Veterinary World, EISSN: 2231-0916
Available at www.veterinaryworld.org/Vol.7/March-2014/12.pdf

RESEARCH ARTICLE
Open Access

Evaluation of various cultural enrichment methods for the detection of selected food borne bacterial pathogens

C. Latha, B. Sunil, V. J. Ajay Kumar, C. J. Anu and J. Deepa

Department of Veterinary Public Health
College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Mannuthy, Kerala, India
Corresponding author: C. Latha, email: latha@kvasu.ac.in
Received: 01-01-2014, Revised: 27-02-2014, Accepted: 03-03-2014, Published online: 26-03-2014

doi: 10.14202/vetworld.2014.172-176
How to cite this article: Latha C, Sunil B, Ajay Kumar VJ, Anu CJ and Deepa J (2014) Evaluation of various cultural enrichment methods for the detection of selected food borne bacterial pathogens, *Veterinary World* 7(3): 172-176.

Abstract

Aim: The study was conducted to evaluate the performance of different enrichment broths such as Tryptic Soy Yeast extract Broth (TSBYE), Brain Heart Infusion broth (BHI), Nutrient Broth (NB), Luria Broth (LB) and Peptone water (PW) for the detection of *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus* and S*almonella enterica* Typhimurium.

Materials and Methods: The bacterial strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh. Growth of these food borne pathogens at two different incubation temperatures (35^{0}C and 37^{0}C) and three different incubation periods (12h., 16h. and 18h.) were studied.

Results: The result of the study showed that enrichment in Tryptic soy broth with yeast extract (TSBYE) and incubation at 37^{0}C for 18h. is superior for the enrichment of all the organisms under study.

Conclusion: TSBYE can be used very effectively as universal enrichment broth in comparison with all other enrichment broths studied for the detection of *L. monocytogenes*, *Y. enterocolitica*, *S. aureus* and S. *enterica* Typhimurium.

Keywords: broth, enrichment, foodborne pathogen, *L. monocytogenes*, *S. aureus*, *S.enterica* Typhimurium, *Y. enterocolitica.*

Introduction

Surveillance of food borne diseases is of utmost priority in the public health agenda worldwide. As per World Health Organization report [1] food borne diseases are responsible for high level of morbidity and mortality among the public. *Listeria monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus* and S*almonella enterica* Typhimurium are agents of major concern because of their association with popular foods such as meat and meat products, dairy products, fruits and vegetables.

Listeria monocytogenes is an emerging food borne pathogen responsible for both sporadic and epidemic cases of Listeriosis associated with a variety of foods including meat products [2]. *Listeria monocytogenes* is responsible for the highest hospitalization rates (91%) amongst all known food borne pathogens worldwide [3]. *Yersinia enterocolitica* is an important food and water borne pathogen causing a variety of gastrointestinal problems such as diarrhoea, abdominal pain and pseudo appendicitis [4, 5]. There is a strong evidence that foods of animal origin especially pork and dairy products are responsible for *Y. enterocolitica* infections in humans [6].

Staphylococcus aureus is one of the most common agents of food poisoning outbreaks with enhanced pathogenicity due to the presence of enterotoxins [7, 8]. According to CDC report [9], it is responsible for 2,41,148 food borne illnesses worldwide. Contamination of food with staphylococci can occur directly from infected food producing animal or at any stages of food production, processing, transportation, storage or retailing [10]. Salmonellosis is recognized as a global zoonosis and food borne disease posing public health risk. It is the most widespread disease in both developed and developing countries and contributes to high morbidity and economic loss [11]. Salmonella infections have been associated with the consumption of raw and under cooked meat products [12].

Reliable detection techniques are a prerequisite for the detection of these pathogenic bacteria in food and food processing plants. Because the conventional culturing technique for detecting pathogens is time consuming, results are frequently not available until the food has been either released to the market or consumed, thus increasing the risk of transmission of pathogens [13].

