Spiking, Selective broth.

INTRODUCTION

Several methods for determining and quantifying the presence of indicator organism particularly coliforms exist. It is classified into (1) cultural or traditional, (2) molecular and (3) enzymatic methods (Rompre *et al*. 2002; Lawaniya *et al*. 2015). Among these methods, molecular and enzymatic methods are more precise and rarely need any confirmation but cultural methods purely depends on confirmation of *E. coli* and Coliforms. Most of the approved methods either ISO or APHA are cultural based and still need to go through confirmation step. Present demand is to have such a selective broth which would be focused largely in *growth* promotion of coliforms and *E. coli* with inhibition of non coliforms.

Among the cultural methods, non-selective Violet Red Bile Agar is used for enumeration of coliforms in food and dairy products which confirms the recommendation given by American Public Health Association (APHA, 1992). The VRBA culture medium allows coliform detection and enumeration in 24 h; however, it does not allow discrimination of *E. coli* from there rest of the coliforms and needs further confirmation (APHA, 2001; Begley *et al*. 2005).

The presumptive test for *E.coli* can be done by using *E.coli* (EC) medium, violet red bile broth and Lauryl tryptose broth where after incubation, small inoculum from the test tube indicating gas formation is transferred to EC broth for confirmation. On routine basis solid medium like Violet Red Bile agar (VRBA) and most probable number are standardized methods for coliform detection (APHA, 2001; Bridson, 2006).

Selective and enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria. While selective media are agar based, enrichment media are liquid in consistency. Various approaches to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these. MacConkey's agar used for *Enterobacteriaceae* members contains Bile salt that inhibits most gram positive bacteria (Jung and Hoilat, 2021).

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Among the various available broths used in scientific community Lactose broth recommended by the American Public Health Association (APHA) is used for the detection of coliform bacteria in water, foods and dairy products. Lactose broth gives presumptive test of coliform bacteria. It specifically has peptic digest of animal tissue and beef extract to supply the essential nutrients to the organisms (APHA, 1985). It is also used in the Most Probable Number (MPN) test for water analysis and for detection of coliforms in food and dairy products (Downes and Ito, 2001; AOAC International, 2002).

Coliform broth is also recommended for isolation and cultivation of coliforms from cream yogurt and raw milk (Atlas, 2004). It contains Sodium lauryl sulphate and bile salts which is inhibitory to many gram positive organisms but not to coliforms (APHA, 1985). While detecting presence of coliforms by chromogenic methods, Tergitol-7 broth is usually chosen. It inhibits gram-positive bacteria and proteus swarming and yields better recovery of coliforms.

MacConkey broth is a modification of MacConkey medium (Allen, 2005). It contains less inhibitory bromocresol purple dye due to which lactose non-fermenting organisms like Salmonella and Shigella do not alter the appearance of the medium (British Pharmacopoeia, 2016; The United States Pharmacopoeia, 2019). Similarly, Violet red bile broth, a modification of MacConkey's original formulation (APHA, 1992; Marshall, 1992) and used to enumeration of coli-aerogens bacterial group. It relies on the use of the selective inhibitory components crystals violet and bile salts and the indicator system lactose and neutral red and it is recommended by APHA (Downes and Ito, 2001; APHA, 2015b). Along with these, Lauryl sulphate broth is also a choice for selective growth as it is designed to obtain rich growth and substantial amount of gas from small inocula of coliform organisms (APHA, 2015b). Another population broth which is non-selective and use for cultivation and enumeration of bacteria is Nutrient broth. It was originally designed for analyzing water and waste water (APHA, 2015a) and also listed in the Official Methods of Analysis of AOAC International (Horwitz, 2007; Difco and BBL Manual, 2009).

Overall scenario of various broth media applicable in analysis of milk products indicating a potential in formulating new selective enrichment broth for coliforms. The new ideal broth should have maximum prospective inhibition of non-coliforms and also lowering down incidences of false positive results as well as time saving in further testing for group or species level confirmation.

