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Chemosystematic of *Enterobacteriaceae* Familia Obtained from Blood Cultures Based on Total Protein Profiles

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Abstract

The purpose of this study was to determine the chemosystematic of 14 strains of bacteria in blood cultures from Semarang using 1 reference strain *S. typhi* NCTC 786, based on the total protein profiles with the similarity relationship analysis based on *Simple Matching Coefficient* (S_{SM}) analysis and algorithm method of *unweighted pair group with averages* (UPGMA) presented in a dendrogram. The results showed that the chemosystematic based on the total protein profiles using SDS-PAGE method can classify the member of bacterial strains of each species. The Clusters respectively consist of 4 strains of *S. typhi* (similarity: 89.7%), 2 strains of *Ser. marcescens* (similarity: 89.7%), two strains of *E. coli*, and one strain of *Salmonella* ssp, *S. typhi* NCTC 786 (similarity: 100%). Those three incorporated clusters had the similarity value of 75.3%. Those four strains of *Ent. cloacae* composed in one cluster (similarity: 100%) are incorporated in a cluster consisting of one strain of *Kleb. pneumoniae* (similarity: 92.9%). Both clusters were incorporated in a cluster consisting of S. *typhi* NCTC 786 (similarity: 67.9%).

Key words: *Enterobacteriaceae*, chemosystematic, blood cultures, protein profile

Introduction

In Semarang, typhoid fever is determined as the third of 10 major diseases after Dengue Fever, diarrhea, and gastroenteritis (Anonymous, 2008). Typhoid fever is a systemic infectious disease caused by *Salmonella typhi* bacteria *(S. typhi),* with no specific clinical symptoms, thus the gold standard diagnosis of this disease should be conducted in laboratory tests (Koharo *et al*., 2010; Ley *et al*., 2010; Fadeel *et al*., 2011).

Widal test is considered as the simplest, easiest, cheapest and fastest laboratory test. However, its sensitivity, specificity and prediction value are varies because anti-O and anti-H can be found in patients infected by the member of *Enterobacteriaceae* familia species beside *S. typhi* (Novianti, 2006; Beig *et al*., 2010). The finding of *S. typhi* in blood cultures or in bone marrow is the gold standard of typhoid fever (Khoharo *et al*., 2010; Ley *et al*., 2010). Blood culture success rate varies from 40% to 89% compared to *S. typhi* isolation success rate. The success of obtaining *S. typhi* isolates from positive Widal blood cultures was 10.74% (Amarantini *et al*.2009). These showed that there are other bacteria types beside *S. typhi*

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such as *Salmonella* ssp., *Escherichia coli (E. coli), Enterobacter cloacae (Ent. cloacae), Serratia marcescens (Ser. marcescens),* and *Klebsiella pneumoniae (Kleb. pneumoniae)* (Darmawati *et al*., 2012).

Identification of species and strains diversity of bacteria can be conducted with polyphasic systematic approach, by combining the systematic of phenetics numeric, and molecular (Sembiring, 2004; Vandamme *et al*., 1996). This approach uses phenotypic and genotypic characteristics. The biochemical and chemical characteristics of total protein profile bacterial cell with *sodium dodecyl sulphate polyacrylamide gel electrophoresis* (SDS-PAGE) method can be used as chemical classification by numerical methods (Berber, 2004; Berber and Yenidunya, 2005; Amarantini, 2010; Yanti, 2011). The Diversity of bacterial species including *Enterobacteriaceae* familia and strain diversity of the member of each species can be classified based on the physiological characteristic differences, such as the ability of fermenting various carbohydrates, and its resistance to many antibiotics (Darmawati *et al*., 2011; Darmawati *et al*., 2012). This shows the existence of genetic variations in both inter-species and inter-individual member of each species.

Thus, the objective of was to classify bacteria of *Enterobacteriaceae* familia members based on its phenetics numeric in positive Widal blood cultures from Semarang based on its total protein profiles using UPGMA algorithm (unweighted pair group method with averages), so it enable to describe the similarities and differences of total protein profiles as genetic expression owned by each individual member of each species of bacteria, including *Enterobacteriaceae* that further can describe its morphology.

