# Analysis of Whole Cell Protein Profiles by SDS-PAGE to Identify Indigenous Cellulose-producer Acetic Acid Bacteria

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**Abstract**

This study was carried out to analyze the suitability of the identification of four indigenous cellulose-producing acetic acid bacterial isolates (ANG29, KRE65, ANG32 and SAL53) based on the analysis of whole cellular protein profiles against identification based on phenotypic traits. Whole cellular protein profiles were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method. The whole cellular protein profiles obtained from sample isolates, were compared with reference isolates for species identification. The results showed that based on visual observations can be determined as much as 12 bands of protein with a molecular weight of 19,099 KDa up to 132.182 KDa. Based on the analysis of protein bands were detected visually, fourth indigenous cellulose- producing acetic acid bacterial isolates in the study had a higher similarity profile to the reference strain *Gluconacetobacter xylinus* BTCC 769 compared with other reference strains namely *G. hansenii* NBRC 14820$^T$. This condition is consistent with the results of the identification of fourth cellulose producing acetic acid bacterial isolates based on phenotypic traits. Thus, the whole cellular protein profiles by SDS-PAGE technique can be used as a one of method to identification of cellulose producing acetic acid bacterial isolates.

**Keywords**: Acetic acid bacteria, bacterial cellulose, identification, SDS-PAGE, whole cellular protein

## Introduction

Acetic acid bacteria (AAB) is a group of bacteria with common characteristics have the ability to oxidize alcohol and sugar, especially oxidize ethanol to acetic acid and have been widely used in the commercial industry such as the production of acetic acid, gluconate, sorbose (Jojima *et al*., 2004), and cellulose (Swissa *et al*., 1980). This bacterial group indicates the Gram negative reactions, ellipsoid to rod cells and some of its members have the ability to produce extracellular cellulose (Hanmoungjai *et al*., 2007). Acetic acid bacteria are known to have good adaptability on liquids that contain lots of sugar and alcohol (Jojima *et al*., 2004), so that the main habitat in nature which include acetic acid, ripe fruits, liquid latex plants, alcoholic drinks and flowers.

Several studies have been conducted to isolate *Gluconacetobacter* of fruits (Kojima *et al*., 1998; Dellaglio *et al*., 2005), flowers, food fermentations (Lisdiyanti *et al*., 2001; Park *et al*., 2003), drinks (Jia *et al*., 2004; Kadere *et al*., 2008), and vinegar (Sokollek *et al*., 1998; Son *et al*., 2002; Hwan *et al*., 2004). Although some other types of bacteria such as *Agrobacterium*, *Pseudomonas*, *Rhizobium*, and *Sarcina* have the ability to produce cellulose, *Gluconacetobacter* is known as cellulose-producing bacteria with the highest capability (Jonas and Farah, 1998; Klemm *et al*., 2005).

Systematically, acetic acid bacteria are classified into Acetobacteraceae family as one evolutionary line branching subclasses of α-Proteobacteria (De Ley *et al*., 1984; Sievers *et*

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*al*., 1994). At first, acetic acid bacteria were classified into three genera, namely *Gluconobacter*, *Acetobacter*, and *Fratueria* (Holt *et al*., 1994). Currently, Acetobacteraceae family consists of 10 genera (Muramatsu *et al*., 2009), namely *Acetobacter*, *Acidomonas*, *Asaia*, *Gluconacetobacter*, *Gluconobacter*, *Granulibacter*, *Kozakia*, *Neoasaia*, *Saccharibacter*, and *Swaminathania*. The latest genus found is *Granulibacter* (Greenberg *et al*., 2006), which is the only member of the Acetobacteraceae family that is pathogenic.

Sodium Dodecyl Sulphate Poliacrylamide Gel Electrophoresis (SDS-PAGE) protein pattern analysis has been successfully used to identify various types of bacteria, including *Listeria* species (Park *et al*., 2006), lactic acid bacteria (Ghazi *et al*., 2009), *Salmonella* serovars (Aksakal, 2010), and to solve problems in the identification of closely related species in the genera *Lactobacillus* and *Leuconostoc* (Dicks *et al*., 1990; Pot *et al*., 1993; Kim *et al*., 2003; Sanchez *et al*., 2003; Jin *et al*., 2008). However, the usefulness of this method for the identification of cellulose producing acetic acid bacteria strains isolated from ripe fruits and nata inoculum has not yet been assessed. In this article, we screened and identified acetic acid bacteria strains using SDS-PAGE of whole-cell proteins. The objective of this study was to analyze the suitability of identification of four indigenous cellulose producing acetic acid bacterial strain (ANG29, KRE65, ANG32 and SAL53) based on the analysis of total cellular protein profiles againts identification based on phenotypic traits.

