Turk J Vet Anim Sci
28 (2004) 753-759


Research Article

# The Use of Novobiocin and Cefixime-Tellurite in Broth, and of Cefixime-Tellurite, Salicin, Rhamnose and MUG in Agar Medium for the Detection of *Escherichia coli* 0157:H7 in Ground Beef

Murat GÜLMEZ *, Ethem BAZ, Abamüslüm GÜVEN
Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Kafkas University, Kars - TURKEY

Received: 25.02.2003

**Abstract:** We aimed to improve the selectivity of lauryl tryptose (LST) broth and HC (hemorrhagic colitis *Escherichia coli* strains) agar for the detection of *E. coli* 0157:H7 in frozen ground beef samples. The selectivity of LST broth was superior to that of LST containing novobiocin (20 mg/l) (LST + n), but LST containing novobiocin, cefixime (0.05 mg/l) and tellurite (2.5 mg/l) (LST + nct) was found to be unsuitable as an enrichment medium. The sorbitol positive strains were significantly more resistant to novobiocin than were the sorbitol negative strains ($P < 0.05$). As a result, the enrichment of ground beef samples in a selective broth without antibiotics such as LST, and then spread plating a subsequent amount from this original enrichment culture and the first three 10-fold serial dilutions onto a rhamnose and sorbitol based selective medium such as cefixime-tellurite HC medium (CTR-HC) was found to be recommendable as a routine cultural method. Transferring the metallic sheen property of eosin methylene blue (EMB) agar to CTR-HC agar can represent an alternative to the available media, and this will shorten the analysis time by 1 day. Use of salicine or 4-methylumbelliferyl-β-D glucuronide appears unnecessary in routine cultural techniques.

Key Words: *E. coli* 0157:H7, rhamnose, salicin, 4-methylumbelliferyl-β-D glucuronide

## Kıymadan *Escherichia coli* 0157:H7 Araştırmak Amacıyla Sıvı Besiyerinde Novobiyosin ve Sefiksim-Tellurit ve Katı Besiyerinde Sefiksim-Tellurit, Salisin, Ramnoz ve MUG'un Kullanımı

**Özet:** Dondurulmuş kıymalarda *Escherichia coli* 0157:H7 araştırmada kullanılan lauryl tryptose (LST) buyyon ve HC (Hemorrhagic colitis *E. coli* strains) agarın selektivitesi artırılmaya çalışıldı. LST buyyonun selektivitesi novobiyosin (20 mg/l) içeren LST (LST + n) buyyondan daha yüksek bulundu, fakat novobiyosin, sefiksim (0,05 mg/l) ve tellurit (2,5 mg/l) içeren LST (LST + nct) buyyon zenginleştirme besiyeri olarak uygun bulunmadı. Sorbitol pozitif suşlar sorbitol negatif suşlara kıyasla novobiyosine anlamlı derecede daha dirençli bulundular ($P < 0,05$). Sonuç olarak, kıymaların LST gibi saplementsiz uygun bir besiyerinde zenginleştirilmesi, ve sonra da bu kültürden ve bu kültürün ilk üç ondalık seri seyreltisinden yeteri miktarda alınarak ramnoz ve sorbitol bazlı sefiksim-telluritli HC agar gibi bir katı besiyerine ekilmesi rutin bir kültür yöntemi olarak önerilebilir nitelikte bulunmuştur. Eosin methylene blue (EMB) agarın metalik yansıma özelliği CTR-HC agara transfer edilebilinirse analiz süresini bir gün kısaltacak olan bu besiyeri mevcut besiyerlerine alternatif olabilir. Salisin veya 4-methylumbelliferyl-β-D glucuronide (MUG)'in rutin kültür yöntemlerinde kullanılması gerekli bulunmadı.

Anahtar Sözcükler: *E. coli* 0157:H7, ramnoz, salisin, 4-methylumbelliferyl-β-D glucuronide

## Introduction

*Escherichia coli* 0157:H7 is a food-borne pathogen, primarily associated with the consumption of contaminated ground beef and represents an important food safety concern worldwide (1). In order to ensure the safety and quality of its products, the food industry needs to institute rapid, simple and inexpensive screening procedures to monitor the presence of this pathogen on a routine basis (2). However, this is a continually developing field and we are still far from having a universally accepted method for this purpose (3).