Materials and Methods

Strains of bacteria

There were 14 bacteria strains used in this study (4 strains of *S. typhi*: BA 07.4, 30.3 KD, KD 30.4, 02.2 SA; 1 strain of *Salmonella* ssp. BA 30.05, 2 strains of *E. coli*: BA and BA 30.1 30.2; 2 strains of *Ser. marcescens*: 08.4 KD, 08.5 KD, 4 strains of *Ent. cloacae*: BA 45.4.1, TG 03.5, KT 16, SA 02.1 and 1 strain of *Kleb. pneumoniae* KD 58.4) using one reference strain of *S. typhi* NCTC 786, from in and outpatient positive widal blood samples from Semarang (Tugurejo Hospital, Semarang Hospital, Sultan Agung Islamic Hospital, Bangetayu and Kedungmundu Public Health Centers). Bacterial identification was conducted by API 20E and API 50CHB /E media (Bio Merieux Inc.).

The soluble total protein isolation and protein separation

The soluble total protein was obtained by growing a colony of bacteria in 100 ml of BHI liquid, incubated at 37˚ C for 18 h. Bacteria cultures were then centrifuged at the temperature of 4˚C, at the speed of 3000 rpm for 20 min. Presipitans were washed by suspending them using 0.1 M PBS (Phosphate buffered saline) at pH 7.4. These washings were conducted 3 times. the presipitans then were re-suspended in 1.5 ml PBS at pH 7.4. The bacterial suspensions were sonicated 6 times in 30 sec with the rest period of 30 sec at 4˚C. *repeating duty cycle* Amplitude used was 0.7. The results of bacterial sonicated suspension were centrifuged at the temperature of 4 ˚C, at the speed of 12 000 rpm for 20 min. Supernatant obtained was the soluble total protein cells which protein concentration was readily measured using the reagent of *Protein Assay CBB solution* (5x) (Nacalai Tesque Inc.: code 29440-44, Kyoto Japan). The soluble protein absorption was then measured at a wavelength of 595 nm and separated using SDS-PAGE methods with ATTO corporation ATTA Tokyo Japan (AE-6530M/AE-6530P), stained with 0.25% *Coomassie Brilliant Blue* R250.

Data analysis

Data coding, unit character coding was done by giving a score. Positive unit character (+) was given score 1 while the negative unit character (-) was given score 0. The edited

data was then analyzed using MVSP program (*Multi-variate Statistical Package*) version 3.1 A The inter-strains similarity relationship was known by using *Simple Matching Coefficients* (S_{SM}). The clusterization is conducted by using *unweighted Pair Group Methode with Averages* (UPGMA) algorithms. The results of the analysis were finally presented in the form of dendrogram using *Adhobe photoshop* program.

