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# THE COMPARISON BETWEEN DIFFERENT ENRICHMENT BROTH MEDIA AND SELECTIVE SOLID MEDIA FOR GROWING OF *Salmonella typhimurium* AND *Listeria monocytogenes*

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## ABSTRACT

The effect of different enrichment media i.e brain heart infusion (BHI broth), tryptic soy broth (TSB), nutrient broth (NB), buffered peptone water (BPW), university of Vermont medium ($UVM_1$) and Listeria enrichment broth (LEB) on the growth of *Salmonella typhimurium* and *Listeria monocytogenes* was stueid. Plating selective media as well as bismuth sulfate, brillient green and xylose lysine deoxycholate (XLD) was also evaluated for isolation of *Salmonella typhimurium*. Effect of modified buffered peptone water for growth of *Salmonella typhimurium* was studied. The influence of acriflavine and nalidixic acid on the growth of *Listeria monocytogenes* was also evaluated. The results revealed that the best enrichment medium for *Salmonella typhimurium* was TSB followed by BHI broth, while BPW was the lowest enrichment medium for *Salmonella typhimurium*. On the contrary BPW was the most suitable one for enrichment of *Listeria monocyto genes*. Bismuth sulfate was the most suitable for isolation of *Salmonella typhimurium*. Modification of dextrose or lactose content (or percent) paid to the ability increasing of BPW for enrichment of *Salmonella typhimurium*. The addition of acriflavine and nalidixic acid to TSB and BHI broth had not the ability to enrichment of *L. monocytogenes.* On the contrast unaddition acriflavine and nalidixic acid to LEB and $UVM_1$ had the ability to enrichment of *L. monocytogenes*.

**Keywords:** *Salmonella, Listeria,* enrichment , medium, ,acriflavine , nalidixic acid, modification.

## INTRODUCTION

*Salmonella typhimurium* is a rod shaped, non spore forming gram-negative bacteria. It is a facultative anaerobic belong to family Enterobacteriaceae family. The optimal growth temperature for *S. typhimurium* is 37C°. Direct plating using selective media was found to be successful in detection and isolating *Salmonella* strains (Dusch and Altwegg, 1995**)**. However the enrichment step was necessary for enhancing the detection and isolation of target pathogens (Fedorka-Cray *et al*., 1998**,** Nam *et al*., 2004**).**

*Listeria monocytogenes* is gram-positive, non-spore forming rods. It is considered a human pathogen. In adults human, its is known to cause meningitis, en cepthalitis, abscesses, and death. For *L. monocytogenes,* it has been well-documented that this organisms is virulence in vivo (Buncic *et al*., 2001**).** *L. monocytogenes* is food-borne pathogen, with a widespread occurrence in, e.g., fresh meat and poultry (Farber and Peterkin, 1991), processed ready-to-eat meats, (Johnson *et al*., 1990**),** seafood (Jorgensen and Huss, 1998), and soft-style cheese, (Loncarevic *et al*., 1998) .Several studies have evaluated the performance of different isolation methods for

their ability to detect low levels of *L. monocytogens*, as well as injured cells (Capita *et al*., 2001**;** Patel and Beuchat, 1995**;** Silk *et al*., 2002**;** Suh and Knabel, 2001). One of the most commonly used enrichment broths is the University of Vermot Medium (UVM), which contains nalidixic acid (suppresses gram-negative bacteria) and acriflavine (suppresses gram-positive bacteria) as selective supplements (Bruhn *et al*., 2005). Gracieux *et al*., (2003), concluded that virulent *L. monocytogenes* strains reached significantly higher cell count on selective agar media such as Palcam, Oxford, Rapid *L. monocytogenes* (RLM), and ALOA Listeria agar than did address any biases of these enrichment procedures.

Nutrient broth is included in a standard methods procedures for testing food, dairy products, and other materials (Vanderzant and Splittstoesser, 1992). Bacteriological Analytical Manual, (1995). The nutritionally rich formula of Brain-Heart Infusion Solids is used to grow a variety of microorganisms. The original Brain-Heart Infusion media are specified in standard methods for multiple applications (Vanderzant and Splittstoesser, 1992; Cunnif, 1995). UVM Modified Listeria enrichment Broth is a modification of the formula described by Donnelly and Baigent, (1986). This formula is used for the selective enrichment of *Listeria* spp. form food (Vanderzant and Splittstoesser, 1992; Lee and McClain, 1994) and clinical specimens (Murray *et al*., 1995). Listeria enrichment Broth, Modified is used for selectively enriching Listeria from raw and pasteurized milk according to the International Dairy Federation (IDF,1995).

Modified Oxford Medium is recommended for isolation and identifying *L. monocytogenes* from processed meat and poultry products (Lee and McClain, 1989). Oxford Medium is recommended for isolating *Listeria* from enrichment broth cultures. The most widely recognized antimicrobial agent combinations are the Oxford Medium formulation (Curtis *et al*., 1989), and the Modified Oxford Medium (Lee and McClain, 1989). Oxford Listeria Agar Base is prepared according to the formulation of Curtis *et al*. (1989).

