Evaluation of Selective Media Containing Iron Source and Alpha-Glucosidase Substrates for Enterobacter sakazakii (Cronobacter spp.) Detection

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Abstract

Enterobacter sakazakii (Cronobacter spp.) causes meningitis, necrotizing enterocolitis, sepsis, and bacteremia in neonates and children and has a high mortality rate. For rapid E. sakazakii detection, various differential and selective media containing α-glucosidase substrates, such as 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside (BCIG) or 4-methylumbelliferyl-α-D-glucoside (α-MUG), have been developed as only E. sakazakii exhibits α-glucosidase activity in the genus Enterobacter. However, Escherichia vulneris (family: Enterobacteriaceae) can also utilize α-glucosidase substrates, thereby resulting in false positives. Various iron sources are known to promote the growth of gram-negative bacteria. This study aimed to develop a selective medium containing α-glucosidase substrates for E. sakazakii detection that would eliminate false positives, such as those of E. vulneris, and to determine the role of iron source in the medium. Three previously developed (TPD) media, i.e., Oxoid, OK, and VRBG, and the medium developed in this study, i.e., NGTE, were evaluated using 58 E. sakazakii and 5 non-E. sakazakii strains. Fifty-four E. sakazakii strains appeared as fluorescent or chromogenic colonies on all media that were assessed. Two strains showed colonies on NGTE medium and not on TPD media. In contrast, the remaining two strains showed colonies on TPD media and not on NGTE medium. None of the non-E. sakazakii strains showed fluorescent or chromogenic colonies on any of the evaluated media except E. vulneris, which showed colonies on TPD media and not on NGTE medium. This study demonstrated that the newly developed NGTE medium was not only equally efficient in promoting the growth of bacterial colonies when compared with the currently available media but also eliminated false positives, such as E. vulneris.

Keywords

Enterobacter sakazakii, selective media, glucose, α-glucosidase substrates, iron

Introduction

Previously referred to as ‘yellow pigmented E. cloacae’, Enterobacter sakazakii (Cronobacter spp.) was reclassified based on differences from E. cloacae in DNA relatedness, pigment production and biochemical reactions [1-5]. E. sakazakii (Cronobacter spp.) caused a severe form of neonatal meningitis with a high mortality rate [2]. A full risk assessment of E. sakazakii (Cronobacter spp.) will require more knowledge of its presence in food for neonates and infants [3]. Dried infant formula has been a vehicle of transmission in outbreaks and sporadic cases of E. sakazakii (Cronobacter spp.) [4]. Healy et al. [5] reported the enzymatic profiles of E. sakazakii (Cronobacter spp.) with specific reference to α-glucosidase reaction and concluded that E. sakazakii
(Cronobacter spp.) isolates produced α-glucosidases in contrast to other Enterobacter isolates. In 2002, US Food and Drug Administration published the isolation and enumeration of E. sakazakii (Cronobacter spp.) from dehydrated powdered infant formula, but this method was very complicated and time-consuming procedures [6,7].

Therefore, to detect E. sakazakii (Cronobacter spp.) rapidly by using α-glucosidase reaction, Iversen et al. [8] made the new chromogenic (5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside) medium, but the false positive showed 16 of 18 Escherichia vulneris strains, 2 of 3 strains of Pantoea spp., and 1 of 8 Citrobacter koseri strains on this agar [8]. Two major differences between E. sakazakii (Cronobacter spp.) and the other Enterobacter species are the presence of α-glucosidase and the absence of phosphoamidase in E. sakazakii (Cronobacter spp.) [8]. A fluorogenic method has been developed using α-glucosidase reaction, which was recognized a single, simple, and rapid test to distinguish E. sakazakii (Cronobacter spp.) from other Enterobacter species [8,9].

Leuschner & Bew [10] reported that a nutrient agar added with 4-methyl-umbelliferyl α-D-glucoside (α-MUG) as a differential-selective medium for the detection of E. sakazakii (Cronobacter spp.) was developed, but E. vulneris represented false positive on this agar. Guillaume-Gentil et al. [11] developed new media with addition of vancomycin to lauryl sulfate tryptose so as to detect E. sakazakii (Cronobacter spp.) in environmental samples, but some strains with yellow colonies were identified as Pantoea spp., Pantoea agglomerans, or E. vulneris.