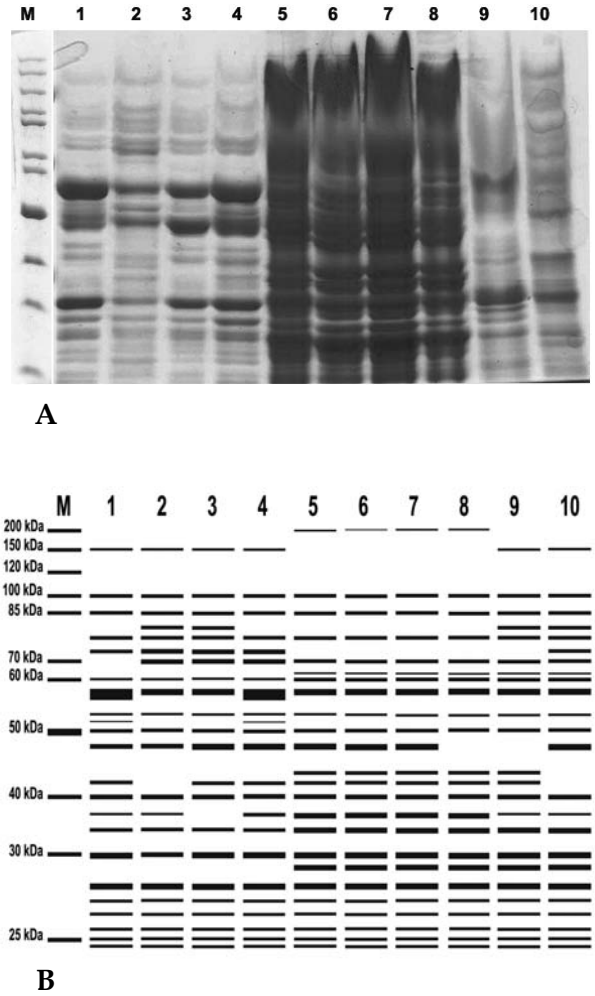

Figure 1.(A). Visualization of soluble total protein profiles on SDS-PAGE (B). Visualization of diagrammatic representation of 9 strain bacteria of *Enterobacteriaceae* familia members and the reference strains of *S. typhi* NCTC 786 M of soluble total protein profiles) protein marker, 1) BA 07.4, 2) KD 30.3, 3) SA 02.2, 4) KD 30.4, 5) *S. typhi* NCTC 786, 6) BA 30.5, 7) BA 30.1, 8) BA 30.2, 9) KD 08.4, 10) KD 08.5

Results and Discussion

The separated soluble total protein bacterial cells by SDS-PAGE (Figure 1A and 2A), was visualized with diagrammatic representation (Figure 1B and 2B). Data was

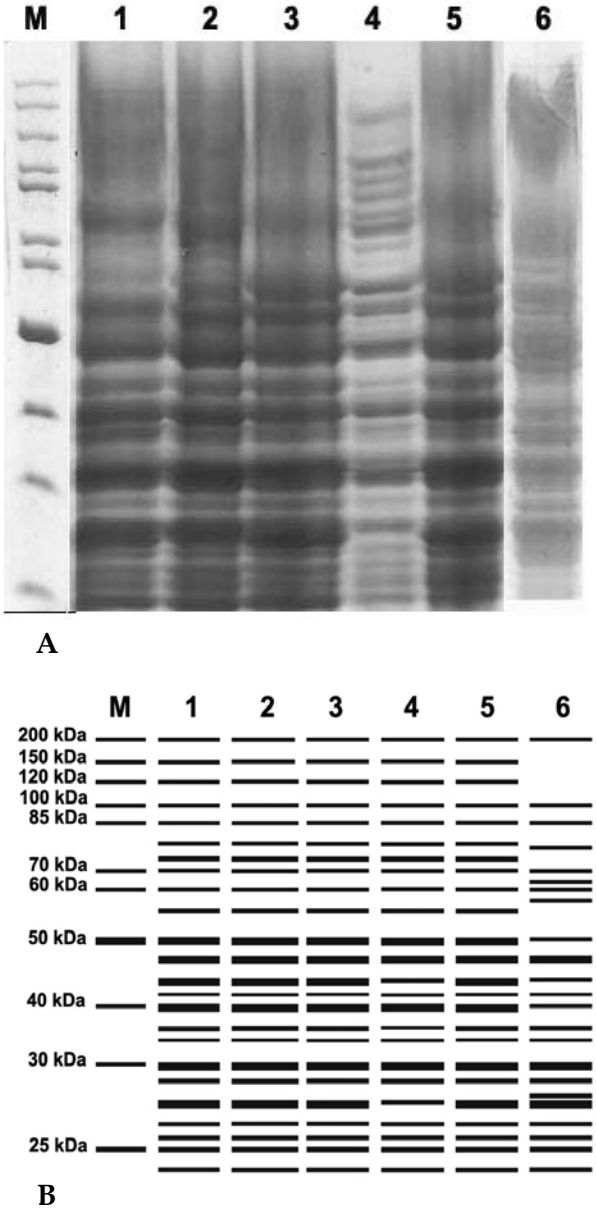

Figure 2. (A). Visualization of soluble total protein profiles on SDS-PAGE (B). Visualization of diagrammatic representation of five strains of *Enterobacteriaceae* familia members and the reference strain of *S. typhi* NCTC 786 M of soluble total protein profiles) Marker protein, 1) BA 45.4.1, 2) TG 03.5, 3) KT 16, 4) SA 02.1, 5) KD 58.4, 6) S. typhi NCTC 786.

then edited and analyzed with the MVSP 3.1A program, the inter-strains similarity relationship was analyzed with S_{SM} and then was classified with UPGMA algorithm. The results of the analysis were presented in a dendrogram by *Adhobe photo shop* program (Figure 3 and 4).