Bismuth Sulphite Agar is a modification of the original selective medium for the isolation and preliminary identification of *Salmonella typhi* and other Salmonellae from pathological material, sewage, water supplies, food and products suspected of containing these pathogens. The use of this medium is advocated by several authorities (Anon, 1981; ICMSF, 1978; Speck, 1984).

Brilliant Green Agar was first described as a selective isolation medium for *Salmonella species* by Kristiansen *et al*., (1925). Brilliant Green Agar corresponds to the medium recommended by the APHA (1976). SS Agar was described for the isolation of *Salmonellae* and shigellae from faeces, foodstuffs and other material.

The main objective of this study was to comparison between different enrichment media and different plating selective media for enrichment and isolation of *Salmonella typhimurium* and *L. monocytogenes*,and also to evaluate the effect of acriflavine and nalidixic acid on growth of *L. monocytogenes.*

## MATERIALS AND METHODS

**Bacterial strains:**

Two pathogenic bacteria strains , *Listeria monocytogenes* and *Salmonella typhimurium* were kindly provided by Dr. Abdel-Salam. A.F., Regional Center for Food and Feed, (ARC) Egypt .

**Maintenance of isolates**

*S. typhimurium* and *L.monocytogenes* strains were maintained through monthly transfers on nutrient agar for *Slamonella typhimurium* and on trypticase soy agar (TSA) + 0.6% yeast extract (YE), oxoid Ltd, Hampshire, UK) for *L. monocytogenes*. The isolates were stored at 4ºC.

**Preparation of bacterial inoculum:**

Standard inoculum was prepared by inoculation of conical flask 100 ml volume containing 50 ml of trypticase soy broth +0.6% yeast extract (pH 7.3) with a loop of *Listeria monocytogenes*, then incubated for 24 hr at 30ºC. Another flask containing 50 ml of 1.0% buffered peptone water (pH 7.2) was inoculated with *Salmonella typhimurium* and then incubated for 24 hr at 37ºC. Cell counts were determined by serial dilution and subsequent enumeration on palcam agar for *L. monocytogenes* and Salmonella shigella agar for *Salmonella typhimurium*.

**The comparison of four pre-enrichment media for growth of *Salmonella typhimurium:***

The four enrichment media selected for evaluation, were Brain Heart Infusion (BHI) broth, trypticases soy broth +0.6% yeast extract (T.S.B.Y.E), buffered peptone water (B.P.W) and nutrient broth (NB).The pH each medium was 7.2. These media were prepared in Erlenmeyer flasks (250 ml), then inoculated with concentration of $35x10^6$ cfu/ml *Salmonella typhimurium*. The flasks were incubated at 37ºC for 24 hours on rotary shaker (100 rpm) and *Salmonella typhimurium* density was determined according to method described by Berrang *et al*., (2001).

**Evaluation of three plating media for isolation of *Salmonella typhimurium*:**

Three bacterial isolation plating media selected for evaluation, were Bismuth sulfate, Brilliant green and X.L.D. After preparing both B.P.W and TSBYE in Erlenmeyer flasks (250 ml) and inoculation with *Salmonella tyhimurium* inoculum containing $24x10^{11}$ cfu/ml, the flasks incubated at 37ºC for 24 hours on rotary shaker (100 rpm). Twenty five ml of both BPW and TSBYE were transferred into a sterile flask and mixed well with 225 ml of sterile peptone water( 0.1)% to make dilution 1:10, and *Salmonella typhimurium* density were determined onto individual Bismuth sulfate, Brilliant green and XLD plates, followed by incubation at 37ºC for 24 hours.

**Effect of modified buffered peptone water medium on growth of *Salmonella typhimurium:***

Buffered peptone water was prepared in Erlenmeyer flasks (250 ml) by addition of different concentrations of lactose and dextrose as follows:

- First flask: control without any modification (as it is),
- Second flask: containing 1.7% casein and 0.25, dextrose

- Third flask: containing 1.7% casein,
- Fourth flask containing 1.7% casein and 0.4% lactose.
- Fifth flask containing 0.4% lactose
- Sixth flask: containing 0.25% dextrose, fourth, flask containing 0.4% lactose.

All flasks were inoculated with *Salmonella typhimurium* inoculum containing $17.3x10^{11}$ cfu/ml. the flasks were incubated on rotary shaker (100 rpm)at 37ºC for 24 hours and *Salmonella typhimurium* density was determined according to method described by Berrang *et al*. (2001).

**The comparison between five enrichment media for growth of *Listeria monocytogenes* :**

Five enrichment media were performed, TSBYE, BHI broth, University of Vermont Medium ($UVM_1$), *Listeria Enrichment* broth (LEB)and Nutrient broth. These media were prepared in Erlenmeyer flasks (250 ml) and inoculated with *L. monocytogenes* inoculum containing $9x10^5$ cfu/ml, the flasks were incubated on rotary shaker (100 rpm) at 30ºC for 24 hours, and *Listeria monoctygenes* density were determined on palcam agar base according to the method described by Berrang *et al*. (2001).

**The comparison between tryptic soy broth and nutrient broth for growth of *Listeria monoctyogenes on oxford agar base*:**

This experimental was carried out with the same method mentioned before in the comparison of five enrichment media for growth of *Listerial monoctyogenes* by palcam agar base.