Oh & Kang [12] reported α-MUG was used as a selective marker to develop a differential medium for E. sakazakii (Cronobacter spp.). And Restaino et al. [13] developed new chromogenic agar with contains two chromogenic substrates that are specific for E. sakazakii (Cronobacter spp.), plus selected sugars, a pH indicator, and inhibitors for both Gram-positive and Gram-negative contaminants all contributing to its selectivity and differential properties. However, Oh & Kang [12] did not study the false positive as Escherichia vulneris, Pantoea spp., and Citrobacter koseri strains on this agar, and also Restaino et al. did not research the false positive as Escherichia vulneris.

Hence, it is necessary to develop the media for enrichment of E. sakazakii (Cronobacter spp.) or for recovery of injured E. sakazakii (Cronobacter spp.) without the false positive such as E. vulneris. It has been shown in numerous studies that supplementation of egg contents with iron such as ferric ammonium citrate (FAC), ferrous sulphate (FS) and ferrioxamine E (FE) can overcome the antimicrobial properties of ovotransferrin and enhance the growth and detection of Salmonella in eggs [14]. No strains of E. coli, the Proteus-Providencia-Morganella-group have an uptake and utilization system of FE [14,15]. However, Ferrioxamine E can be used by Citrobacter spp., Klebsiella spp., Pseudomonas spp., Enterobacter spp., and Yersinia enterococlitica [15]. Hence, Ferri-oxamine E is considered a selective iron source because it is utilized by E. sakazakii (Cronobacter spp.), but not by E. coli. Also, the type of carbohydrate was related with α-glucosidase reaction and production of yellow pigment.

This study was performed to distinguish E. sakazakii (Cronobacter spp.) from non-E.
Selective Media for Detecting Enterobacter sakazakii (Cronobacter spp.) was grown on 4 different selective media for E. sakazakii (Cronobacter spp.), and to remove false positive such as E. vulneris and to compare the colony morphology of E. sakazakii (Cronobacter spp.) and non-E. sakazakii (Cronobacter spp.) on various modified selective media for E. sakazakii (Cronobacter spp.) made by combining iron sources and carbohydrate sources.

Materials and Methods

1. Strains

Strains of E. sakazakii (Cronobacter spp.) were obtained from a range of culture collections. A list of all strains with their origin is collected in Table 1. All strains were grown on tryptic soy broth (TSB: Becton Dickinson, USA) at 37°C. Purity of the culture was confirmed using a biotyping kit (API 20 E: bioMerieux) and real-time PCR.

Table 1. Chromogenic or fluorogenic reaction of 4 different media supplemented with α-glucosidase substrates in 58 strains of E. sakazakii (Cronobacter spp.)

| Species        | Strain no. | Source | Chromogenic or fluorogenic reaction |
|----------------|------------|--------|-----------------------------------|
|                |            |        | OXOID                                  |
| E. sakazakii   | FSM 145    | UGA    | +                                  |
| E. sakazakii   | FSM 261    | NRC    | +                                  |
| E. sakazakii   | FSM 262    | NRC    | +                                  |
| E. sakazakii   | FSM 265    | NRC    | +                                  |
| E. sakazakii   | FSM 270    | NRC    | +                                  |
| E. sakazakii   | FSM 271    | NRC    | +                                  |
| E. sakazakii   | FSM 272    | NRC    | +                                  |
| E. sakazakii   | FSM 273    | NRC    | +                                  |
| E. sakazakii   | FSM 274    | NRC    | +                                  |
| E. sakazakii   | FSM 275    | NRC    | +                                  |
| E. sakazakii   | FSM 287    | NRC    | +                                  |
| E. sakazakii   | FSM 290    | NRC    | +                                  |
| E. sakazakii   | FSM 292    | NRC    | +                                  |
| E. sakazakii   | FSM 293    | NRC    | +                                  |
| E. sakazakii   | FSM 294    | NRC    | +                                  |
| E. sakazakii   | FSM 295    | NRC    | +                                  |
| E. sakazakii   | FSM 297    | NRC    | +                                  |
| E. sakazakii   | FSM 298    | NRC    | +                                  |
| E. sakazakii   | FSM 299    | NRC    | +                                  |
| E. sakazakii   | FSM 300    | NRC    | +                                  |
| E. sakazakii   | FSM 302    | NRC    | +                                  |
| E. sakazakii   | FSM 303    | NRC    | +                                  |
| E. sakazakii   | FSM 318    | NRC    | +                                  |
| E. sakazakii   | FSM 321    | NRC    | –                                  |
| E. sakazakii   | FSM 324    | NRC    | +                                  |
| E. sakazakii   | 1          | UGA    | +                                  |
| E. sakazakii   | 1.91       | UGA    | +                                  |
| E. sakazakii   | 2          | UGA    | +                                  |
| E. sakazakii   | 2.39       | UGA    | +                                  |
| E. sakazakii   | 2.40       | UGA    | +                                  |
| E. sakazakii   | 2.41       | UGA    | +                                  |
| E. sakazakii   | 2.42       | UGA    | +                                  |
Table 1. Continued