Since plating samples directly onto selective agar medium is not appropriate, due to the additional complication of competing flora, an enrichment procedure is commonly needed (4-6). Lauryl tryptose (LST) broth and HC (hemorrhagic colitis *E. coli* strains) agar are 2 of the many commercially available media.

* mgulmez@hotmail.com.

Recovering low but significant levels of *E. coli* O157:H7 from foods by cultural methods is difficult due to the outgrowth of sorbitol negative, β-galactosidase negative and salicin negative colony populations other than the target organisms (1,5,7). Another problem is that sorbiol positive colonies (atypical colonies) outgrow on selective agar plates and so may mask the typical colony color of target colonies (6,8-10). Therefore, it is necessary to restreak the separated colonies onto another selective agar plate such as sorbitol MacConkey agar (SMAC) containing cefixime-tellurite and salicin (CT-SSMAC) (11). However, this procedure requires an additional 24 h incubation period. Therefore, the purpose of the present study is to evaluate the use of novobiocin and cefixime-tellurite in lauryl tryptose (LST) broth, and of cefixime-tellurite, salicin, rhamnose and 4-methylumbelliferyl-β-D glucuronide (MUG) in HC medium (hemorrhagic colitis *E. coli* strains) for the detection of *E. coli* O157:H7 in ground beef with the best suppression of the sorbitol positive colony population. It also aims at determining a simple, fast and accurate cultural isolation method.

## Materials and methods

*E. coli* O157:H7 inoculum preparation: One strain of *E. coli* O157:H7 (strain no. 937) provided by Dr. Y. Özbaş (Hacettepe University,Ankara, Turkey) and 7 other *E. coli* O157:H7 strains isolated from ground beef samples in our laboratory were used as reference strains in the experiments. Each strain was separately maintained on tryptic soy agar with 0.6% yeast extract (Difco) at 4 °C with monthly transfers and was transferred to tryptic soy broth with 0.6% yeast extract (Difco) and grown for 20 h at 37 °C before use.

**Media.** Lauryl tryptose (LST) broth (Oxoid), LST containing novobiocin (Oxoid) (LST + n) and LST containing novobiocin and cefixime-tellurite (Oxoid) (LST + nct) were used for enrichment. HC (hemorrhagic colitis *E. coli* strains) agar, HC agar containing CT (CT-HC), and HC agar containing CT, salicin (S, 5 g/l, Sigma), rhamnose (R, 5 g/l, Difco), and MUG (M, 100 mg/l, Difco) (CTSRM-HC) were used as plating medium. All media and supplements were used as stated in the Bacteriological Analytical Manual (12), except for HC and CT-HC media, which did not include MUG. The final pH of all media used in this study was adjusted to 7.0 ± 0.2.

**Sample preparations.** Twenty ground beef samples (1 kg each) were obtained during November 2002 from local retailers in Kars, Turkey, and were delivered to our laboratory in 1 h under cold storage. Each sample was hand kneaded separately in a sterile bag, and then a 100 g from each was transferred to another sterile bag and freeze stored at –20 °C for 2 months. After this freezing period, each sample was defrosted at 4 °C for 12 h and then kneaded and pummeled for 2 min with the addition of 900 ml of LST broth. After a resuscitation period at 25 °C for 2 h, each sample was filtered with cheesecloth in a sterile Erlenmayer flask. From this resuscitated culture, three 250 ml portions were transferred to other separate flasks. The test protocol is as follows:

**Resuscitation**: Static incubation at 25 °C for 2 h.

**Portioning:** (3 portions of 200 ml each)

**Supplementation**:

A (not supplemented, LST)

B (supplemented with novobiocin (20 µg/ml, LST + n),

C (supplemented with novobiocin (20 µg/ml), cefixime (0.05 µg/ml) and tellurite (0.25 µg/ml); LST + nct).