**Effect of acriflavine and nalidixic acid on growth of *Listeria monoctyogenes*:**

$UVM_1$ and LEB were prepared in Erlenmeyer flasks (250 ml) with addition of different concentrations of acriflavine (8, 10, 15 mg/L) with fixed the concentration of nalidixic at 0.04 mg/L, and then prepared LEB and $UVM_1$ without addition of acriflavine and nalidixic acid. Also, TSBYE and BHI broth were prepared with addition of acriflavine and nalidixic acid with the same concentration of original formulae of LEB and $UVM_1$ without changes.The media were inoculated with *L.monocytogenes* inoculum containing $3x10^2$ cfu/ml, the flasks were incubated on rotary shaker (100 rpm) and *L. monocytogenes* density was determined according to method described by Berrang *et al*. (2001).

## RESULTS AND DISCUSSION

From the results illustrated in figure (1) it is obvious that TSB was the best enrichment medium for increasing counts of *S. typhimurium* following by BHI broth where the counts increased from $35x10^6$ cfu/ml to $52x10^{10}$ and $34.0x10^{10}$ cfu/ml respectively.

On the other hand, BWP was the lowest enrichment broth medium for encourage of *Salmonella typhimurium* counts which led to increasing of *S. typhimurium* counts from $3.5x10^7$ cfu/ml to $2.5x10^9$ cfu/ml using S.S agar (selective agar medium) for enumeration.

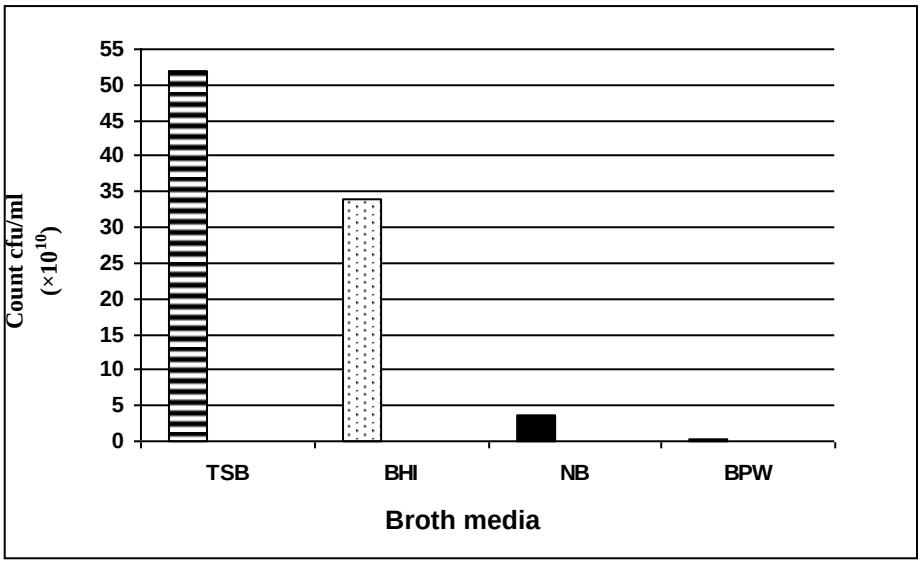



**Figure (1): Effect of four pre-enrichment media on the growth of *Salmonella typhimurium* .**
*** The use inoculation of *Salmonella typhmurium* was 35x10$^{6}$cfu/ml.**

Data in table (1) show the effect of tryptic soy broth (TSB) and buffered peptone water (BPW) on the proliferation of S. typhimurium .Results revealed that the TSB showed higher bacterial count on bismuth sulfate then other tested media followed by BPW where the counts increased from 2.4x10$^{12}$ (initial inoculum ) to 5.6x10$^{17}$ cfu/ml,1.9x10$^{17}$ cfu/ml respectively .The lowest count was recorded in the case of XLD medium using BPW as enrichment medium being 1.1x10$^{15}$ cfu/ml .It means that the TSB medium containing some nutrient and growth factors such as vitamins and amino acid which supported Salmonella growth then BPW.

**Table (1): Evaluation of three plating media for isolation of *Salmonella typhimurium* ( x10$^{16}$ cfu/ml)**.

| Medium | Total count colony forming united (cfu/ml) | | |
|---|---|---|---|
| | Bismuth sulfate | Brilliant green | XLD |
| **TSB** | 56.0 | 2.3 | 0.29 |
| **BPW** | 19.0 | 1.0 | 0.11 |

*** The use inoculation of *Salmonella typhmurium* was 24x10$^{11}$ cfu/ml.**

The obtained results in figure (2) elucidated that the modification of dextrose (0.25%) or lactose (0.4%) led to increasing the ability of BPW for enrichment of *S. typhimurium* and following by increasing of *S.typhimurium* density from 17.3x10$^{11}$ cfu/ml to 24x10$^{17}$ and 12x10$^{17}$ cfu/ml respectively.On the contrary casein or casein with dextrose or lactose caused decreasing the ability of BPW in enrichment of *S. typhimurium* and following by logarithmic decreased of *S. typhimurium* density comparison (control) using B.S agar for enumeration. 1This study was supported with those following results which

demonstrated that the importance of a suitable pre-enrichment medium for the recovery of heat-injured *Salmonella* prior to selective enrichment has been demonstrated previously (Clark and Ordal 1969**;** Edel and Kampelmacher, 1973). The difference in performance between commercial preparations of the same medium type during recovery of heat-injured cells supports the findings of Stephens *et al*. (1997). Differences in pre-enrichment media of up to 3 log 10 cycles between the worst and best medium were reported by Stephens *et al*. (1997). The influence of medium components on the recovery and survival of damaged bacteria has been reviewed previously (Harris, 1963).