| Species       | Strain no. | Source | Oxoid | OK | VRBG | NGTE |
|---------------|------------|--------|-------|----|------|------|
| *E. sakazakii*| 2.43       | UGA    | –     | –  | +    |      |
| *E. sakazakii*| 2.44       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.45       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.46       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.47       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.68       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.69       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.70       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.71       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.72       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.73       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.74       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.75       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.76       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.77       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.78       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.79       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.80       | UGA    | +     | +  | +    | –    |
| *E. sakazakii*| 2.81       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.82       | UGA    | +     | +  | +    | –    |
| *E. sakazakii*| 2.83       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 2.84       | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 3          | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 5          | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 6          | UGA    | +     | +  | +    | +    |
| *E. sakazakii*| 7          | UGA    | +     | +  | +    | +    |

Oxoid, BRILLIANCE ENTEROBACTER SAKAZAKII AGAR (DFI formulation, Code: CM1055).
OK, Manufacture media using the method of Oh & Kang (2004) [12].
VRBG, violet red bile glucose.
NGTE, The newly developed medium in this study.
UGA, Dr. Jeffrey Kornacki, University of Georgia, Athens, GA; NRC, Dr. John Marugg, Nestle Research Center, Lausanne, Switzerland.
+, *E. sakazakii* (Cronobacter spp.); -, non-*E. sakazakii* (Cronobacter spp.).

2. Medium

Four different media were prepared to detect *E. sakazakii* (Cronobacter spp.) and non-*E. sakazakii* (Cronobacter spp.) by use of the enzyme substrate, 5-bromo-4-chloro-3-indolyl-α-D-glucopyranoside (BCIG) or 4-methyl-umbelliferyl α-MUG (Sigma-Aldrich, USA) and of various iron sources, FAC, FS, and FE (Table 2). When the agar reached a temperature of 50°C–60°C, enzyme substrate and iron sources were added to the agar.

3. Reaction of α-glucosidase substrates in 4 different media

The reaction of α-glucosidase substrates in 4 different media was determined by change of colony color under normal light or colony fluorescence under UV light after incubation at 37°C for 24 h. Sensitivity was defined as the number of true positives divided by the sum of true positives plus false negatives, expressed as a percentage.
Selective Media for Detecting Enterobacter sakazakii (Cronobacter spp.)

Table 2. Media used in this study

| Reaction of E. sakazakii (Cronobacter spp.) | Oxoid | OK | VRBG+α-MUG | NGTE+α-MUG |
|--------------------------------------------|-------|----|-------------|------------|
| Normal light                               | Blue-green | Yellow | Pink | Yellow |
| UV light                                   | N/A | Fluorescent | Fluorescent | Fluorescent |
| Ingredients                                |       |       |       |       |
| 5 g tryptone                               | 20 g tryptone | 7 g peptone | 5 g sodium chloride |
| 5 g soya peptone                           | 1.5 g bile salts no. 3 | 3 g yeast extract | 10 g glucose |
| 5 g sodium chloride                        | 1.0 g sodium thiosulfate | 1.5 g bile salts no. 3 | 20 g tryptone |
| 1 g ferric ammonium citrate                | 1.0 g ferric citrate | 10 g lactose | 200 mg/mL Fe |
| 1 g sodium desoxycholate                   | 50 mg α-MUG | 5.0 g sodium chloride | 50 mg α-MUG |
| 1 g sodium thiophosphate                   | 15 g agar | 0.03 g neutral red | 15 g agar |
| 0.1 g chromogen                            |       | 0.002 g crystal violet |       |       |
| 15 g agar                                  |       | 50 mg α-MUG |       |       |
|                                           |       | 15 g agar |       |       |

NGTE, The newly developed medium in this study.