The Dendrogram showing the similarity relationship between 9 strains of *Enterobacteriaceae* familia members and 1 reference strain *S. typhi* NCTC 786 (Figure 3) was composed into two clusters. The first cluster consists of four strains of *S. typhi* composed of 3 incorporated sub-clusters

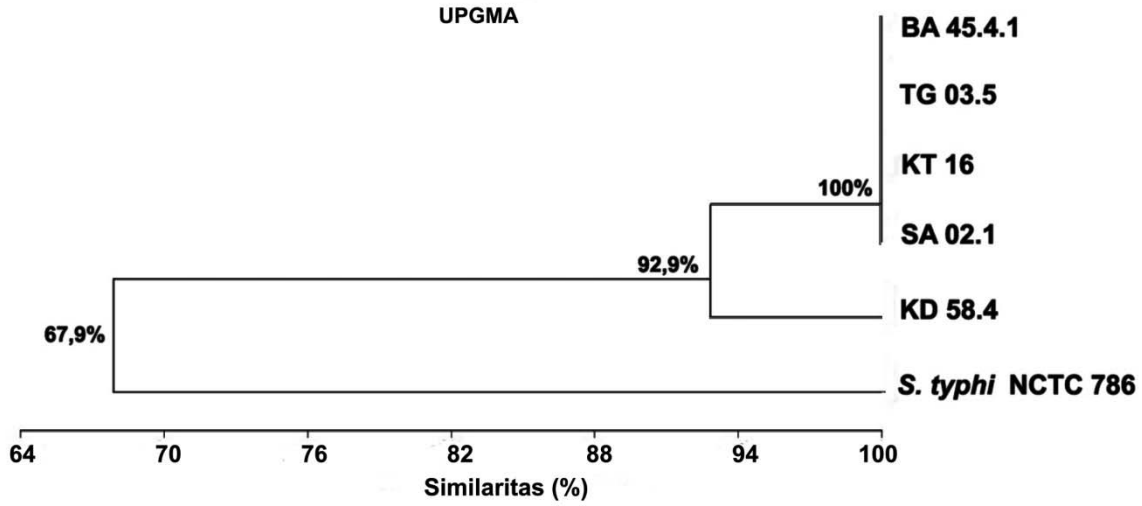

Figure 3. Dendrogram showing the similarity relationship between 9 strains of *Enterobacteriaceae* familia members (*S. typhi* SA 02.2; *S. typhi* KD 30.3; *S. typhi* BA 07.4; *S. typhi* KD 30.4; *Ser. Marcescens* KD 08.4; *Ser. Marcescens* KD 08.5; *Salmonella ssp.* BA 30.5; *E. coli* BA 30.2; *E. coli* BA 30.1) with reference strains of *S. typhi* NCTC 786 based on total protein profiles based on *Simple Matching Coefficient* analysis and UPGMA algorithm