MATERIAS AND METHODS

The study under investigation was planned to compare and evaluate the Formulated selective coliforms broth. It was conducted in Department of Dairy Microbiology, SMC College of Dairy Science, Kamdhenu University, Anand in the year 2020. This selective broth for coliforms growth was developed (Gawai, 2020) by optimizing three factors while keeping some base ingredients constant at fixed level. In this developed broth addition of Sodium lauryl sulphate salt @ 0.2 g, Gentamicin sulphate + Amoxycillin (1:1 ratio) @ 10 µl and Cefsulodin @ 312.5 µl per 100 ml, with desirability of 0.92 showed strong inhibition of targeted organisms like *Salmonella typhi* ATCC 14028, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 while promoted the growth of coliforms. The final composition and photograph of the formulated selective coliform broth is given in Table 1.

The cocktail of coliform culture was prepared by mixing contents from three positive tubes of MacConkey's broth from Most Probable Number (MPN) experiment conducted on milk sample. This culture was propagated in nutrient broth medium and incubated at 37°C for 24 h and then stored at 5±2°C. Sub culturing was done at an interval of 7 days during the course of the study.

Escherichia coli ATCC 25922 and *Salmonella typhimurium* ATCC 14028, were procured from Hi-media Laboratories, Mumbai and stored at 5±2°C. These cultures were maintained by routine sub-culturing in 20 ml test tubes containing 5 ml of sterilized recommended broth and on agar slant tubes.

Formulated selective broth was compared with commercially available broths namely Lactose broth, Coliform broth, Tergitol-7 broth, MacConkey's broth, VRB broth, lauryl Sulphate Tryptose broth and Nutrient broth. Target organism was added in respective broth spiked @ 10 cells/10 ml of broth. Growth response of the Formulated selective broth and commercially available broth was compared by plating method on recommended agar media at an interval of 0, 6, 12 and 24 h at incubation temperature at 37°C. For spiking specific cells of microbes a spiking protocol suggested by Gawai *et al*. (2017) was used. The counts obtained on recommended agar media plates were measured and statistically analyzed by 2-factorial CRD.

RESULTS AND DISCUSSION

Results of comparison study of test organisms in various broth medium are mentioned below. The counts obtained

Table 1: Optimized final formulation for preparation of selective broth for coliforms.

Ingredients	Quantity per 100 ml
Bile salt	0.25 g
Sodium chloride	0.25 g
Di-sodium phosphate	0.24 g
Mono sodium phosphate	0.15 g
Tergitol	0.01 g
Yeast extract	0.30 g
Lactose	1.00 g
Sodium lauryl sulphate salt	0.25 g
Cefsulodin 10 mg/1 vial	312.5 µl
Gentamicin sulphate + Amoxycillin (1:1 ratio)	10 µl (5+5 µl)
pH adjustment	7.4 (adjusted with 0.1 N HCl)

after specific intervals were organized, log transformed and statistically compared.

Growth patterns of *Escherichia coli* ATCC 25922 and Coliforms in various broth medium

It was observed that *E.coli* ATCC 25922 showed good growth with all broths tested. Highest growth was reported with Formulated broth *i.e.* 9.60 log cfu/ml and least growth was in MacConkey broth *i.e.* 8.60 log cfu/ml after 24 h of incubation. Tergitol broth also showed good growth *i.e.* up to 9.55 log cfu/ml after 24 h of incubation (Table 2). As data reported in Table 3, Coliform cocktail culture showed best result in case of Formulated selective broth and in Tergitol broth *i.e.* 9.40 log cfu/ml after 24 h of incubation. Least growth showed in nutrient broth after 24 h of incubation *i.e.* 8.40 log cfu/ml. When compared from the average of all time intervals, Nutrient broth and Lactose broth were at par with mean value of 4.85 log cfu/ml.

Bindschedler *et al*. (1981) evaluated Brilliant green bile broth, Lauryl sulfate tryptone broth, Lactose glutamic acid broth, Enteric enrichment broth and Lactose broth for enumeration of coliforms, *E. coli* and *Enterobacteriaceae* in several varieties of dairy products (fresh and dehydrated) and dehydrated cocoa products. They reported Lactose glutamic acid broth have highest sensitivity in coliforms detection while Brilliant green bile, lactose or enteric enrichment broth were significantly less sensitive than other two broth media. They also highlighted that Lauryl sulfate broth was at par with other media and Lactose glutamic acid broth was significantly better in detection of *E. coli*.