**Enrichment**: at 42 °C for 22 h.

**Selective plating:** After enrichment, samples were kept in icy water for 2 min, serially diluted using 0.85% saline, and then kept at 4 ± 2 °C during plating. The HC was spread plated from the third to the sixth 10-fold serial dilutions of each enrichment culture (50 µl from each), and CT-HC and CTSRM-HC were spread plated from the original enrichment culture and the first three 10-fold serial dilutions of each culture (50 µl).

**Colony counting and selection:** Plates were incubated aerobically at 42 °C for 18-24 h, and then the typical (colorless) and atypical colonies (yellow) grown on HC and other HC based media were separately counted.

Each reference strain was streaked onto each separate HC agar and test medium for the determination of the typical colony morphology and color pattern. To determine *E. coli* O157 in the samples analyzed, 5 typical colonies, if present, were picked from the plates of each selective enrichment/plating series (HC, CT-HC and CTSRM-HC) and streaked onto CT-HC medium to investigate both the purity and CT resistance patterns of selected colonies. The CT resistant pure colonies were

separately streaked onto EMB, salicin (S, 5 g/l) HC (SHC), rhamnose (R, 5 g/l) HC (RHC), MUG (M, 100 mg/l) and HC (MHC) agar plates, and triple sugar iron (TSI, Oxoid) agar tubes. In addition, indol tests were applied to all selected CT resistant strains. All of the methods used in this study for the isolation and identification of *E. coli* O157 are described by the FDA Bacteriological Analytical Manual (12). All the selective medium plates used in this study were incubated at 42 °C for 18-24 h. The MUG tests were repeated using LST-MUG medium and a UV lamp (366 nm) (Merck). After incubation, typical colonies (nonfermentation of S, R, and MUG, colonies with a metallic sheen on EMB, acid butt and acid slope with gas production but no $H_2S$ production in TSI and indol production in tryptone broth) were identified by agglutination and serological tests for *E. coli* O157:H7.

The presence of the O157 antigen was investigated by the latex agglutination test using the *E. coli* O157 test kit (Oxoid). Antisera contained in a commercially available O:H stereotyping kit (*Escherichia coli* antisera, SEIKEN, Denka Seiken Co. Ltd., Tokyo, Japan) were utilized for O:H serotyping following the manufacturer's specifications.

**Data analysis.** Enumerated typical and atypical colony populations grown on 4 different selective agar media were normalized to an area basis (CFU/ml) and transformed to $\log_{10}$ values. ANOVA was applied to determine the differences between bacterial counts in different enrichment and selective plating combinations, and significance was based on 5% and 1% levels ($P < 0.05$ and $P < 0.01$).

## Results

The best growth and morphological patterns of colonies were detected on HC medium when this medium was spread plated from the fifth or sixth 10-fold serial dilution tubes of LST or LST + n enrichment cultures. However, the original enrichment culture and also the first 3 dilution tubes of the same enrichment were the cultures of choice for making a good inoculation onto CT-HC medium. While no typical colonies were detected using LST + nct/CT-HC and LST + nct/CTSRM-HC protocols, only 4 typical colonies from 1 sample and another 3 typical colonies from another sample (2 samples) could be determined using LST + nct/HC medium protocol (Table 1). The detailed results of typical and atypical colony populations counted using 3 different selective plating media from each 50 µl sample of three different LST based enrichment broths used for the enrichment of the 20 frozen beef samples are given in Table 1.

