Journal of Microbiological Methods 115 (2015) 34–41

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Development of selective and differential medium for *Shigella sonnei* using three carbohydrates (lactose, sorbitol, and xylose) and X-Gal

G.N. Na, S.A. Kim, O.C. Kwon, M.S. Rhee *

Department of Food Bioscience and Technology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea

ARTICLE INFO

Article history:
Received 21 February 2015
Received in revised form 19 May 2015
Accepted 19 May 2015
Available online 21 May 2015

Keywords:
Shigella sonnei
Selective and differential medium
X-Gal
Lactose
Sorbitol
Xylose

ABSTRACT

The aim of this study was to develop a new selective and differential medium for isolating *Shigella sonnei* (designated 3SD medium). The new medium was based on three carbohydrates (lactose, sorbitol, and xylose) and a chromogenic substrate (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, X-Gal). *S. sonnei* cannot ferment lactose, sorbitol, or xylose, but can ferment X-Gal, which generates turquoise-blue colonies with rough edges. Other bacteria (54 strains of foodborne pathogens and spoilage bacteria) produced visually distinct colonies on 3SD medium (colorless or pink–violet colonies), or their growth was inhibited on 3SD medium. The optimum concentration of 50 mg/L X-Gal was selected because it yielded the highest level of morphological discrimination between *S. sonnei* and other bacteria, and this concentration was cost-effective. Bile salt concentration optimization was performed using healthy, heat-injured, and acid-injured *S. sonnei*. The recovery rate differed significantly depending on the bile salt concentration; media containing >1.0 g/L bile salt showed significantly lower recovery of stress-injured cells than medium containing 0.5 g/L bile salt ($P < 0.05$). Growth of all Gram-positive bacteria was inhibited on medium containing 0.5 g/L bile salt; therefore, this concentration was used as the optimal concentration. Previous media used to isolate *Shigella* spp. (MacConkey, xylose lysine desoxycholate, and *Salmonella–Shigella* agar) showed poor performance when used to support the growth of injured *S. sonnei* cells, whereas 3SD medium supported a high growth rate of injured and healthy cells (equivalent to that obtained with nutrient-rich tryptic soy agar). To validate the performance of 3SD medium with real specimens, *S. sonnei* and other bacteria were spiked into samples such as untreated water, carrot, salad, and oyster. 3SD medium showed superior specificity (100%) and sensitivity (100%) for *S. sonnei*, and yielded no false-positive or false-negative results. Thus, the novel 3SD medium described herein is a powerful tool for the rapid and efficient selective isolation of *S. sonnei* in research and clinical laboratories, and the food industry.

1. Introduction

Shigella is a member of the family Enterobacteriaceae. It is a fastidious Gram-negative, facultative-anaerobic, rod-shaped bacterium, which is non-motile and does not form spores. *Shigella* species are classified by the following four serogroups: *Shigella dysenteriae*, serogroup A, serotypes 1–15; *Shigella flexneri*, serogroup B, serotypes 1–8; *Shigella boydii*, serogroup C, serotypes 1–19; and *Shigella sonnei*, serogroup D, serotype 1 (Warren et al., 2006). *Shigella* is the causative agent of shigellosis (bacillary dysentery), and primarily causes waterborne (water polluted with feces) or foodborne outbreaks throughout the world (Echeverria et al., 1991; International Commission on Microbiological Specifications for Foods, 1996; Smith, 1987). *Shigella* infection is the third most common cause of enteric bacterial infection in the United States (Scallan et al., 2011). This illness has diverse clinical manifestations including abdominal pains, cramps, fever, vomiting, nausea, and diarrhea that sometimes contains blood (Nygren et al., 2013). Shigellosis is highly virulent because it is easily transmitted from person to person via the fecal–oral route (Wachsmuth and Morris, 1989). Shigellosis outbreaks are also associated with contaminated raw foods or inappropriately prepared processed foods (Cowden et al., 1995; Dunn et al., 1995; Frost et al., 1995; Gaynor et al., 2009; Hosseini and Kaffashian, 2010; Kapperud et al., 1995; Kuo et al., 2009; Lee et al., 1991; Lew et al., 1991; Terajima et al., 2004; Yagupsky et al., 1991).

