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BIOCHEMICAL PROFILES OF *SALMONELLA* SPP ISOLATED FROM FRESH MILK PRODUCTS

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Abstract

Salmonella is still remain an important enteric bacteria that affects human health by the consumption of contaminated food products. The diseases is called as salmonellosis and transmitted by fecal-oral route. The genus *Salmonella* consists of several spesies. This study aimed to employ biochemical assays to detect and characterized *Salmonella* in food sample, especially in fresh milk products in Yogyakarta. In order to detect *Salmonella*, we used the selective enrichment procedure followed by confirmation test in Triple Sugar Iron Agar and Urea broth medium. Candidates of *Salmonella* isolates were characterised by using API kits.Microbiological analysis of the isolated strains based on biochemical assays confirmed the finding of *Salmonella* sp in fresh milk products. These isolates were identified as *Salmonella* spp and *Salmonella* Typhi.

Keywords: *Salmonella,* fresh milk products, biochemical pofile, API

INTRODUCTION

Yogyakarta is well known as a student city. Fresh milks are sold by street vendors and café in Yogyakarta as most of the students consume itdaily. Fresh milk is milk from cows which is processed by heating like pasteurization process but it is not yet perfectly kill the harmful bacteria. Milk is easy to be contaminatedby bacteria and serves as an ideal medium for bacterial growth, which may cause various food borne diseases (Fadei, 2014). Although pasteurization eliminates pathogens and consumption of non-pasteurized dairy product is uncommon, dairy-associated disease outbreaks continue to occur. According to analysis by the Centers for Disease Control and Prevention (CDC) between 1993 and 2006, more than 1,500 people in the United States became sick from drinking raw milk or eating cheese made from raw milk (Langer *et al*., 2012). During 2007-2012, a total of 81 outbreaks associated with non-pasterurized milk were reported from 26 states in the US. These outbreaks resulted in 979 illness and 73 hospitalizations. The causative agent was reported,*ie*. *Campylobacte*r spp, was the most common pathogen, causing 81% outbreaks, followed by Shiga toxin-producing *E. coli* (17%), *Salmonella enterica* serotype Typhimurium (3%) and *Coxiella burnetii* (1%) (Mungai *et al*., 2015). *Salmonella* is the most recognized as an important food-borne pathogen and food poisoning. These pathogens are known as zoonotic pathogens that cause infections both in humans and animals (Aksakal, 2010; Boughton *et al.* 2004; Rathinasabapathi, 2004; Silva and Gibbs 2012).

In accordance with that mentioned in the Approved List of Bacterial Names (Skerman *et al*., 1980), the genus *Salmonella* consists of five species, namely *Salmonella arizonae* with

the type strain ATCC 13314; *Salmonella choleraesuis*, type strain ATCC 13312; *Salmonella enteritidis*, type strain ATCC 13076; *Salmonella typhi*, type strain ATCC 19430 and *Salmonella typhimurium*, type strain ATCC 13311. Kauffmann-White scheme classified *Salmonella* into more than 2,500 serovars based on the basis of somatic (O), flagellar (H), and capsular (Vi) antigens in the cell walls (Aksakal, 2010). Even though all the member of the genus *Salmonella* recognized as major zoonotic pathogens, it is known that only *Salmonella* Typhi caused infection in humans.

The aim of this study is to evaluate the rate of *Salmonella* contamination from the fresh milk using the API 20E and API 50CHE systems. The biochemical profile of these isolates will be classified and analyzed using numerical systematic approach along with the *S. typhi*.

RESEARCH METHOD

Sample. A total of 125 samples of fresh milks (milk collection station) were collected from different places of Yogyakarta. At each location, samples were taken aseptically by sterile glass bottle.

Enrichment culture. Pre-enrichment and enrichment culture were done accord to the recommended prosedures by Taskila *et al*. (2012)and Bacteriological Analytical Manual FDA (Andrew *et al*., 2014). Buffer peptone water (BPW) used for pre-enrichment culture and Rappaport-Vassiliadis Soy broth (RVS) used for enrichment media. Screening of suspected colonies was done in selective medium Salmonella Shigella Agar (SSA) and Chromocult Salmonella Agar (CCA) at 37°C for 24-48 hours. The suspected colonies was confirmed using the Urea broth and Triple Sugar Iron Agar as the defferential media. All of the isolates were grown at 30ºC for 24 h on Brain Heart Infusion (BHI) agar before used (Taskila *et al*., 2012; Turner *et al*.,2000).