Table 1. The detailed results of typical and atypical colony populations determined from 20 frozen beef samples using 3 different enrichment and selective plating protocols.

| Enrichment/ plating combination | Colony numbers (Log10 CFU/50 µl enrichment culture) | | | | | | Total sample numbers* | |
|---|---|---|---|---|---|---|---|---|
| | Minimum | | Maximum | | Mean ± SD. | | | |
| | Typical | Atypical | Typical | Atypical | Typical | Atypical | Typical | Atypical |
| 1a: LST/HC | 0 | 4.30 | 9.57 | 9.68 | 9.54 ± 8.92 | 9.52 ± 8.95 | 13 | 20 |
| 1b: LST/CT-HC | 0 | 0 | 4.35 | 5.15 | 3.36 ± 3.37 | 4.35 ± 4.58 | 8 | 17 |
| 1c: LST/CTSRM-HC | 0 | 1.78 | 3.90 | 5.58 | 2.93 ± 3.40 | 4.89 ± 5.12 | 8 | 20 |
| 2a: LST + n/HC | 0 | 0 | 8.48 | 8.84 | 7.2 ± 6.80 | 8.30 ± 8.58 | 13 | 20 |
| 2b: LST + n/CT-HC | 0 | 0 | 3.17 | 4.90 | 2.1 ± 2.56 | 4.68 ± 4.00 | 9 | 18 |
| 2c:LST + n/ CTSRM-HC | 0 | 0.78 | 2.78 | 5.83 | 1.85 ± 2.3 | 5.04 ± 5.31 | 4 | 20 |
| 3a: LST + nct/HC | 0 | 0 | 0.60 | 3.60 | 0.54 ± 0.56 | 2.38 ± 2.96 | 2 | 12 |
| 3b:LST + nct/CT-HC | 0 | 0 | 0 | 5.32 | 0 | 4.09 ± 1.67 | 0 | 3 |
| 3c. LST + nct/CTSRM-HC | 0 | 0 | 0 | 3.32 | 0 | 2.44 ± 2.81 | 0 | 8 |

* represents the sample numbers in which typical or atypical colonies were counted.

From a total of 251 typical colonies, only 15 (5.97%) were found to be biochemically identical to *E. coli* O157. Thirteen of these colonies gave positive agglutination results in the latex O157 test. Nine of the 13 colonies were picked from Samples 1, 6, and 8 using the LST/CT-HC protocol. The other 4 colonies were picked from Sample 1 using the LST/CTSRM-HC protocol. Both protocols contained no novobiocin in LST, but contained CT in HC medium (Table 2). In contrast the LST + n enrichment protocol yielded no latex test positive *E. coli* O157 colony. Only 7 of these 13 strains were found to be positive in the O157 antiserum tests, and 6 strains were found to be positive in the H7 antiserum test. Therefore, only 6 (2.39%) out of 251 typical colonies, all of which were isolated from Sample 1, were identified as *E. coli* O157:H7. The detailed results of each phenotypical colony and serological tests for the identification of *E. coli* O157:H7 are given in Table 2.

Table 2. Biochemical and serological test results used for the isolation and identification of *E. coli* O157:H7 from frozen ground beef.

| Enrichment/ plating media combination | E. coli O157:H7 | | | NonO157 typical colony groups and colony numbers* | | | | | | | | | | Total** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Latex O157 a(b)c | Antisera O157 | Antisera H7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| 1a: LST/HC | | | | 9 | 11 | 2 | 8 | 12 | 1 | 2 | 1 | 3 | 1 | 50 |
| 1b: LST/CT-HC | 5(4)8 | --- | --- | | 11 | 3 | 3 | 12 | 3 | 8 | | | | 40 |
| | 4(4)1 | 4 | 4 | | | | | | | | | | | |
| | 1(1)6 | --- | --- | | | | | | | | | | | |
| 1c: LST/ CTSRM-HC | 4(4)1 | 3 | 2 | 15 | | | 5 | | | | | | | 20 |
| 2a: LST + n/HC | | | | 17 | 10 | 19 | 2 | 1 | | | 1 | | | 50 |
| 2b: LST + n/CT-HC | | | | 8 | 14 | 5 | 13 | | 8 | | 2 | | | 50 |
| 2c: LST + n/CTSRM-HC | 1(0)8 | --- | --- | 26 | | | | | | | | | | 26 |
| Test/colony numbers | 15(13) | 7 | 6 | 75 | 46 | 29 | 31 | 25 | 12 | 10 | 4 | 3 | 1 | 236 |
| Indol (6.36%)*** | | + | | | | | | | + | | | + | | 15 |
| EMB | | + | | | | | | + | | | | + | | 28 (11.86%) |
| MUG (M) | | - | | - | - | - | - | - | - | - | - | - | - | 236 (100%) |
| Salicin (S) | | - | | - | - | - | - | - | - | - | - | - | - | 236 (100%) |
| Rhamnose (R) | | - | | - | | | - | | | - | - | | | 120 (50.85%) |
| TSIA Total | | + | | + | + | | | + | + | | + | + | | 165 (69.91%) |
| TSIA Butt | | + | | + | + | + | + | + | + | + | + | + | + | 236 (100%) |
| TSIA Slope | | + | | + | + | | | + | + | | + | + | | 166 (70.34%) |
| TSIA Gas | | + | | + | + | + | + | + | + | | + | + | + | 226 (95.76%) |
| TSIA H2S | | - | | - | - | - | - | - | - | - | - | - | - | 236 (100%) |
| Test combinations: EMB + TSIA | | | | | | | | + | | | | + | | 28 (11.86%) |
| Test combinations: Rhamnose + TSIA | | | | + | | | | | | | + | | | 79 (33.47%) |
| Test combinations: EMB + Indol | | | | | | | | | | | | + | | 3 (1.27%) |
| Test combinations: Rhamnose + Indol | | | | | | | | | | | | | | 0 |
| Test combinations: Rhamnose + EMB | | | | | | | | | | | | | | 0 |