Various selective and differential media have been used to detect and isolate *Shigella*. These media are grouped into the following three classes: 1) low-selectivity media, including MacConkey agar and eosin methylene blue agar; 2) intermediate-selectivity media, including desoxycholate citrate agar and xylose lysine desoxycholate agar; and 3) high-selectivity media, including *Salmonella–Shigella* agar and Hektoen enteric agar (Uyttendaele et al., 2001; van der Zee, 2003; Warren et al., 2006). Low- and intermediate-selectivity media are not stringent enough to isolate *Shigella* spp. (Warren et al., 2006). By contrast, high-selectivity media

Abbreviations: 3SD medium, *Shigella sonnei* selective and differential medium.

* Corresponding author.
E-mail address: rheems@korea.ac.kr (M.S. Rhee).

http://dx.doi.org/10.1016/j.mimet.2015.05.019
0167-7012/

are too stringent to promote growth of stress-injured *Shigella* spp. cells and are thus not recommended for the primary isolation of *Shigella* spp. (Lampel, 2001; Uyttendaele et al., 2001). Isolation of *Shigella* spp. remains challenging, and at least two or three different selective media should be used to improve the probability of an effective isolation. The currently used media do not separately identify the four *Shigella* species.

Shigellosis is primarily a disease of developing countries, and is caused by poor hygiene and overcrowded conditions (Huang et al., 2005). Among the four *Shigella* species, *S. sonnei* is the most common pathogen and is responsible for disease outbreaks in many parts of the world (Wachsmuth and Morris, 1989). *S. sonnei* accounts for 90% of infections caused by *Shigella* spp. (Sack et al., 2001). The majority of shigellosis infections in the USA during 2000–2010 were caused by *S. sonnei* (86%), followed by *S. flexneri* (13%), *S. boydii* (0.7%), and *S. dysenteriae* (0.5%) (Shiferaw et al., 2012). *S. sonnei* is also the most common *Shigella* species reported in China and Thailand (Mao et al., 2013; von Seidlein et al., 2006). The development of effective isolation media that differentiate *Shigella* species from other bacteria and accurately discriminate *S. sonnei* from other *Shigella* species is crucial to ensure food and water safety, and public health. Currently, no selective and differential medium with such precise targeting properties has been developed. The optimum medium also should effectively support the recovery and growth of stress-injured *Shigella* cells.

The present study developed a new selective and differential medium for the detection and isolation of *S. sonnei* (designated 3SD medium). The medium is based on three carbohydrates (lactose, sorbitol, and xylose) and a chromogenic enzyme substrate (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside, X-Gal). 3SD medium showed high selectivity and sensitivity for *S. sonnei*. The composition of the medium was developed based on the selection and growth patterns of ten *S. sonnei* strains, ten *Shigella* spp. strains, and 44 strains of non-*Shigella* organisms (primarily foodborne pathogens and spoilage bacteria). The capacity of 3SD medium to promote the recovery and growth of stress-injured (heat and acid) and healthy *S. sonnei* was verified.

2. Materials and methods

2.1. Bacterial strains

This study used 64 bacterial strains (Table 1), all of which were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA), National Culture Collection Pathogens (NCCP; Osong, Republic of Korea), the Korean Culture Center of Microorganisms (KCCM; Seoul, Republic of Korea), or the Food Microbiology Culture Collection at Korea University (Seoul, Republic of Korea). All bacterial genera and species were confirmed by biochemical (VITEK 2 system) or molecular (PCR) tests. Tested strains were allocated into five groups (A to E) as follows: group A, *S. sonnei* ($n = 10$); group B, other *Shigella* spp. ($n = 10$); group C, strains that were difficult to distinguish from *Shigella* spp. due to similar biological characteristics ($n = 5$); group D, Gram-negative strains belonging to Enterobacteriaceae, major foodborne pathogens, or spoilage bacteria ($n = 25$); and group E, Gram-positive strains ($n = 14$).