Media preparation particularly autoclaving and over heating is an important aspect of culture media that is often overlooked out which may adversely affect medium performance and reliability. During the autoclaving process, auto-oxidation of phosphate buffers and sugars may potentially occur (Baylis *et al*., 2000). Selectively of enrichment conditions play determinant role in the successful recovery of Salmonellae in high but not in low moisture foods (D'Aoust *et al*., 1980; Gabis and Silliker, 1974; Silliker and Gabis, 1974). *Salmonella* isolation methodology has been evaluated in many studies (Khox *et al*., 1942; Vassiliadis *et al*., 1974, Vassiliadis *et al*., 1976, Vassiliadis *et al*., 1978; Cox *et al*., 1982; Davies and Wray, 1994; Peplow *et al*., 1999). Some research has focused on development of rapid methodologies such as polymerase chain reaction (Huang *et al*., 1999**;** Peplow *et al*., 1999), whereas, others have concentrated on improvements to conventional methods (Davies and Wary, 1994; Read *et al*., 1994; Hammack *et al*., 1999). The reasons for failure of the some enrichment media may be attributed to the composition inculusion of inhibitory substances, physical composition, or both (Skjerve and Olsvik, 1991; Davies *et al*., 2000). Enrichment broth may be toxic for some *Salmonella* strains (Patil and Parhad, 1986).

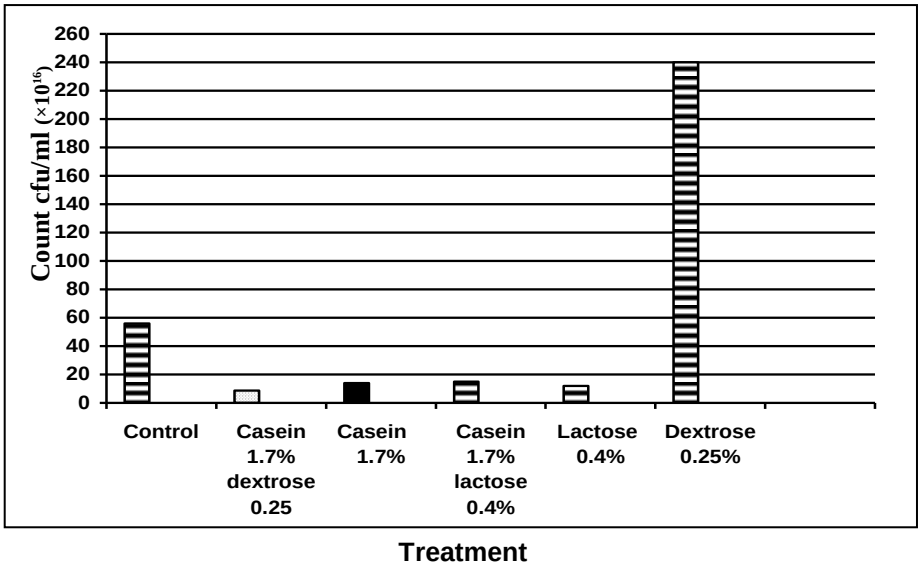
The results in figure (3) clearly showed that NB was most suitable for enrichment and cultivation of *L. monocytogenes* then followed by BHI broth such as increased *L*. *monocytogenes* counts from $9x10^5$ cfu/ml to $26x10^7$ and $5.7x10^7$ cfu/ml respectively, using palcam agar for enumeration. On the contrary both $UVM_1$ and LEB induced inactivating and controlling of *L. monocytogenes*. These results are in agreement with those recorded by Hassouba (1997).

From the summarized results recorded in Table (2) it is evident that using oxford agar for enumeration after enrichment in TSB or NB instead of palcam was most efficient selective medium than palcam agar which led to increase of *L. monocytogenes* counts from $2.5x10^6$ cfu/ml to $17x10^8$ and $47x10^8$ cfu/ml respectively, compared with increasing *L. monocytogenes* counts using palcam agar with the same enrichment broth media (TSB, NB).

**Table (2): The comparison between TSB and N.B for growth of *Listeria monoctyogenese* on oxford agar ( $x10^8$ cfu/ml)**