Grabow and Preez (1979) compared the total coliform counts obtained by means of standard membrane filtration techniques, using MacConkey agar, m-Endo LES agar, Teepol agar and pads saturated with Teepol broth. They applied many combinations of treatments over 490 samples

Table 2: Growth patterns of *Escherichia coli* ATCC 25922 in selected broth media.

Broth (B)	Log cfu/ml, Period (P)				
	0 h	6 h	12 h	24 h	Broth mean
Formulated broth	1.00	4.40	8.10	9.60	5.77^{f}
MacConkey broth	1.00	3.90	6.10	8.60	4.90^{a}
Violet red bile broth	1.00	3.80	6.30	9.20	5.07^{c}
Lactose broth	1.00	3.90	7.10	8.90	5.22^{d}
Coliforms broth	1.00	4.00	6.30	9.00	5.07^{c}
Lauryl sulphate tryptose broth	1.00	3.80	6.20	9.30	5.07^{c}
Nutrient broth	1.00	4.00	6.10	9.00	5.02^{b}
Tergitol broth	1.00	4.30	8.00	9.55	5.71^{e}
Period mean	1.00^{a}	4.01^{b}	6.77^{c}	9.14^{d}	
	B		P		B×P
S.Em.	0.03		0.02		0.06
C.D. (0.05)	0.09		0.06		
0.19					
CV %	1.70				

Table 3: Growth patterns of coliform cocktail in selected broth media.

Broth (B)	Log cfu/ml, Period (P)				
	0 h	6 h	12 h	24 h	Broth mean
Formulated broth	1.00	4.80	8.00	9.40	5.80^{g}
MacConkey broth	1.00	3.90	6.30	8.70	4.97^{b}
Violet red bile broth	1.00	4.50	6.20	8.90	5.15^{d}
Lactose broth	1.00	4.10	5.80	8.50	4.85^{a}
Coliforms broth	1.00	4.20	6.10	9.00	5.07^{c}
Lauryl sulphate tryptose broth	1.00	4.40	6.20	9.25	5.21^{e}
Nutrient broth	1.00	4.10	5.90	8.40	4.85^{a}
Tergitol broth	1.00	4.70	7.90	9.40	5.75^{f}
Period Mean	1.00^{a}	4.33^{b}	6.55^{c}	8.94^{d}	
	B		P		B×P
S.Em.	0.03		0.02		0.06
C.D. (0.05)	0.08		0.06		0.17
CV %	1.51				

Table 4: Growth patterns of *Salmonella typhi* ATCC 14028 in selected broth media.

Broth (B)	Log cfu/ml, Period (P)				
	0 h	6 h	12 h	24 h	Broth mean
Formulated broth	1.00	1.30	1.70	2.10	1.52[a]
MacConkey broth	1.00	3.50	6.80	8.20	4.87[b]
Violet red bile broth	1.00	4.90	6.90	9.30	5.52[f]
Lactose broth	1.00	4.20	7.20	9.20	5.40[e]
Coliforms broth	1.00	4.50	6.00	7.90	4.85[b]
Lauryl sulphate tryptose broth	1.00	4.10	6.80	9.20	5.27[d]
Nutrient broth	1.00	5.00	6.30	8.10	5.10[c]
Tergitol broth	1.00	4.10	7.40	8.20	5.17[c]
Period Mean	1.00[a]	3.95[b]	6.13[c]	7.77[d]	
		B	P		B×P
S.Em.		0.03	0.02		0.09
C.D. (0.05)		0.09	0.06		0.18
CV %			2.40		

of river water and city wastewater after different stages of conventional purification and reclamation processes. It was found that Endo agar generated the highest average counts for all tested samples. Teepol agar had reported higher counts in MacConkey agar. Among 871 positive isolates *Aeromonas hydrophila* was the most commonly detected while species of *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter* represented 55% of isolates. In another study, Chen and Wu (1992) found that recovery of control or freeze stressed *Escherichia coli* on faecal coliform agar (FCA) was about 1 log cycle lower than that with nonselective media. A similar comparison study on the same line of work under present investigation was done by Lawaniya *et al.* (2015). They evaluated recovery of different strains of *E. coli* on different selective enrichment media like Violet red bile agar (VRBA), Eosin methylene blue (EMB), Tergitol-7 (T-7), Difco MI broth and MacConkey broth. They spiked targeted organisms both in broth and milk and reported that the efficacy of T-7 medium was better in *E. coli* detection over medium like Violet red bile broth (VRBB), Eosin methylene blue (EMB) and MacConkey broth. This study also highlighted that T-7 broth could inhibited the background microflora to a more extent and concurrently exhibited good recovery of target organism.