Though sensitivity of many modern detection methods such as PCR have improved significantly, enrichment protocol is necessary to improve detection efficiency and to avoid false results because pathogens are often present in very low numbers in food samples rendering the recovery of target organisms difficult [14]. Development of multi pathogen detection in a single assay not only reduces the cost for testing but also provides data on the presence of different pathogens in a single experiment. Furthermore, multi-pathogen detection is a rational approach since many foods such as milk and milk products, meat and poultry,

Table-1. Growth of selected bacterial food borne pathogens at various incubation temperatures after 18h incubation in various broths

Incubation temperature	Media	Mean bacterial counts (Log10cfu/ml)± SE			
		L. monocytogenes	*Y. enterocolitica*	*S. aureus*	*S. enterica* Typhimurium
35 °C	TSBYE	9.3±0.04^{b}	8.3±0.02^{bc}	9.27±0.08^{b}	9.3±0.20^{cd}
	BHI	8.3±0.03^{c}	8.3±0.07^{bc}	7.6±0.13^{cd}	8.4±0.14^{e}
	NB	7.6±0.19^{de}	7.8±0.08^{cd}	7.0±0.10^{d}	8.3±0.09^{ef}
	LB	7.4±0.12^{e}	7.3±0.02^{de}	7.2±0.10^{d}	8.1±0.03^{ef}
	PW	6.7±0.16^{f}	6.9±0.04^{e}	7.1±0.08^{d}	7.8±0.25^{f}
37 °C	TSBYE	10.3±0.27^{a}	9.5±0.20^{a}	11.0±0.21^{a}	10.4±0.14^{a}
	BHI	8.6±0.20^{bc}	8.6±0.40^{b}	9.2±0.08^{b}	10.1±0.06^{ab}
	NB	8.3±0.22^{c}	8.2±0.27^{bc}	8.9±0.36^{b}	9.7±0.39^{bc}
	LB	8.2±0.46^{cd}	7.8±0.36^{cd}	7.9±.36^{c}	8.1±0.09^{ef}
	PW	6.6±0.22^{f}	7.7±0.37^{cd}	8.1±0.11^{c}	8.9±0.19^{d}

Values with different superscripts in the same column differ significantly, $p<0.05$.

fruits and vegetables are common carriers of these food borne pathogens [15]. Hence, the present study was carried out to evaluate different enrichment techniques for the isolation of *L. monocytogenes*, *Y. enterocolitica*, *S. aureus* and *S. enterica* Typhimurium.

Materials and Methods

Bacterial strains: The reference strains of bacterial pathogens, *L. monocytogenes* (MTCC 1143), *Y. enterocolitica* (MTCC 3234), *S. aureus* (MTCC 1144) and *S. enterica* Typhimurium (MTCC 98) were procured from Microbial type culture collection and Gene bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. Maintenance of pure cultures was carried out by regular sub culturing onto Nutrient Agar slants at 25 days interval.

Comparison of different enrichment methods for isolation: Five different enrichment broths including Tryptic Soy Yeast extract Broth (TSBYE), Brain Heart Infusion broth (BHI), Nutrient Broth (NB), Luria Broth (LB) and Peptone water (PW), were used for the isolation of *L. monocytogenes*, *Y. enterocolitica*, *S. aureus* and *S. enterica* Typhimurium, respectively. A loopful of individual bacterial culture was inoculated into five different broths. Two different incubation temperatures (35^{0}C and 37^{0}C) and three different incubation time (12h., 16h. and 18h.) were studied. The inoculated tubes were incubated at different incubation conditions. Duplicate tubes were used for each enrichment condition. After the period of incubation, optical density (OD) was measured using spectrophotometer at 600nm (Perkin Elmer, Lambda 25).

Isolation and tdentification of organisms: For the isolation and identification of organisms, serial dilution was made and selected dilutions were plated onto selective agar plates. For the isolation of *L. monocytogenes*, 0.1 ml of inoculum from selected dilution was transferred to Polymyxin- Acriflavin-Lithium Chloride- Ceftazidime- Aesculin- Mannitol (PALCAM) agar plates and incubated at 37^{0}C for 48 h. After incubation, colonies with graygreen colour with a sunken centre and halo were counted. Yersinia identification agar was used for the isolation of *Y. enterocolitica*. After incubation at 37^{0}C for 24 h., red bull's eye colonies were counted. For the isolation of *S.aureus*, Baird Parker (BP) agar plates were used and incubated at 37^{0}C for 48 h. After incubation, grey black to jet black colonies with light coloured margin surrounded by an opaque zone were counted. From selected dilution, 0.1 ml of the inoculum was transferred to Brilliant Green Sulpha agar plates for isolating *S. enterica* Typhimurium and incubated at 37^{0}C for 24 h. After incubation, pink coloured colonies were counted [16]. Selected colonies were then subjected to a series of biochemical tests for confirmation. The entire protocol was repeated for six times.

Statistical analysis: All data were analysed by ANOVA and Duncan's multiple range test (DMRT) using SPSS, Version 22.0.