| Medium | TSB | N.B |
|---|---|---|
| **Count cfu/ml** | 17.0 | 47.0 |

*** The use inoculation of *L. monocytogenes* was $2.5x10^6$ cfu/ml.**




**Figure(2): Effect of modified buffered peptone water medium on growth of *Salmonella typhimurium.***

*** The use inoculation of *Salmonella typhmurium* was 17.3x10$^{11}$ cfu/ml.**

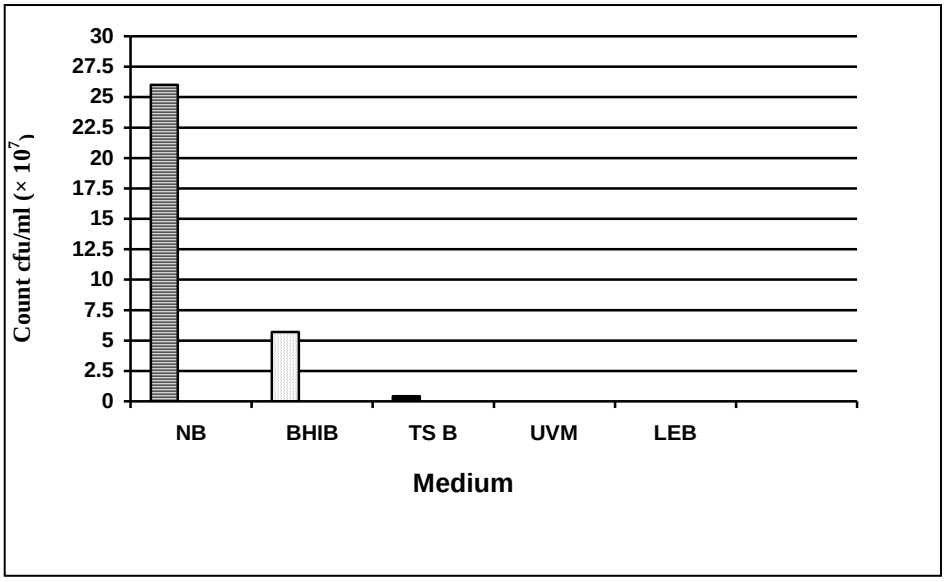



**Figure (3): The comparison between five enrichment media for growth of *Listeria monoctyogenes.***

*** The use inoculation of *L. monocytogenes* was 9x10$^{5}$ cfu/ml.**

Data in Table (3) observed that unaddition of acriflavine and nalidixic acid to LEB and $UVM_1$ paid to increaseof its ability for enrichment and cultivation of *L. monocytogenes* compared with using the same media with its concentration, traces or high of acryflavine and nalidixic acid. On the contrary the addition acriflavine and nalidixic acid to both TSB and BHI broth paid to never its ability for enrichment of *L. monocytogenes* compared with using the same media without addition acriflavine and nalidixic acid .

Nearly similar results cleared that *L. monocytogenes* strains were inhibited by acriflavine where these strains varied in their sensitivity to acriflavine ,also nalidixic acid in UVM is used to suppress the growth of gram-negative bacteria and *Bacillus spp* but has no effect on the growth of *L. monocytogenes***,** also, acriflavine is used in UVM to suppress non *Listeria* gram-positive bacteria. Acriflavine is used as the only supplement to suppress gram-positive bacteria in other *Listeria* selective enrichments medium (Silk *et al*., 2002). Many gram-positive bacteria such as lactic acid bacteria produced bacteriocins that are inhibitory against *L. monocytogenes* (Nes and Hole, 2000; Ross *et al*., 2002) and it has been shown that *L. inocua* can produce a bacteriocin(s) which inhibits *L. monocytogenes* (Yokoyama *et al*., 1998). Bacteriocin-negative lactic acid bacteria may be inhibitory to both lineage 1 and 2 *L.monocytogenes* strains and it has been suggested that this is partly due to nutrient competition and that an interaction of this type could also take place between two *L. monocytogenes* strains (Buchanan and Bagi, 1997; Nilsson *et al*., 2004). *Listeria monocytogenes* was affected by the selective agents present in UVM and LEB and the growth was inhibited compared to growth in BHI broth and TSB. This effect is in agreement with other studies (Cornu *et al*., 2002; Macdonald and Sutherland, 1994). The selective antibiotics inhibit the growth of background resident microorganisms (Kim, 2006). The ability of selective enrichment broth to resuscitate temperature-, preservate-, salt, and acid stressed cells was discussed by meny investigaters (Abdul-Raouf *et al*., 1993; Benjamin and Datta, 1995; Koutsoumanis and Sofos, 2004; Liao and Fett, 2005).

From the obtained results observed that no addition acriflavine and nalidixic to LEB and $UVM_1$ paid to increasing of its ability for growth of *L. monocytogenes.* On the contrary observed that addition acriflavine and nalidixic to T.S.B and B.H.I.B paid to never its ability for growth of *L.monoctyogenes*.

**Table (3): Effect of acriflavine (mg/L) and nalidixic acid (mg/L) on growth of *Listeria monocytogenes*.**

| Medium | LEB | | | $UVM_1$ | | | LEB | $UVM_1$ | B.H.I.B. | | B.H.I.B | | T.S.B | | T.S.B | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Acriflavine | | | | | | Without N or A | | N | A | N | A | N | A | N | A |
| | (mg/L) | | | | | | | | (mg/L) | | | | | | | |
| | 8 | 10 | 15 | 8 | 10 | 15 | | | 0.02 | 0.012 | 0.04 | 0.015 | 0.02 | 0.012 | 0.04 | 0.015 |
| Counts cfu/ml | Zero | | | | | | $72x10^5$ | $52x10^5$ | Zero | | | | | | | |

**The use inoculation of *L. monocytogenes* was $3\times10^2$.**
**A: acriflavine**
**N: nalidixic acid**

## REFERENCES

Abdul-Raouf, U.M., L.R. Beuchat and M.S. Ammar (1993). Survival and growth of *Escherichia coli* O157: H7 in ground, roasted beef as affected by pH, acidulantes and temperature. Applied Environ. Microbiol., 59: 2364-2368.