Growth patterns of *Salmonella typhi* ATCC 14028 in various broth medium

Formulated coliforms broth showed best reduction of *Salmonella typhi* ATCC 14028 as reported in Table 4 which was 2.1 log cfu/ml after 24 h of incubation. Rest of all broths did not show strong reduction of *Salmonella typhi* ATCC 14028 at all studied time intervals. Maximum growth was observed in Violet red bile broth *i.e.* 9.3 log cfu/ml after 24 h indicating that VRB broth is least efficient in reduction of *Salmonella typhi* ATCC 14028. Similarly Tergitol broth and Nutrient broth were non-significant in their performance in all time intervals with broth means of 5.10 and 5.17 respectively.

Growth patterns study on five commercial media was performed by Lawaniya *et al.* (2015). They spiked *Salmonella abony* NCTC 6017 @ 1.28 log cfu/ml different broth medium and observed that its counts in Tergitol-7 increased up to 7.87 log cfu/ml, while reported 8.95, 9.10, 8.97 and 8.23 log cfu/ml on EMB, VRBB, Difco MI and MacConkey broth respectively after 24 h of incubation. In their study, they reported growth as well as inhibitory behavior of *S. flexneri*, *Y. enterocolitica* and *S. marcescens* on these commercial media. In our case, results for inhibition of *Salmonella typhi* ATCC 14028 was much better, showed marked reduction in Formulated selective broth (Table 4).

Lawaniya (2014) studied the growth pattern in *E.coli* Selective Medium (EC-SM) and T-7 by spiking a log *i.e.* 10 cells of *S. aureus* in sterilized skim milk. The partial inhibition of *S. aureus* growth was achieved up to 4.88 log cells/mL after 12.0 h of exposer in the developed broth. This indicated the bacteriostatic action in EC-SM broth. In Tergitol-7 broth, fair growth of *S. aureus* was observed. It was 2.58 log cfu/ml after 12.0 h on incubation indicating that T-7 broth also not able to strongly inhibit *S. aureus* populations. Along with this in same work, Lawaniya (2014) studied the growth pattern of *E. faecalis*. He observed that maximal growth inhibition for *E. faecalis* was achieved up to 5.15 log cfu/mL in EC-SM, as the reduction was observed up to 3.32 log cfu/mL after 12.0 h of incubation. Whereas, in Tergitol-7 broth the growth inhibition for *E. faecalis* was up to 2.26 log cfu/mL after 12.0 h of incubation. These results were in accordance with the study under present investigation.

Present study made clear that Formulated selective coliforms broth is more selective for *E.coli* and coliforms and do not support growth of test pathogens. Performance wise, Tergitol-7 broth is also good medium which showed promising and comparable results with Formulated selective coliforms broth.

CONCLUSION

The growth pattern study of *Escherichia coli* ATCC 25922, Coliforms cocktail and *Salmonella typhimurium* ATCC 14028 on eight commercial broth media revealed that Formulated coliform broth and Tergitol broth promoted the growth of *E.coli* ATCC 25922 and Coliforms. However, Formulated coliforms

broth showed better inhibition of *Salmonella typhi* ATCC 14028, *i.e.* 2.10 log cfu/ml after 24 hrs of incubation which the best when compared with other tested commercial broths.

Commercial broths which are generally being used for growth of coliforms in water, dairy and food products analysis and most of these are recommended by the recognized scientific bodies. Performance was Tergitol broth was more or less similar with Formulated broth. Both these could act as best alternative when focus is on detection of true lactose fermenters *i.e.* coliforms. Furthermore for developing methods like enzyme substrate based chromogenic or flourogenic, this formulated selective coliform broth could be the best as these methods require homogeneous population.

Conflict of interest: None.

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