Results

The growth of individual organisms in different broths at different incubation conditions were analyzed after the period of incubation. The optical density was measured using Spectrophotometer at 600nm. The counts obtained against each OD values were compared as per McFarland Standards [17].

The mean OD values of different organisms after incubation at 12h., 16h.and 18h. are given in Figures 1-4. Repeated measures two factor ANOVA was carried out for comparing OD values of different broths in different periods at 37^{0}C. Results showed that the OD values of different broths significantly increased from 12h. to 18h ($p \leq 0.01$). A highly significant difference was observed in OD values between all enrichment broths and enrichment conditions ($p \leq 0.01$).

The mean counts of individual organisms at two different incubation temperatures, *viz.* 35^{0}C and 37^{0}C are shown in Table-1. Lowest mean count was observed for all organisms at 35^{0}C in PW except for *S. aureus*. At 37^{0}C, lowest count was observed for *L. monocytogenes* and *Y. enterocolitica* in PW and in LB for *S. aureus* and *S. enterica* Typhimurium.

A significant difference in mean count was found

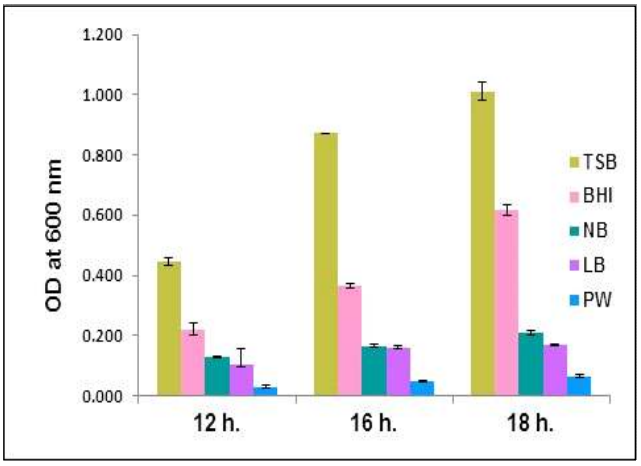

Figure-1: OD values of *L. monocytogenes* in different broths

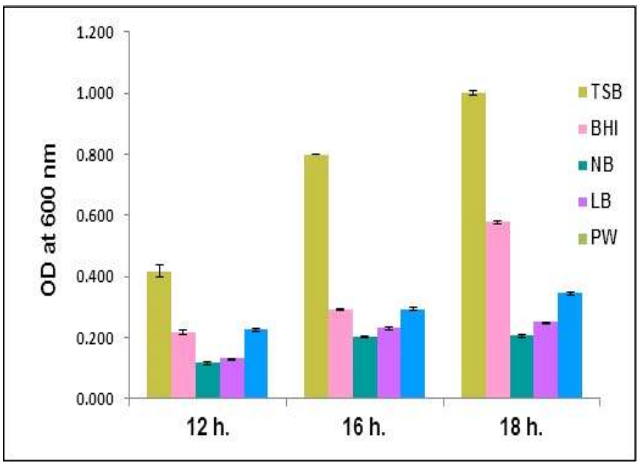

Figure-2: OD values of *Y. enterocolitica* in different broths

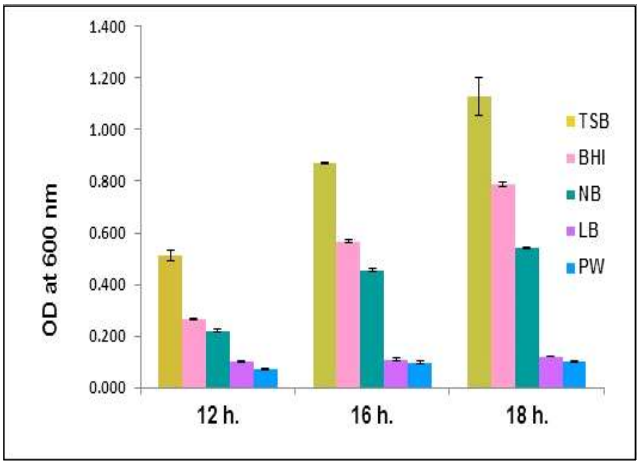

Figure-3: OD values of *S. aureus* in different broths

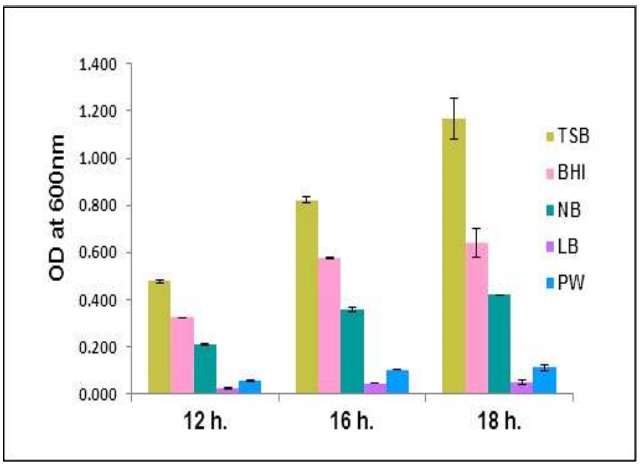

Figure-4: OD values of *S. enterica* Typhimurium in different broths

Table-2. Growth of different organisms at different incubation periods at 37°C update as before

Incubation time	Media	Mean bacterial counts (Log10cfu/ml)± SE			
		L. monocytogenes	*Y. enterocolitica*	*S. aureus*	*S. enterica* Typhimurium
12h.	TSBYE	6.89±0.29^{de}	6.49± 0.02^{fg}	7.069±0.03^{f}	6.85±0.05^{g}
	BHI	6.40±0.19^{ef}	5.99±0.06^{gh}	6.82±0.11^{f}	6.46±0.02^{j}
	NB	5.93±0.33^{fg}	5.80±0.16^{h}	4.57±0.16^{h}	5.89±0.03^{k}
	LB	5.69±0.26^{g}	5.35±0.13^{h}	6.64±0.14^{f}	5.73±0.22^{k}