## Materials and Methods

### *Acetic acid bacteria strains*

Four strains indigenous cellulose-producing acetic acid bacteria used in this study namely ANG29, KRE65, ANG32 and SAL53 were potentially cellulose producing strains isolated from ripe fruits and Nata de Coco inoculum. The reference strains used in this research were *Gluconacetobacter hansenii* NBRC 14820$^T$ obtained from NITE Biological Rensource Center (NBRC) Japan and *Gluconacetobacter xylinus* BTCC 769 obtain from Biotechnology Culture Collection (BTCC) LIPI Cibinong, Indonesia.

### *Extraction of total proteins*

Identification of four selected strains with a chemosystematic approach is based on the protein finger printing. Proteins were analyzed in the dissolved form and whole cell proteins were visualized using SDS-PAGE. Furthermore, whole protein fingerprint profiles were obtained from all selected strains and reference strains analyzed numerically to determine the value of similarity.

Cell protein extraction and electrophoresis were carried out by modification of the procedure developed by Scopes (1987) and Aksakal (2010). Four strains indigenous cellulose-producing acetic acid bacteria (ANG29, KRE65, ANG32 and SAL53) and two reference strains (*G. xylinus* 769 BTCC and *G. hansenii* NBRC 14820T) each was grown in 100 ml of Hestrin-Schramm (HS) medium and then incubated aerobically in a shaker incubator at 100 rpm, 30°C for 72 hours. Suspension cultures of each bacteria centrifuged at 15,000 rpm, 4°C for 15 minutes. Cell pellets were suspended in 10 ml of buffer solution of 0.1 M phosphate buffer saline pH 7.4. One ml suspension transferred to a 1.5 ml microsentrifuge tube and centrifuged at 15,000 rpm for 15 min at 4°C. The precipitate entrained resuspended in 10 mL solution of 10% SDS and 10 mL of loading buffer solution [0,125M Tris (hydroxymethyl) aminomethane, 4% SDS, 10% 2-mercaptoethanol, 0.2% Bromophenol blue; pH 6.8]. The mixture was then homogenized with a vortex, then the sample was boiled for 10 minutes at 100°C, centrifuged for 1 minute (15,000 rpm at 20°C). Supernatant obtained was taken and used as a protein sample. The protein content of the sample was measured by the method of Bradford (Bradford, 1976) using Bio-rad protein assay.

### *Electrophoresis*

Samples of protein (supernatant) as much as 30 mL was added on to electrophoresis loading buffer 5X (6 mL) and then incubated at a temperature of 100°C for 2 minutes and as soon as may be stored at 0°C. The

concentration of protein samples to be electrophoresed synchronized by means of dilution..

Soluble protein profiles of bacterial cells visualized by SDS-PAGE. The gel used is discontinuous gel consisting of 5% stacking gel and 10% resolving gel. The concentration of protein samples were loaded into the wells, the concentration was adjusted to 35 µg/ml. Marker protein used is a product of Fermentas (Fermentas life sciences). Gels were stained with a solution of 1% coomassie brilliant blue destaining then performed with a mixed solution of methanol and glacial acetic acid and distilled water at a ratio of 5:1:4, until the protein bands clearly visible.

The molecular weight of each protein band in the gel of each strain of bacteria is determined by measuring the molecular mobility of proteins in polyacrylamide gel based protein molecular weight standard curve of reference. Reference (marker) protein that known molecular weight, electrophoresed along with the tested bacterial protein, and mobility (retention factor) was calculated using the formula:

$$Rf = \frac{Distance\ movement\ protein\ bands\ from\ a\ starting\ \ place}{Distance\ movement\ tracker\ color\ from\ a\ starting\ place}$$

The standard curve of reference protein molecular weight was made to the linear regression equation of the relationship between the retention factor (X-axis) and the logarithm of the molecular weight (Y-axis).

***Data analysis of protein profiles***

Data were analyzed using MVSP Plus software-version 3.1 (Kovach, 2007) to determine the similarity between strains. Similarity value is determined using simple matching coefficient (SSM), the grouping is done by algorithms UPGMA (Unweighted Pair Group Method with Arithmetic Averages) (Sneath & Sokal, 1973). The results of the analysis were presented as dendogram using the Corel Draw X5. Dendogram produced subsequently used as a basis to determine the similarity between strains. Identification of indigenous cellulose-producing acetic acid strain conducted by comparing the similarity index between tested strains with reference strains.