American Public Health Association, APHA, (1976). Compendium of Methods for the Microbiological Examination of Foods. Washington D.C.

Anon, 1981. International Standard ISO 6579-1981. International Organization for Standardization, Geneva.

Bacteriological Analytical Manual (1995). Association of Official Analytical Chemists. 8th Edn., BAM Inc., Gaithersburg, Maryland.

Baylis, C.L., S. Macphee and R.P. Betts (2000). Comparison of two commercial preparations of buffered peptone water for the recovery and growth of *Salmonellas bacteria* from foods. J. Applied Microbiol., 89: 501-510.

Benjamin, M.M and A.R. Datta (1995). Acid tolerance of enterohemorrhagic *Escherichia coli* Appl. Environ. Microbiol., 61: 1669-1672.

Berrang, M.E., S.R. Ladely and R.J. Buhr (2001). Presence and level of *Campylobacter, Coliform, Escherichia coli* and total aerobic bacteria recorded from broiler parts with and without skin. J. Food Prot., 64(2): 184-188.

Buchanan, R.L. and L.K. Bagi (1997). Microbial competition: Effect of culture conditions on the suppression of *Listeria monocytogenes* scott a by *Carnobacterium piscicola*. J. Food Prot., 60: 254-261.

Buncic, S., S.M. Avery, J. Rocourt and M. Dimitrijevic (2001). Can food-related environmental factors induce different behaviour in two key serovars, 4b and 1/2a, of *Listeria monocytogenes.* International Journal of Food Microbiology, 65: 201-212.

Bruhn, J.B., B.F. Vogel and L. Gram (2005). Bias in the *Listeria monocytogenes* enrichment procedure: Lineage 2 strain outcompete lineage 1 strains in University of Vermont selective enrichment. Applied Environ. Microbiol., 71: 961-967.

Capita, R., C. Alonso-Calleja, M. Prieto, M. Garcia-Fernandez and B. Moreno (2001). Comparison of PALCAM and modified oxford plating media for isolation of *Listeria*species in poultry meat following UVM II or Fraser secondary enrichment broths. Food Microbiol., 18: 555-563.

Clark, C.W and Z.J. Ordal (1969). Thermal injury and recovery of <I>*Salmonella typhimurium*</I> and its effect of enumeration procedures. Applied Microbiol., 18: 332-336.

Cornu, M., M. Kalmokoff and J.P. Flandrois (2002). Modelling the competitive growth of *Listeria monocytogenes* and *Listeria innocua* in entrichment broths. Int. J. Food Microbiol., 73: 261-274.

Cox, N.A., J.S. Bailey and J.E. Thomson (1982). Effect of various media and incubation conditions on recovery of inoculated *Salmonella* from poultry feed. Poult. Sci., 61: 1314-1321.

Cunnif, P., (1995). Official Methods of Analysis. 16th ed. AOAC International, Arlington, V.A.

Curtis, G.D.W., R.G. Mitchell, A.F. King and M.J. Griffin (1989). A selective differential medium for the isolation of *Listeria monocytogenes*. Lett. Applied Microbiol., 8: 95-98.

D'Aoust, J.Y., C. Maishmeny, D. M. Bmmgener, D.R. Conley and A. Roit (1980). Detection of *Salmonella*in refrigerated pre-enrichment and enrichment broth cultures. J. Food Prot., 43: 343-345.

Davies, P.R., P.K. Turkson, J.A. Funk, M.A. Nichols, S.R. Ladley and P.J. Fedorks-Cray (2000). Comparison of methods for isolating *Salmonella* bacteria from faeces of naturally infected pigs. J. Applied Microbiol., 89: 169-177.

Davies, R.H. and C. Wray (1994). Evaluation of a rapid cultural methods for identification of *Salmonellas* in naturally contaminated veterinary samples. J. Applied Bacteriol., 77: 237-241.

Donnelly, C.W. and G.J. Baigent (1986). Method for flow cytometric detection of *Listeria monocyogenes*in milk. Applied Environ. Microbiol., 52: 689-695.

Dusch, H. and M. Altwegg (1995). Evaluation of five new plating media for isolation of *Salmonella* species. J. Clin. Microbiol., 33: 802-804.

Edel, W. and E.H. Kampelmacher (1973). Comparative studies on the isolation of sublethally injured *Salmonella* in nine European laboratories. Bull. World Health Org., 48: 167-174.

Farber, J.M. and P.I. Peterkin (1991). *Listeria monocytogenes* a food-borne pathogen. Microbiol. Rev., 55: 476-511.

Fedorka-Cray, P.J., D.A. Dargatz, L.A. Thomas and J.T. Cray (1998). Survey of *Salmonella serotypes* in feedlot cattle. J. Food Prot., 61: 525-530.

Gabis, D.A. and J.H. Silliker, 1974. ICMSF methods studies. VI The. Influence of selective enrichment and incubation temperatures on the detection of *Salmonella* in dried foods and feeds. Can. J. Microbiol., 20: 1509-15011.