	PW	5.29±0.09^{g}	5.80±0.12^{h}	5.21±0.10^{g}	5.4±0.32^{l}
16h.	TSBYE	9.17±0.11^{b}	8.17±0.06^{c}	9.26±0.10^{b}	9.26±0.07^{d}
	BHI	8.60±0.17^{bc}	6.88±0.14^{ef}	8.64±0.17^{c}	8.80±0.02^{e}
	NB	8.40±0.19^{c}	7.41±0.22^{de}	7.83±0.27^{e}	8.39±0.08^{f}
	LB	7.32±0.07^{d}	6.65±0.37^{f}	7.89±0.24^{e}	7.34±0.18^{h}
	PW	6.73±0.10^{de}	7.02±0.24^{ef}	5.98±0.14^{f}	6.77±0.14^{i}
18h.	TSBYE	10.03±0.18^{a}	9.28±0.1^{a}	11.09±0.20^{a}	10.37±0.08^{a}
	BHI	8.87±0.07^{bc}	8.93±0.33^{ab}	9.37±0.16^{b}	10.01±0.2^{b}
	NB	8.42±0.19^{c}	8.51±0.17^{bc}	8.57±0.15^{cd}	9.71±0.15^{c}
	LB	8.29±0.46^{c}	8.12±0.30^{c}	8.05±0.19^{e}	8.16±0.05^{g}
	PW	6.89±0.11^{de}	7.87±0.30^{cd}	8.16±0.05^{de}	8.72±0.15^{e}

Values with different superscripts in the same column differ significantly, $p<0.05$.

between TSBYE and all other enrichment broths for *L. monocytogenes*, *S. aureus* and *S. enterica* Typhimurium at 35°C, but the bacterial counts in BHI, LB, NB and PW did not differ significantly except for *L. monocytogenes*. At 37°C, no significant difference was found between all the counts in NB and BHI. All the organisms had maximum bacterial counts in TSBYE and showed significant difference from BHI except for *S. enterica* Typhimurium. Among the different temperatures studied, incubation at 37°C showed better results than 35°C and so, 37°C was selected as the incubation temperature for further study.

Table-2 represents the growth of different organi-

sms at different incubation periods viz. 12h., 16h. and 18h.at 37^{0}C. At 16 h. lowest count was observed for *L. monocytogenes*, *S. aureus* and *S. enterica* Typhimurium in PW and in LB for *Y. enterocolitica*. Highest bacterial count was found in TSBYE followed by BHI and NB after 18h. of incubation.

The results showed that variation in bacterial counts significantly increased from 12h. to 18h. After 12 h. incubation, mean counts of *L. monocytogenes* and *Y. enterocolitica* in different enrichment broths did not show any significant difference. Whereas at 18h. of incubation significant difference was found between TSBYE and other enrichment broths except for *Y. enterocolitica*.