Results

Fifty-eight strains of E. sakazakii (Cronobacter spp.) were analyzed using 4 different media supplemented with α-glucosidase substrates, BCIG or 4-methyl-umbelliferyl α-MUG. All strains formed colonies and the chromogenic or fluorogenic reaction are shown as Table 1. Fifty four E. sakazakii (Cronobacter spp.) strains appeared as fluorescent or chromogenic colonies on all of the media tested.

Two strains were negative on Oxoid, OK, and VRBG media while showing positive on newly developed medium (NGTE) medium. Interestingly, the other two strains were observed vice versa showing positive on Oxoid, OK, and VRBG media, but negative on NGTE medium (Table 3). The detection rate of E. sakazakii (Cronobacter spp.) on each medium expressed over 96% recovery (Table 4). The comparison of colony size on the media with addition of various iron sources showed in Table 5.

In 58 E. sakazakii (Cronobacter spp.) tested, all 4 different media showed over 96% recovery rate (Table 4). Each colony of each media inoculated E. sakazakii (Cronobacter spp.) was verified as E. sakazakii (Cronobacter spp.) by using API 20E biochemical systems and the oxidase test (Data not shown). FSM 321, 2.43, 2.80, and 2.82 were

Table 3. Chromogenic or fluorogenic reaction of 4 different media supplemented with α-glucosidase substrates in non-E. sakazakii (Cronobacter spp.)

| Species          | Strain No. | Source | Oxoid | OK | VRBG | NGTE |
|------------------|------------|--------|-------|----|------|------|
| E. hermanii      | FSN 324    | UGA    | -     | -  | -    | -    |
| Salmonella spp.  | 111        | FDA    | -     | -  | -    | -    |
| E. cloacae       | SP 118     | NRC    | -     | -  | -    | -    |
| E. coli          | K12        | FDA    | -     | -  | -    | -    |
| E. vulneris      | 102-4a     | NRC    | +     | +  | +    | +    |

UGA, Dr. Jeffrey Komacki, University of Georgia, Athens, GA; FDA, Culture collection of U.S. Food and Drug Administration, College Park, MD; NRC, Dr. John Marugg, Nestle Research Center, Lausanne, Switzerland.

+, E. sakazakii (Cronobacter spp.); -, non-E. sakazakii (Cronobacter spp.).
Table 4. Sensitivity of 4 different media with supplemented with \(\alpha\)-glucosidase substrates, 5-bromo-4-chloro-3-indolyl-\(\alpha\)-D-glucopyranoside (BCIG) or 4-methyl-umbelliferyl \(\alpha\)-D-glucoside (\(\alpha\)-MUG), in 58 strains of *E. sakazakii* (Cronobacter spp.) at 37°C for 24 h

| Media     | Total of *E. sakazakii* | Positive of *E. sakazakii* | Negative of *E. sakazakii* | Percentage of recovery (%) |
|-----------|-------------------------|---------------------------|---------------------------|---------------------------|
| Oxoid     | 58                      | 56                        | 2                         | 96.6                      |
| OK        | 58                      | 56                        | 2                         | 96.6                      |
| VRBG      | 58                      | 56                        | 2                         | 96.6                      |
| NGTE      | 58                      | 56                        | 2                         | 96.6                      |

Percentage of recovery = \(\text{[Positive / Total of *E. sakazakii* (Cronobacter spp.)}] \times 100\).