The column a(b)c, represents tested colony numbers (latex O157 positive colony numbers); sample code * represents tested biochemically typical nonO157 positive colony groups and the distribution of 236 colonies in these groups; ** represents the total colony numbers selected from different enrichment/selective plating combinations; *** represents positive colony numbers (%) in 236 nonO157 typical colonies after each test or test combination.

## Discussion

All of the 8 test strains grew easily and produced identical colonies agreeing with the well known coloration pattern on all selective plating media (12). Selected colonies were also colorless on HC medium and other HC based media because of their inability to ferment sorbitol, salicin, rhamnose and MUG. Atypical colonies were easily distinguished from typical colonies by their distinct yellow color on the same media. In addition, typical colonies appeared as greenish-metallic in reflected light, and with a blue-black center in transmitted light on EMB.

There was an unwanted outgrowth of atypical colonies on HC medium compared to typical colonies ($P < 0.05$). On the other hand, none of the enrichment broth/selective agar combinations demonstrated more typical colony populations than they did atypical colonies (Table 1). When outgrowths of atypical sorbitol positive colonies appeared on selective media, the atypical yellow colors of colonies on HC and other HC based media turned light blue, the same color as that of the media. Many other researchers have also reported such problems (5,7,10). We overcame this problem by drying the plates more effectively than usual (for example, 3 days at 20 $^{o}$C), selecting the most appropriate 10-fold serial dilution tube for spread plating for the growth of 10 to 150 colonies per plate, and by preventing accumulation of excess humidity in incubators during the incubation period.

Methods for the detection and isolation of *E. coli* O157:H7 with a summary of comparative enrichment media and plating media studies have been well documented by De Boer and Heuvelink (13) and Chapman (3). The conditions for growth of *E. coli* O157:H7 in different enrichment media vary considerably due to the selective agents used for the selective recovery of this pathogen from ground beef (1,2,14). It has been recommended that a nonselective broth such as modified buffered peptone water or mEC broth should be used for the detection of injured *E. coli* O157:H7 strains in foods (15-17). In addition, antibiotic supplements may lead to the reporting of false negative sample statements (1) and/or time consuming and economically impractical labor. Restaino et al. (6) demonstrated the superiority of modified buffered peptone water over mEC containing novobiocin. We also demonstrated that LST broth was superior to LST + n broth ($P < 0.05$). The suppressive effect of novobiocin was more pronounced on typical colony populations than on atypical colony populations in the samples (Table 1) ($P < 0.05$). MacRae et al. (1) also demonstrated that the sensitivity of *E. coli* O157 to CT supplement has led to an extended lag time of 10 to 16 h for strains, but that enrichment for 24 h has tolerated that sensitivity within an important range. In the present study, the typical colonies grew well on CT supplemented agar plates when inoculated from LST or LST + n enrichment cultures, except for LST + nct (Table 1).