Discussion

The study revealed that the TSBYE supported the growth of the four pathogens significantly within 18h. at 37^{0}C compared to the other enrichment broths used in the study.The use of TSBYE has a number of advantages over individual selective media used for the isolation of each pathogen in the study. Selective media are often inhibitory and fail to recover cells which may have been injured during food preservation processes [18]. In addition, some of the selective agents are expensive and toxic [19]. However, TSBYE is more economical and easy to handle as an enrichment media.

The ability of TSB in the recovery of *L. monocytogenes*, *Y.enterocolitica*, *S. aureus* and *S. enterica* Typhimurium has been reported by many workers. Kim and Bhunia [14] developed an enrichment broth (SEL) containing Tryptic soy broth, yeast extract and antimicrobial agents which are proven to support the growth of healthy and injured foodborne pathogens. The recovery of *L. monocytogenes* in TSBYE was reported by Amoako *et al* [20]. The present study revealed that TSBYE produced two fold higher cell density for *L. monocytogenes* after 18h. incubation at 37^{0}C compared to BHI followed by NB and LB. A fourfold difference was found between TSBYE and PW for the growth of the organism. Singh and Virdi [21] used TSB for the semi selective enrichment of *Y. enterocolitica*. Balakrishna *et al*. [22] also reported the use of TSB for the growth of *Y. enterocolitica*. In the study, growth of *Y. enterocolitica* in TSBYE was found significantly different from BHI, NB, LB and PW.

In the present study, a one fold significant increase in cell count was found for *S. aureus* in TSBYE compared to NB, which was in agreement with the report by Bocher *et al*. [23] who stated that the sensitivity of TSB was significantly higher compared to NB for the growth of Methicillin resistant *S. aureus*. In another study, Khueankhancharoen and Thipayarat [24] studied the growth kinetics of *salmonella* in different traditional enrichment broths such as NB, LB, BPW and TSB and reported that TSB was superior to LB and NB for the isolation of *S. enterica* Typhimurium. The log scale increase from the initial count to 5 log cfu/ml was achieved within 10 h. while in the present study, an increase of 10 log cfu/ml was achieved in TSBYE within 18 h.

Conclusion

It is evident from this study that TSBYE promoted the growth of four major food borne pathogens, *L. monocytogenes*, *Y. enterocolitica*, *S. aureus* and *S. enterica* Typhimurium, significantly. Based on the data obtained in this study, TSBYE can be used very effectively as universal enrichment broth in comparison with all other enrichment broths studied for the detection of these food-borne pathogens by all techniques including cultural and molecular methods.

Authors' contributions

CL designed the study and initiated the research. VJA and CJA carried out collection of samples, standardisation of procedure, draft and revision of manuscript. BS and JD helped in interpretation of results. All authors read and approved the final manuscript.

Acknowledgements

Kerala State Council for Science Technology and Environment, India is duly acknowledged for the financial support extended for this work.

Competing interests

The authors declare that they have no competing interests.

References

1. WHO. (2013) Report on food borne disease surveillance. http://www.who.Int/foodborne _ disease/en/html. Accessed 05-12-2013.
2. Sunil, B., Latha, C., Remya, R., Ajaykumar, V.J. and Vrinda Menon, K. (2012) Comparison of different enrichment methods for the detection of *L.monocytogenes* from milk samples. *J. Ind. Vet. Assoc., Kerala.* 10 (2):20-21.
3. Jemmi, C.T. and Stephan, R. (2006) *L. monocytogenes*: Food borne pathogen and Hygiene indicator. *Rev. Sci. Tech .Eff. Int. Epiz*. 25(2):571-580.
4. Bottone, E.J. (1999) *Yersinia enterocolitica*: Overview and epidemiologic correlates. *Microbes Infect.* 1:323-333.
5. Boghenbor, K.K., Stephen, L.W.O., Branko, K., Andreas, B., Trudy, M.W., Matthias, W., Beatrice, B.C. and Joachim, F. (2006) Genotyping of Human and Porcine *Y. enterocolitica, Y. intermedia* and *Y. bercovieri* strains from Switzerland by Amplified Fragment Length Polymorphism analysis. *Appl. Environ. Microbiol*. 72 (6): 4061-4066.
6. Kwaga, J. and Iversen, J.O. (1992) Laboratory investigation of virulence among strains of *Yersinia enterocolitica* and related species isolated from pigs and pork products. *Can. J. Microbiol.* 38: 92-97.
7. Pelliser, M.R., Klein, C.S., Ascoli, K.R., Zotti, T.R., Arisil, A.C.M. (2009) Occurrence of *Staphylococcus aureus* and multiplex PCR detection of classic enterotoxin genes in cheese and meat products. *Braz. J. Microbiol*. 40: 145-148.
8. Akineden, O., Hassan, A.A., Schneider, E., Usleber, E. (2008) Enterotoxigenic properties of *Staphylococcus aureus* isolated from goat's milk cheese. *Int. J. Food Microbiol*. 124: 211-216.
9. CDC. (2011) CDC estimates of food borne illness in United States. http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. Accessed on 21-11-2013.
10. Normanno, G., La Salandra, G., Dambrosio, A., Quaglia, N.C., Corrente, M., Parisi, A., Santagada, G., Firinu, A., Crisetti, E., Celano, G.V. (2007) Occurrence, Characteri-