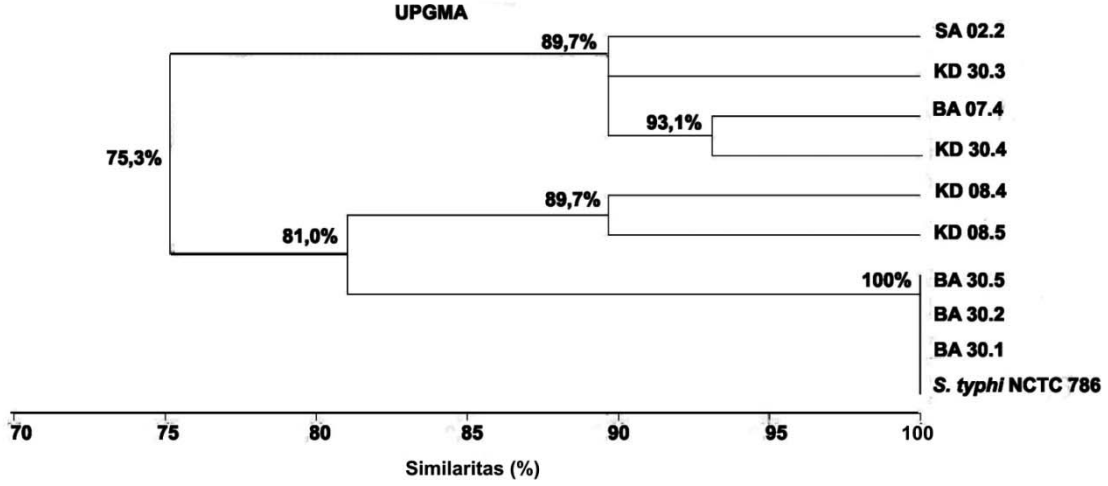

Figure 4. Dendogram showing the similarity relationship between the 5 strains of *Enterobacteriaceae* familia members (*Ent. cloacae* BA 45.4.1; *Ent. Cloacae* TG 03.5; *Ent. Cloacae* KT 16; *Ent. Cloacae* SA 02.1; *Kleb. Pneumoniae* KD 58.4)) with the reference strain of *S . typhi* NCTC 786 based on total protein profiles based on *Simple Matching Coefficient* analysis and UPGMA algorithm.

with the similarity value of 89.7%. The first sub-cluster consists of two strains (KD 30.3 and BA 07.4) with the similarity value of 93.1%, the second and third sub-clusters each respectively consists of 1 strain; KD 30.3 and 02.2 SA. The second cluster was composed of two incorporated sub-clusters with the similarity value of 81.0%. The first sub-cluster consists of two *S.marcescens* strains (KD 08.4 and KD 08.5) with the similarity value of 89.7%, and the second sub-cluster was composed of four strains of bacteria consisting of two *E. coli* strains (BA 30.1 and BA 30.2), 1 *Salmonella* ssp. strain BA 30.5 and one reference strain of *S. typhi* NCTC 786 with incorporated similarity value of 100%. The incorporated similarity value of the first and second clusters is 75.3%.

The similarity relationship between 5 strains of *Enterobacteriaceae* familia members and 1 reference strain of *S. typhi* NCTC 786 is shown in the dendrogram (Figure 4) composed of two incorporated clusters with the similarity value of 67.9%. The first cluster is composed of two sub-clusters; the first sub-cluster consists of 4 strains of *Ent. cloacae* (BA 45.4.1, TG 03.5, KT 16, SA 02.1) with the similarity value of 100%, while the second sub-cluster consists of one strain of *Kleb. pneumoniae* KD 58.4.

Dendrogram (Figures 3 and 4) may indicate the diversity of bacteria species of *Enterobacteriaceae* familia members in clusters or sub-clusters levels. The strain members of *S. typhi, Ser. marcescens, E. coli* and *Salmonella* ssp. have higher similarity (75.3%) upon the reference strain of *S. typhi* NCTC 786 than the strain members of *Ent. cloacae* and *Kleb. pneumoniae* upon the same reference strains (67.9%). Both dendrogram composed based on the total protein profile has a similar topology with the dendrogram based on phenotypic characters characterized using API 20E and API 50CHB/E media (Darmawati *et al*., 2012). The results of this study are also in accordance with several other studies that the total protein profile analysis using SDS-PAGE method is identical to identification and classification results based on the biochemical characteristics (Amarantini, 2010; Yanti, 2011).

Vandamme *et al*. (1996) states that the total protein profiles obtained with SDS-PAGE method can be used to compare and classify a number of strains. In addition, some studies have also shown that the total protein profiles have a high similarity with the results of DNA-DNA hybridization.

The results of classification based on biochemical characteristics seem congruent with the classification results based on the total protein profiles. These because biochemical characteristics are the enzymatic activity reflections that enzyme is composed of protein while protein is genome reflection, thus the classification based on biochemical characteristics and total protein profiles will result an accurate classification.

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