All the latex positive *E. coli* O157:H7 strains were isolated from media containing CT (Table 2). For this reason it may be stated that in contrast to CT-HC, selecting the target colonies on a CT free selective plating medium inoculated from the fifth and sixth serial 10-fold dilution of enrichment culture will probably lead to false negative sample statements due to false colony selection from plates plated from high serial dilutions of enrichment cultures. Furthermore, selection of suspect colonies among CT resistant colonies on CT-HC medium will not require an additional CT resistance test. Thus, the analysis time can be shortened by at least 1 day, with attendant economic benefits for the isolation of *E. coli* O157:H7 in ground beef samples. Supplementation of selective media with some chromogenic and/or fluorogenic substances can also improve selectivity (12).

At the end of this study, 236 of the 251 CT resistant sorbitol negative colonies presented negative growth profiles in biochemical tests; these colonies were divided into 8 separate groups (Groups 1 and 8, and Groups 3 and 10 were the same) using their test results while they were presented in 10 groups by using their morphological properties on the plating media. Each enrichment/plating protocol gave rise to different groups with different colony numbers in each group (Table 2). It is interesting that all 20 colonies from the LST/CTSRM-HC protocol were present in only 2 groups (Groups 1 and 4), and 26 colonies from the LST + n/CTSRM-HC protocol were present in only 1 group (Group 1). When these 46 strains were tested separately for the ability to ferment MUG or salicin, they were all found to be non-fermenters of these sugars. Tarr et al. (18) also demonstrated that the MUG test has eliminated few sorbitol negative colonies from consideration, and this technique was thus not used to evaluate isolates. The usefulness of salicin in selective plating media for the detection of *E. coli* O157:H7 in raw vegetables has been demonstrated (7). It was found to be more important for

the further identification protocol that all of these 46 colonies were eliminated by EMB or indol tests, but not by MUG or salicin tests. This demonstrates that there may be no need for the incorporation of MUG or salicin into the selective plating medium. When combined test results were taken into consideration there were no rhamnose negative or indol positive colonies, or rhamnose negative or EMB positive colonies among the 236 nonO157 CT resistant typical colonies. Almost half of these strains fermented rhamnose and they could thus be eliminated from consideration.

Since we could not determine any false negative results among the 251 strains tested in this study, the repetition of MUG and sorbitol fermentation tests in broth media appeared to be unnecessary in the identification of *E. coli* O157:H7, because these tests require extra effort and time but have very low productivity rates. If necessary, the application of these tests to serologically confirmed strains only may be more economic and time saving.

The selection of one sorbitol based agar medium over another depends on the researcher's choice, but using a selective enrichment broth without antibiotic supplement in combination with a sorbitol and rhamnose based plating medium containing CT such as CTR-HC medium can enhance the isolation rate of presumptive *E. coli* O157 in ground beef on a routine basis. Nevertheless, our results should be evaluated in comparison with many other enrichment broths and selective agar media on many retail ground beef samples for the recommendation of an improved routine cultural method for *E. coli* O157.

In conclusion, a combination of the metallic sheen property of EMB with sorbitol and rhamnose negativity in a plating medium supplemented with CT may be an alternative to available selective plating media. If this can be achieved, isolation of *E. coli* O157 from beef samples can be completed in 24 h after enrichment by using this new selective plating medium and rapid test kits such as latex agglutination tests and others.