zation and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and Dairy products. *Int. J. Food Microbiol*. 115: 290-296.
11. Antoine, S.T., Annaelle, K., Anne, B. (2008) Epidemiological analysis of*Salmonella enterica* from beef sampled in the slaughter house and retailersin Dakar (Senegal) using pulsed field gel electrophoresis and antibiotic susceptibility testing. *J. Food Microbiol*. 123:191-197.
12. Robles, G.M., Loredo, A., Ojeda, G., Vega, A., Chew, Y., Velarde, S., Fratamico, P. (2008) Identification of *Salmonella* serovars isolated from Cantaloupe and Chile pepper production systems in Mexico by PCR-RFLP. *J. Food Protect*.71 (11): 2217-2222.
13. Kawasaki, S., Fratamico, P.M., Horikoshi, N., Okada, Y., Takeshita, K., Sameshima, T. and Kawamoto, S. (2009) Evaluation of a multiplex PCR system for simultaneous detection of *Salmonella*, *E.coli* O157:H7, and *L. monocytogenes* in foods and in food subjected to freezing. *Foodborne Path. Dis*. 6(1):81-88.
14. Gracias, K. S., and Mc Killip, J. L. (2004) A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Can. J. Microbiol*. 50**:**883-890.
15. Kim, H. and Bhunia, A.K. (2008) SEL, a selective enrichment broth for simultaneous growth of *Salmonella enterica*, *E. coli* O157:H7, and *L.monocytogenes. Appl. Environ. Microbiol*. 74 (15): 4853-4866.
16. APHA. (2001) Compendium of methods for the Microbiological examination of foods (4thed.) Washington DC, pp 343-428.
17. Mcfarland, J. (1907) The nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *J. Am. Med. Assoc*. 14:1176-1178.
18. Busch, S.V., Donnelly, C.W. (1992) Development of a repair enrichment broth for the resuscitation of heat injured *L. monocytogenes* and *L.innocua*. *Appl. Environ. Microbiol*. 58(1): 14-20.
19. Cloak, O.M., Duffy, G., Sheridan, J.J., Blair, I.S., Mc Dowell, D.A. (1999) Isolation and detection of *Listeria* spp, *Salmonella* spp and *Yersinia* spp using a simultaneous enrichment step followed by a surface adhesion immune fluorescent technique. *J. Microbiol. Methods*. 39(1): 33-43.
20. Amoako, E.B., Syed, T., Richard, F.A. and Jim, S. (1992) Evaluation of the ability of primary selective enrichment to resuscitate heat-injured and freeze-injured *Listeria monocytogenes* cells. *Appl. Environ. Microbiol*. 58(9): 3177-3179.
21. Singh, I and Virdi, J.S. (2011) Production of Yersiniastable toxin (YST) and distribution of *yst*genes in biotype 1A strains of *Yersinia enterocolitica*. *J. Med. Microbiol*. 53:1065-1068.
22. Balakrishna, K., Murali, H.S., Batra, H.V. (2010) Cloning, expression and characterization of attachment invasion locus protein (*ail*) of *Y. enterocolitica* and its utilization in rapid detection by immunoassays. *Letters Appl. Microbiol*. 50: 131-137.
23. Bocher, S.B., Middendorf, H., Westh, A., Mellmann, K., Becker, R., Skov, R. and Friedrich, A.W. (2010) Semi-selective broth improves screening for methicillin-resistant *Staphylococcus aureus*.*J. Antimicrob. Chemother*. 65: 717–720.
24. Khueankhancharoen, J and Thipayarat, A. (2011) Application of modified drop plate technique (MDPT) and logistic model to optimize non-selective substrates for *Salmonella typhi* resuscitation. *Asian. J. Food Ag-Ind*. 4(06): 349-358.

****