2.2. Bacterial culture and cell suspension

The preparation of cultures and cell suspensions was performed as previously described (Kim and Rhee, 2011). The stock culture of each strain was maintained at −20 °C in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) containing 20% glycerol. Each stock culture was subcultured at monthly intervals. Each tested bacterial strain was separately enriched as stipulated by the Food and Drug Administration (FDA) isolation protocol and cultured by performing inoculating-loop transference of stock culture to the enrichment broth. Each enriched culture was harvested by centrifugation (Centra-CL2, IEC, Needham Heights, MA, USA) for 15 min at 3000 × *g*. After the supernatant was decanted, the pellet was washed twice in sterile 0.2% peptone and then resuspended in sterile 0.2% peptone.

2.3. Carbohydrate (lactose, sorbitol, and xylose) and X-Gal fermentation tests

All Gram-negative strains (groups A–D) were tested to determine whether they utilized carbohydrates or X-Gal for fermentation. Gram-positive strains (group E) cannot grow in medium containing bile salt; therefore, carbohydrate and X-Gal fermentation tests were not performed for group E. The basal broth for these tests contained 10 g/L peptone (Difco), 5 g/L sodium chloride (Junsei Chemical Co., Ltd., Tokyo, Japan), and distilled water (US FDA, 2011). For the carbohydrate fermentation tests, 10 g of each carbohydrate [lactose (Sigma, St. Louis, MO, USA), D-sorbitol (Sigma), or D-xylose (Sigma)] and 18 mg of phenol red (used as a pH indicator; Sigma) were added to the basal broth. To test X-Gal fermentation, 50 mg/L X-Gal (Thermo Fisher Scientific Inc., Waltham, MA, USA) was dissolved into the basal broth. Ten milliliters of sterile broth was then transferred into sterile screw-cap tubes. The experimental broths for each set of tests were prepared at the same time and under the same conditions. Bacterial suspension (0.1 ml) was inoculated into 10 ml of prepared broth, which was then incubated at the optimum temperature for 24 h. Fermentation results were determined by observing the broth color. Broths containing X-Gal and carbohydrate substrates were originally cream and red, respectively; fermentation changed the color to blue and yellow (positive results), respectively.

2.4. Preparation of basal medium

The basal medium contained 5 g/L beef extract (Difco), 10 g/L casitone (Difco), 5 g/L lactose, 5 g/L sorbitol, 5 g/L xylose, 5 g/L sodium chloride (Junsei Chemical), 15 g/L bacto agar (Difco), and 30 mg/L neutral red (Sigma). The concentrations of X-Gal and bile salt in the basal medium were as designated in the experiments (see Sections 2.5. and 2.6. below). To prepare the medium, all ingredients were added to distilled water and completely dissolved using heat (boiling) and agitation. The medium was cooled slowly in a dry oven at 50 °C, and then poured into Petri dishes (9 cm in diameter) in a laminar flow bench.

2.5. Optimization of X-Gal concentration based on S. sonnei colony morphology

Various concentrations of X-Gal (5, 10, 25, 50, and 100 mg/L) were tested in the basal medium for optimization of *Shigella* fermentation. Cell suspensions of *S. sonnei* were streaked onto media containing these X-Gal concentrations using a sterile loop, and then incubated at 37 °C for 12, 18, 24, 36, and 48 h. The plates were inspected for colony morphologies such as color, size, shape, and general appearance.

2.6. Optimization of bile salt concentration based on the growth of injured and healthy S. sonnei cells

The bile salt concentration can affect the growth of bacteria; therefore, it was optimized by evaluating the recovery and growth rates of healthy, heat-injured, and acid-injured *S. sonnei* cells. Heat-injured *S. sonnei* suspensions were prepared by preheating 9.9 ml of sterile 0.2% peptone to 60 °C in a water bath (Vision Scientific Co., Ltd, Daejeon, Korea). Next, 0.1 ml of cell suspension was added to the preheated 0.2% peptone, and the cells were held at 60 °C for 2 min. Acid-injured *S. sonnei* suspensions were prepared by inoculating 0.1 ml of healthy cells into 9.9 ml of citrate buffer (pH 3.5) and incubating for 10 min. Suspensions of healthy and injured cells were immediately subjected to 10-fold serial dilution in sterile 0.2% peptone. Aliquots (0.1 ml) of the serial dilutions were plated in duplicate onto the basal medium + 50 mg/L of X-Gal containing 0.5, 1.0, and 1.5 g of bile salt (Difco). The plates were