Table 5. The comparison of colony size on the media with addition of various iron sources

| Iron source | Oxoid | Oxoid2 | Oxoid3 | OK | C1 | NGTE | NGTE8 |
|-------------|-------|--------|--------|----|----|------|-------|
| Carbohydrate | FAC   | FE     | FS     | FC | X  | FE   | FS    |
| \(\alpha\)-Glucosidase substrates | Chro   | Mug    | Mug    | Mug | Mug | Glu   | Glu    |
| *E. sakazakii* (Cronobacter spp.) | MC     | MC     | SC not growth well | MC | LC | LC    | VSC not growth well |
| *E. cloacae* | LC    | LC     | No grow | MC | LC | LC    | VSC    |
| *E. coli* K12 | LC    | MC     | No grow | MC | LC | MC    | No grow |
| *E. hermanii* | MC    | MC     | MC     | MC | MC | MC    | SC     |
| *Salmonella* spp. | MC (black) | MC (black) | MC (black) | MC (black) | MC | MC    | No grow |
| *E. vulneris* | MC    | SC     | No grow | SC | MC | SC    | No grow |

Oxoid 2 and 3, substitution by \(\alpha\)-MUG or Iron source in Oxoid.
NGTE 8, substitution by Iron source in NGTE.
FAC, ferric ammonium citrate; FE, ferroxamine E; FS, ferrous sulphate; X, blank; Lac, Lactose; Glu, Glucose; MC, medium colony; LC, large colony; SC, small colony; VSC, very small colony.

determined by *E. sakazakii* (Cronobacter spp.) (Table 6).

To raise the sensitivity of *E. sakazakii* (Cronobacter spp.) in FSM 321, 2.43, 2.80, and 2.82, which showed partial positive or negative on 4 different media, we tried to compare the media with various iron source, FAC, FS, and FE, respectively (Data not shown). There is no significant difference in type of iron source (Table 7). However, media with FE was more ease to distinguish positive or negative under UV light because the intensity of fluorescence was stronger than media with FAC or FS (data not shown).

None of the non-*E. sakazakii* (Cronobacter spp.) strains showed fluorescent or chromogenic colonies on any of the media tested except *E. vulneris*, showing false positive results.

Table 6. Differences of strain identification using API 20E biochemical profiles in *E. sakazakii* (Cronobacter spp.) FSM 321, 2.43, 2.80, and 2.82, respectively

| Species     | Strain No. | Source | Oxidase | API20E (%) |
|-------------|------------|--------|---------|------------|
| *E. sakazakii* | FSM 321   | NRC    | -       | *E. sakazakii* (59.5) |
| *E. sakazakii* | 2.43      | UGA    | -       | *E. sakazakii* (98.4) |
| *E. sakazakii* | 2.80      | UGA    | -       | *E. sakazakii* (98.4) |
| *E. sakazakii* | 2.82      | UGA    | -       | *E. sakazakii* (97.3) |

NRC, Dr. John Marugg, Nestle Research Center, Lausanne, Switzerland; UGA, Dr. Jeffrey Kornacki, University of Georgia, Athens, GA.
positive on Oxoid, OK, and VRBG media and negative on NGTE medium (Table 3). It is assumed that the negative of *E. vulneris* was caused by glucose (Table 8).

**Discussion**

Various selective media for *E. sakazakii* (*Cronobacter* spp.) have been developed by using of the fluorogenic or chromogenic enzyme substrates so as to eliminate the need for biochemical tests or subculture to confirm the identification of microorganisms [8-12,16,17]. Among the α-glucosidase substrates, 4-nitrophenyl-α-D-glucopyranoside and 4-methy-umbelliferyl-α-glucoside were used as possible markers [18]. 4-nitrophenyl-
α-D-glucopyranoside formed yellow-colored colonies, but has limitations because the yellow breakdown product, 4-nitrophenol, was easily diffusible on agar, making it difficult to read, but 4-methylumbelliferyl-α-glucoside produced distinct, not easily diffusible, and fluorescent colonies under UV irradiation.