Characterization of *Salmonella* isolates. Characterization and identification was done based on morphological characters using selective differential medium and gram staining. *Salmonella*, *Shigella*, and *Yersinia* show clear transparent to blue light colonies depends on the expression of β-glucoronidase enzyme on CCA (Turner *et al*.,2000). Lactose non-fermenting organisms grow as translucent colourless colonies with or without black centres. *Salmonella* is uninhibited and appears as colourless colonies with black centres resulting from H_2S productionon CCA. *Shigella* species also grow as colourless colonies which do not produce H_2S on SSA (Nesa*et al*., 2011). All the suspected colonies were confirmed on urea broth, and H_2S productionon Triple Sugar Iron Agar (TSIA) (WHO,2003). Biochemical testwere done using API systems (20E and 50 CHE) (bioMerieux). The API systems were used to determined biochemical profile of the isolates.

Classification based on numerical systematic analysis. The biochemical profiles as the result of the test were converted into negative or positive values and presented in form of an n x t matrix. N is the quantity of isolates and reference strains being analyzed; t is the quantity of the phenotypic character. The data were edited using Programmer's File Editor (PFE) software and analyzed using the Multi-Variate Statistical Package (MVSP) Plus-Version 2.0 (Kovach 1990) based on the S_{SM} (*Simple Matching Coefficient*) coefficient. Classification was achieved using the UPGMA algorithm (Sneath and Sokal, 1973).

RESULT AND DISCUSSION

Monitoring of *Salmonella* must be routinely done because these bacteria become a serious problem in the field of public health, especially with the increase in the consumption of fresh milk. In an effort to ensure the safety of fresh milk sold in the café or street vendors,we conducted the isolation and identification of *Salmonella* from fresh milk. This study has

successfully detected suspected *Salmonella* colonies. Typical *Salmonella* colonies showed light blue to turquoise colonieson CCA or colorless with black center on SSA (Hoorfar and Baggesen, 1998; Taskila*et al*., 2012). Candidate of *Salmonella* isolates were further selected on Urea Agar and TSIA. The identification was done using the API 20E and 50CHE systems. Finally, we obtained four *Salmonella* isolates. Table 1 showed the biochemical profile of four *Salmonella* isolates. Based on the API tests, it is known that three isolates with the coding number S-35, S-37 and S-69 were identified as *Salmonella* spp at a confidence level greater than 99%.Interestingly, one isolate (Salmonella S-3) appeared to have similarities with *Salmonella typhi* although it only has 72.1% confidence level.

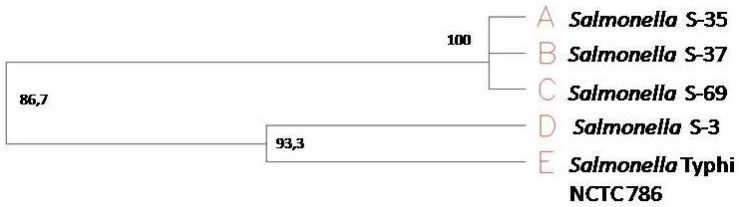

Figure 1. Relationship betwen *Salmonella* isolates based on biochemical characters.

In order to understand the taxonomic relationships among these isolates, it is needed to use numerical systematic analysis in the classificationattemp. Result of grouping based on biochemical characters showed in Figure 1. Two distinct clusters were obtained with UPGMA algorithm. The first cluster consists of three isolates (S-35, S-37 andS-69) with the similarity value of 100%. The second cluster consists of one isolate(S-3). This isolate belong to the same groupwith *Salmonella typhi* NCTC 786. All of the isolates belonged to one group with a similarity index value of 86.7%. This result is also supported by the results of a systematic analysis using numerical taxonomy (Table 2). The data indicated that the numerical systematic analysis provide an effective approach to investigate taxonomic relationships within *Salmonella* strains. The biochemical profiles combined with a systematic numerical analysis able to provide sufficient data to develop a typing and identification. Our results showed that all isolates were belonged to one group with the similarity index value of 86,7%. These included *S. typhi* NCTC786 as a reference strains. Thus it can be concluded that all the isolates tested were a member of the species of *Salmonella typhi* even though they formed different center of diversity. These value is in agreement with the minimum value of classification (0.7) proposed by the taxospecies concept. As high similarity was observed between *Salmonella* spp isolated from marketed fresh milk with *S. typhi*, measures need to be done to ensure its safety. Heating of fresh milk sold at thecafe and street vendors should be able to kill *Salmonella* spp so its safe for consumption.