Gracieux, P., S.M. Roche, P. Pardon and P. Velge, 2003. Hypovirulent *Listeria monoctygoenes* strains are less frequently recovered than virulent strains of PALCAM and rapid'L. mono media. Int. J. Food Microbiol., 83: 133-145.

Hammack, T.S., R.M. Amaguana, G.A. June and P.S. Sherrod, 1999. Relative effectiveness of selenite cystine broth, tetrathionate broth and rappaport-vassiliadis medium for the recovery of *Salmonella spp.* From foods with a low microbial load. J. Food Prot., 62: 16-21.

Harris, N.D., 1963. The influence of the recovery medium and the incubation temperature on the survival of damaged bacteria. J. Applied Bacteriol., 26: 387-397.

Hassouba, M.M., 1997. *Listeria* species in meat, meat products and chicken giblets. M.Sc. Thesis, Faculty of Vet., Medicine, Cairo University.

Huang, H., M.M. Garcia, B.W. Brooks, K. Nielsen and S.P. Ng, 1999. Evaluation of culture enrichment procedures for use with *Salmonella* detection immunoassay. Int. J. Food Microbiol., 51: 85-94.

ICMSF, 1978. Micro-Organisms in Food 1. 2nd Ed., University of Toronto Press, Ontario.

International Dairy Federation (2005). Milk and Milk Products-Detection of *L. monocytogenes.*International Dairy Federation, Brussels, Belgium.

Johnson, J.L., M.P. Doyle and R.G. Cassens (1990). *Listeria monocytogenes* and other *Listeria spp.* in meat and meat products- a review. J. Food Prot., 53: 81-91.

Jorgensen, L.V. and H.H. Huss (1998). Prevalence and growth of *Listeria monocytogenes* in naturally contaminated seafood. Int. J. Food Microbiol., 42: 127-131.

Khox, R., P.H. Gell and M.R. Pollock (1942). Selective media for organisms of the *Salmonella* group. J. Pathol. Bacteriol., 54: 469-483.

Kim, H. (2006). A Selective Entrichment Medium for Simultaneous Growth and Detection of *Escherichia coli* O157: H7, *Listeria monocytogenes* and *Salmonella enteritidis* from Food. Purdue University, West Lafayeette, IN.

Koutsoumanis, K.P. and J.N. Sofos (2004). Comparative acid stress response of *Listeria monoctyogenes, Escherichia coli*O157:H7 and *Salmonells tphimurium* after habituation at different pH conditions. Lett. Applied Microbiol., 38: 321-326.

Kristiansen, M., V. Lester and A. Jurgens (1925). On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol., 6: 291-297.

Lee, W.H. and D. McClain (1989). Laboratory Communication. United State Department of Agiculture, Beltsville, M.D.

Lee, W.H. and D. McClain (1994). Laboratory Communication. United State Department of Agiculture, Beltsville, M.D.

Liao, C.H. and W.F. Fett (2005). Resuscitation of acid-injured *Salmonella* in entrichment broth in apple juice and on the surface of fresh-cut cucumber and apple. Lett. Applied Mcirobiol., 41: 487-492.

Loncarevic, S., E. Bannerman, J. Bille, M.L. Danilesson-Tham and W. Tham, (1998). Characterization of *Listeria strains* isolated from soft and semi-soft cheeses. Food Microbiol., 15: 521-525.

MacDonald, F. and A.D. Sutherland (1994). Important differences between the generation times of *Listeria monoctygoenes* and *Listeria innocua* in two *Listeria* enrichment broths. J. Dairy Res., 61: 433-436.

Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (1995). Manual of Clinical Microbiology. 6$^{th}$ Edn., ASM Press, Washington, D.C.

Nam, H.M., S.E. Murinda, L.T. Nguyen and S.P. Oliver (2004). Evaluation of universal pre-enrichment broth for isolation of *Salmonella spp.*, *Escherichia coli* $O_{157}$: $H_7$ and *Listeria monocytogenes* from dairy farm environmental samples food borne pathogen. Foodborne Pathogens Dis., 1: 37-44.

Nes, I.F. and H. Hole (2000). Class II antimicrobial peptides from lactic acid bacteria. Biopolymers, 55: 50-61.

Nilsson, L., Y.Y. Ng, J.N. Christiansen, B.L. Jorgensen, D. Grotinum and L. Gram (2004). The contribution of bacteriocin to inhibition of *Listeria monocytogenes* by *Carnobacterium piscicola* strains in cold-smoked salmon systems. J. Applied Microbiol., 96: 133-143.

Patel, J.R. and L.R. Beuchat (1995). Evaluation of enrichment broths for their ability to recover heat-injured *Listeria monocytogenes.* J. Applied Bacteriol., 78: 366-372.

Patil, M.D. and N.M., Parhad (1986). Growth of *Salmonellas* in different enrichment media. J. Applied Bacteriol., 61: 19-24.

Peplow, M.O., M. Correa-Prisant, M.E. Stebbins, F. Jones and P. Davies, (1999). Sensitivity, specificity and predictive values of three *Salmonella* rapid detection kits using fresh and frozen poultry environmental samples versus those of standard plating. Applied Environ. Microbial., 65: 1055-1060.