Recently, Leuschener & Bew [10] combined the two characteristics of yellow pigmentation and enzymatic activity by supplementing nutrient agar with 4-methylumbelliferyl-α-glucoside. Isolates that are able to metabolize the substrate yield a yellow fluorescent product and can be identified as E. sakazakii (Cronobacter spp.). Also, the α-glucosidase hydrolyses the chromogenic substrate, 5-bromo-4-chloro-3-indoly α-D-glucopyranoside, producing blue-green colonies on this pale yellow medium. These two methods are a time-saving alternative to detect E. sakazakii (Cronobacter spp.). Unfortunately, the detection rate of E. sakazakii (Cronobacter spp.) did not reach 100%

Restaino et al. [13] recently developed R & F® Enterobacter sakazakii Chromogenic Plating Medium, which showed blue-black raised to domed colonies 1-2.0 mm diameter ± clear halos in E. sakazakii (Cronobacter spp.). But it needed 2nd step to inoculate R & F® Enterobacter sakazakii (Cronobacter spp.) screening biplating medium consisted of melibiose and sucrose so as to confirm E. sakazakii (Cronobacter spp.). Similar results were observed in this study showing the maximum detection rate of 96%. It appeared that at least two media should be combined to detect 100%.

Furthermore, previous studies fail to distinguish between E. sakazakii (Cronobacter spp.) and E. vulneris because both strains showed positive by using fluorogenic or chromogenic enzyme substrates [8,19]. E. vulneris is a gram-negative, oxidase-negative, fermentative, motile rod with the characteristics of the family Enterobacteriaceae, and two-thirds of the strains produced yellow pigment and no acid form D-sorbitol [19]. Farmer [20] reported that the false positive result with E. vulneris was expected since this species was reported to be 25% positive for α-glucosidase and 50% positive for yellow pigment production. Similarly, in our study showed that E. vulneris also utilizes α-glucosidase substrates resulting in false positives. Three previously developed media, Oxoid, OK, and VRBG, showed false positive for E. vulneris, but the NGTE media with glucose used in this study was negative for E. vulneris.

It is unclear but may be due to reason that E. vulneris was difficult to hydrolyse the fluorogenic or chromogenic enzyme substrates under the glucose-containing media [19]. Hence, E. vulneris did not produce the fluorescent colonies on media as negative reaction [19]. Since Butterworth et al. [21] reported that the difference of β–ribosidase activity was due to different rates of substrate uptake through the cell envelop or differences in the substrate affinity of the β–ribosidase enzyme within different species.

Lehner & Stephan [22] published that two different morphologies are exhibited by E. sakazakii (Cronobacter spp.) ATCC 29544 when grown on different plates: the rubbery colonies (Blood agar and Brain heart infusion agar) and the smooth colonies (Plate count agar), and also the intensity of the pigmentation may vary from stain to stain. Also this study demonstrated that the colonies formed on media of Oxoid and QX showed the rubbery, but the one of VRBG and NGTE showed smooth with slime (Data not shown).
It may due to complicated reaction between various iron sources and carbohydrates. In comparison of colony morphology, we observed that colonies of Ferrioxamine E containing media showed were smaller than those of FAC on E. vulneris strain, and also produced larger colony on Enterobacter species than on Escherichia species and Salmonella. It may due to Ferrioxamine E, because it is utilized by E. sakazakii (Cronobacter spp.), but not by E.vulneris as Escherichia species. Also, FE could help not only distinguish E. sakazakii (Cronobacter spp.) from strains but also selective growth.

**Conclusion**

In conclusion, this study demonstrates the NGTE enabled not only detection of an equivalent number of positive colonies but also excluded false positives such as E. vulneris when compared with currently available media. We found out that only glucose could take important role of removal for E. vulneris as false positive on the media. After compared with the colony size and growth on the media, media with addition of FAC and FE revealed better than that of FS. In comparison of colony of E. vulneris strain, it was smaller on media with addition of FE than that of FAC. Ferrioxamine E containing media produced larger colonies in Enterobacter species than in Escherichia species and Salmonella. Furthermore, FE containing media had strong intensity of fluorescence under UV light than other iron sources. Therefore, NGTE with FE, Glucose, and α-MUG showed the best result of detecting E. sakazakii (Cronobacter spp.) and of eliminating false positive as E. vulneris in this study. Further study is required to determine the mechanism between glucose and α-glucosidase enzyme of E. vulneris, and also to improve the recovery percentage of injured E. sakazakii (Cronobacter spp.) by using of various iron sources. Furthermore, we try to study the usability of FE to detect E. sakazakii (Cronobacter spp.) as selective growth factor.

**Conflict of Interest**

The authors declare no potential conflict of interest.

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