Table 1. Biochemical profiles of *Salmonella*isolates based on API 20E and API 50 CHE systems.

No	Test	A	B	C	D	E	No	Test	A	B	C	D	E
1	Glycerol	+	+	+	+	+	31	D-Saccharose	-	-	-	-	-
2	Erythritol	-	-	-	-	-	32	D-Trehalose	+	+	+	+	+
3	D-Arabinose	-	-	-	-	-	33	Inuline	-	-	-	-	-
4	L-Arabinose	+	+	+	-	-	34	D-Melezitose	-	-	-	-	-
5	D-Ribose	+	+	+	+	+	35	D-Raffinose	-	-	-	-	-
6	D-Xylose	+	+	+	+	+	36	AmiDon/AMD	-	-	-	-	-
7	L-xylose	-	-	-	-	-	37	Glycogen	-	-	-	-	-
8	D-Adonitol	-	-	-	-	-	38	Xylitol	-	-	-	-	-

9	Methyl-ßD-Xylopyranoside	-	-	-	-	-	39	Gentiobiose	-	-	-	-	-
10	D-Galactose	+	+	+	+	+	40	D-Turanose	-	-	-	-	-
11	D-Glucose	+	+	+	+	+	41	D-Lyxose	-	-	-	-	-
12	D-Fructose	+	+	+	+	+	42	D-Tagatose	-	-	-	-	-
13	D-Mannose	+	+	+	+	+	43	D-Fucose	-	-	-	-	-
14	D-Sorbose	-	-	-	-	-	44	L-Fucose	+	+	+	-	-
15	L-Rhamnose	+	+	+	-	-	45	D-Arabitol	-	-	-	-	-
16	Dulcitol	+	+	+	-	-	46	L-Arabitol	-	-	-	-	-
17	Inositol	-	-	-	-	-	47	Potassium gluconate	+	+	+	+	+
18	D-Manitol	+	+	+	+	+	48	Potassium 2-Cetogluconate	-	-	-	+	-
19	D-Sorbitol	+	+	+	+	+	49	Potassium 5-Cetogluconate	+	+	+	-	-
20	Methyl-αD-Mannopyranoside	-	-	-	-	-	50	ß-galactosidase	-	-	-	-	-
21	Methyl-αD-Glucopyranoside	-	-	-	-	-	51	Arginine dihydrolase	+	+	+	+	-
22	N-AcetylGlucosamine	+	+	+	+	+	52	Lysine decarboxylase	+	+	+	+	+
23	Amygdalin	-	-	-	-	-	53	Ornithine decarboxylase	+	+	+	+	-
24	Arbutin	-	-	-	-	-	54	Utilization of citrate	+	+	+	-	-
25	Esculin ferric citrate	+	+	+	+	+	55	H_2S	+	+	+	+	-
26	Salicin	-	-	-	-	-	56	Urease	-	-	-	-	-
27	D-cellobiose	-	-	-	-	-	57	Tryptophan deaminase	-	-	-	-	-
28	D-Maltose	+	+	+	+	+	58	Indole	-	-	-	-	-
29	D-Lactose (bovine origin)	-	-	-	-	-	59	Acetoin	-	-	-	-	+
30	D-Melibiose	+	+	+	+	+	60	Gelatinase	-	-	-	-	-

Note :
A =*Salmonella* S-35 (*Salmonella*spp) D= *Salmonella* S-3 (*Salmonella* Typhi)
B= *Salmonella* S-37 (*Salmonella*spp) E= *S.* Typhi NCTC 786
C= *Salmonella* S-69 (*Salmonella*spp)

Table 2. Clustering analysis of*Salmonella* isolatesusing unweightedpairgroupmethod.

Node	Group 1	Group 2	Similarity	In fused group
1	A	B	1.000	2
2	NODE 1	C	1.000	3
3	D	E	0.933	2
4	NODE 2	NODE 3	0.867	5

Note: see Table 1.

CONCLUSION AND SUGGESTION

We observed the diversity of *Salmonella* isolates from fresh milks. These isolates have variations in their ability to use the carbon sources. All isolates has a tendency to fall into *Salmonella typhi* with asimilarity of 86,7%. Biochemical profiles could provide a fast and easy way to identify *Salmonella* spp. So, it need further research such as molecular study to clarify the findings. Nevertheless, based on these findings,it is necessaryto prudent the processing ofmilkbefore consumption.

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