Read, S.C., R.J. Irwin, C. Poppe and J. Harris (1994). A comparison of two methods for isolation of *Salmonella* from poultry litter samples. Poult. Sci., 73: 1617-1621.

Ross, R.P., S. Morgan and C. Hill (2002). Preservation and fermentation: Past, present and future. Int. J. Food Microbiol., 79: 3-16.

Silk, T.M., T.M.T. Roth and C.W. Donnelly (2002). Comparison of growth kinetics for healthy and heat-injured *Listeria monocytogenes* in eight enrichment broths. J. Food Prot., 65: 1333-1337.

Silliker, J.H. and D.A. Gabis (1974). ICMSF methods studies. V. The influence of selective enrichment media and incubation temperatures on the detection of *Salmonellae* in raw frozen meats. Can. J. Microbiol., 20: 813-816.

Skjerve, E. and O. Olsvik (1991). Immunomagnetic separation of *Salmonella* from foods. Int. J. Food Microbiol., 14: 11-17.

Speck, M.L. (1984). Compendium of Methods for the Micro-Biological Examination of Foods. 2nd Edn., American Public Health Association, Washington D.C.

Stephens, P.J., J.A. Joynson, K.W. Davies, R. Holbrook, H.M. Lappin-Scott and T.J. Humphrey (1997). The use of an automated growth analyser to measure recovery times of single heat-injured *Salmonells* cells. J. Applied Microbiol., 83: 446-455.

Suh, J.H. and S.J. Knabel (2001). Comparison of different enrichment broths and background flora for detection of heat-injured *Listeria monocytogenes* in whole milk. J. Food Prot., 64: 30-36.

Vanderzant, C. and D.F. Splittstoesser (1992). Compendium of Methods for the Microbiological Examination of Foods. 3rd ed. American Public Health Association, Washington, D.C.

Vassiliadis, P., D. Trichopoulos, A. Kalandidi and E. Xirouchaki (1978). Isolation of *Salmonellae*from sewage with a new procedure of enrichment. J. Applied Bacteriol., 44: 233-239.

Vassiliadis, P., E. Pateraki, J. Papadakis and D. Trichopoulos (1974). Evaluation of the growth of *Salmonellae* in rappaport's broth and in muller-kauffmann's tetrathionate broth. J. Applied Bacteriol., 37: 411-418.

Vassiliadis, P., J.A. Papadakis, D. Karalis and D. Trichopoulos (1976). Enrichment in muller-kauffmanns broth and rappaports broth from buffered peptone water in the isolation of *Salmonellae* from minced meat. J. Applied Bacteriol., 40: 349-354.

Yokoyama, E., S. Maruyama and Y. Katsube (1998). Production of bacteriocin-like-substance by *Listeria innocua* against *Listeria monocytogenes.* Int. J. Food Microbiol., 40: 133-137.

## مقارنة بين بيئات الإغناء المختلفة وبيئات العزل الصلبة على نمو وعزل كلاً من ميكروب السالمونيلا والليستيريا

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**المركز الإقليمى للأغذية والأعلاف – مركز البحوث الزراعية –وزارة الزراعة**

الهدف من هذه الدراسة هو إجراء مقارنة بين بيئات الإغناء المختلفة مثل brain heart, tryptic soy broth (TSB), nutrient broth (NB), infusion (BHI broth) buffered peptone water (BPW), university of Vermont medium ($UVM_1$) Listeria enrichment broth (LEB) وكذلك بيئات العزل المتخصصة على نمو وعزل كل من *S. typhimurium, L. monocytogenes* ، كذلك دراسة التعديل فى بيئة Buffered peptone water بإضافة نسب مختلفة من سكر اللاكتوز والدكستروز و بروتين الكازين وتأثيرهم على نمو وعزل ميكروب *S. typhimirium* وأيضاً تم دراسة تأثير تواجد كل من Acriflavine والـ Nalidixic acid فى البيئة وتأثيرهم على نمو وعزل ميكروب *L. monocytogenes*.

وقد أوضحت النتائج أن أفضل بيئة إغناء لميكروب *S. typhimurium* كانت بيئة TSB يتبعها بيئة BHI بينما أقل بيئة لإغناء نفس الميكروب كانت بيئة BPW وعلى العكس كانت بيئة BPW أكثر بيئة إغناء ملائمة لإنماء وعزل ميكروب الـ *L.monocytogenes* . وكانت بيئة الـBismuth sulfate هى أكثر البيئات الصلبة ملائمة لعزل ميكروب *S. typhimurium*. وقد وجد أن التعديل فى بيئة كلاً من اللاكتوز والدكستروز أدى إلى زيادة قدرة بيئة الـBPW على إغناء ميكروب *S. typhimurium* . وأن إضافة كلاً من Acriflavine and nalidixic acid إلى كلاً من بيئة الـTSB ، الـ BHI لم يؤثر فى زيادة قدرة البيئة على إغناء ميكروب الـ *L. monocytogenes*، بينما أدى عدم اضافتهم إلى كلاً من بيئة الـLEB، الـ $UVM_1$ إلى زيادة قدرتهم على إغناء ميكروب ال *L. monocytogenes*

**قام بتحكيم البحث**

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