## References


1. MacRae, M., Rebate, T., Johnson, M., Ogden, I.D.: The sensitivity of *Escherichia coli* O157 to some antimicrobials by conventional and conductance assays. Lett. Appl. Microbiol., 1997; 25: 135-137.
2. Blais, B.W., Booth, R.A., Phillippe, L.M., Yamazaki, H.: Effect of temperature and agitation on enrichment of *Escherichia coli* O157:H7 in ground beef using modified EC broth with novobiocin. Int. J. Food Microbiol., 1997; 36: 221-225.
3. Chapman, P.A.: Methods available for the detection of *Escherichia coli* O157 in clinical, food and environmental samples. World J. Microbiol. Biotechnol., 2000; 16: 733-740.
4. Stephens, P.J., Joynson, J.A.: Direct inoculation into media containing bile salts and antibiotics is unsuitable for the detection of acid/salt stressed *Escherichia coli* O157:H7. Lett. Appl. Microbiol., 1998; 27: 147-51.
5. Johnson, J.L., Brooke, C.L. Fristchel, S.J.: Comparison of the BAX for screening/*E. coli* O157:H7 method with conventional methods for detection of extremely low levels of *Escherichia coli* O157:H7 in ground beef. Appl. Environ. Microbiol., 1998; 64: 4390-4395.
6. Restaino, L., Castillo, H.J., Stewart, D., Tortorello, M.L.: Antibody-direct epifluorescent filter technique and immunomagnetic separation for 10-h screening and 24 h confirmation of *Escherichia coli* O157:H7 in beef. J. Food Protect., 1996; 59: 1072-1075.
7. Fujisawa, T., Sata, S., Aikawa, K., Takahashi, T., Yamai, S., Shimada, T.: Evaluation of sorbitol-salicin MacConkey medium containing cefixime and tellurite (CT-SSMAC medium) for isolation of *Escherichia coli* O157:H7 from raw vegetables. Int. J. Food Microbiol., 2002; 74: 161-163.
8. Kang, D.H., Fung, D.Y.: Development of a medium for differentiation between *Escherichia coli* and *E. coli* O157:H7. J. Food Protect., 1999: 62: 313-317.
9. Feldsine, P.T., Forgey, R.L., Falbo-Nelson, M.T., Brunelle, S.L.: *Escherichia coli* O157:H7 visual immunoprecipitate assay: A comparative validation study. J. AOAC Int., 1997; 80: 43-48.
10. Weagant, S.D., Bryant, J.L., Jinneman, K.G.: An improved rapid technique for isolation of *Escherichia coli* O157:H7 from foods. J. Food Protect., 1995; 58: 7-12.
11. Chikthimmah, N., Knabel, S.J.: Survival of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* in and on vacuum packaged Lebanon bologna stored at 3.6 and 13.0 °C. J. Food Protect., 2001; 64: 958-963.
12. Hitchins, A.D., Feng, P., Watkins, W.D., Rippey, S.R., Chandler, L.A.: Bacteriological Analytical Manual, Edition 8, Revision A, Chapter 4. U.S. Food & Drug Administration Center for Food Safety & Applied Nutrition, 1998.
13. De Boer, E., Heuvelink, A.E.: Methods for the detection and isolation of Shiga toxin –producing *Escherichia coli*. J. Appl. Microbiol., 2000; 88: 133-143.
14. Manafi, M., Kremsmaier, B.: Comparative evaluation of different chromogenic/fluorogenic media for detecting *Escherichia coli* O157:H7 in food. Int. J. Food Microbiol., 2001; 30: 257-262.
15. Okrend, A.J.G., Rose, B.E., Bennett, B.: A screening method for the isolation of *Escherichia coli* 0157:H7 from ground beef. J. Food Protect., 1990; 53: 249-252.

16. Sata, S., Osawa, R., Asai, Y., Yamai, S.: Growth of starved *Escherichia coi* O157 cells in selective and non-selective media. Microbiol. Immunol., 1999; 43: 217-227.

17. Silveria, N.F.A., Silva, N., Contreras, C., Miyagusku, L., Baccin, F., Koono, E., Beraquet, N.J.: Occurrence of *Escherichia coli* 0157:H7 in hamburgers produced in Brazil. J. Food Protect., 1999; 62: 1333-1335.

18. Tarr, P.I., Tran, N.T., Wilson, R.A.: *Escherichia coli* 0157:H7 in retail ground beef in Seattle: Results of a one-year prospective study. J. Food Protect., 1999; 62: 133-139.