**Results and Discussion**

Dendrogram constructed based on 54 phenotypic characters to identify all strains in the species level by numerical systematic analysis showed that cellulose-producing acetic acid bacteria strains in this study more closely the similarity to the reference strain *G. xylinus* BTCC 769 compared to the other two strains of reference namely *G. hansenii* NBRC 14820$^T$ and *G. liquefaciens* NBRC 12388$^T$ (data not shown). The whole soluble protein profiles of cellulose-producing acetic acid bacteria strains along with reference strains visualized by SDS-PAGE method is shown in Figure 1. Representative diagram shown in Figure 2.

Construction the representative diagram of dissolved whole cell protein profiles done using the Corel Draw X5, then the data on the protein bands representative diagram estimated molecular weight is determined by molecular weight protein markers (Table 1). Data molecular weight protein bands in Table 1 were then analyzed by numerical systematic method based on the Simple Matching Coefficient (SSM) and the algorithm Unweighted Pair Group Method with Arithmetic Average Encryption (UPGMA) was then constructed in the form of dendrogram (Figure 3).

Analysis of whole cell protein extracts against four strains of indigenous cellulose-producing acetic acid bacteria showed differences between the tested strains. Four tested and two reference strains showed 12 protein bands with three predominant protein bands i.e protein with a molecular weight of 102.015 kDa; 81.750 kDa and 69.168 kDa.

Among the 12 occurring protein bands, there are six protein bands shared by all strains i.e protein band with a molecular weight of 102.015 kDa; 81.750 kDa; 69.168 kDa; 49.515 kDa; 43.681 kDa and 19.099 kDa. Reference strain *G. xylinus* BTCC 769 had eleven protein bands so that there was one protein band that was not owned by the 132.182 kDa. Strain of ANG 29 had nine protein bands so that there

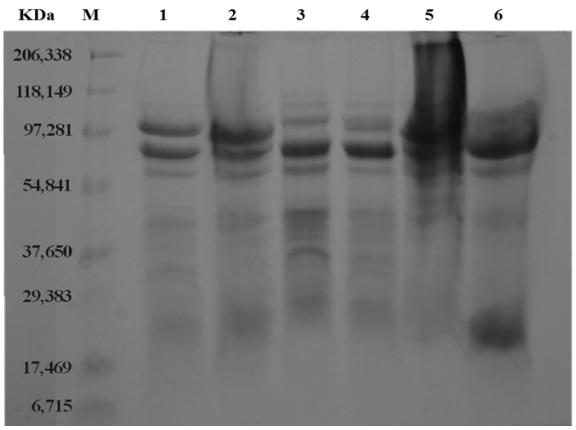


**Figure 1**. Profile of total soluble protein from selected acetic acid bacteria and reference strains using SDS PAGE. Lane 1 (*G. xylinus* BTCC 769), lane 2 (ANG 29 isolates), lane 3 (isolate ANG 32), lane 4 (isolate SAL 53), lane 5 (isolate KRE 65), lane 6 (*G. hansenii* NBRC 14 820 ), M (marker molecular weight proteins).

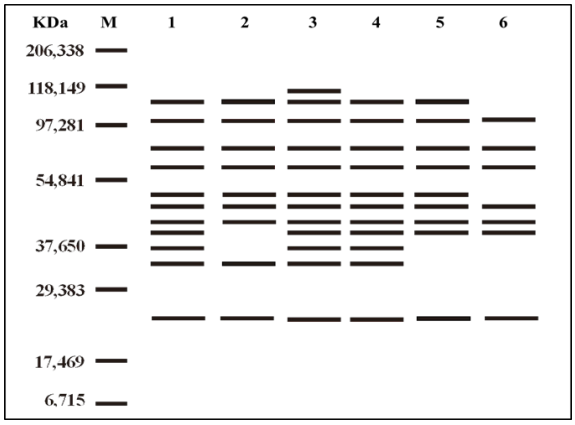


**Figure** 2. Representative diagram of total protein SDS-PAGE of selected acetic acid bacteria and reference strains. Lane 1 (*G. xylinus* BTCC 769), lane 2 (ANG 29 isolates), lane 3 (isolate ANG 32), lane 4 (isolate SAL 53), lane 5 (isolate KRE 65), lane 6 (*G. hansenii* NBRC 14 820 ), M (marker molecular weight proteins).

were three protein bands did not appear, namely 132.182 kDa; 39.845 kDa and 34.858 kDa. Strain of ANG 32 showed twelve protein bands, so that to become strain with highest number of protein bands. SAL 53 strain showed eleven protein bands together with a reference strain *G. xylinus* BTCC 769, so just less one protein band that is 132.182 kDa. KRE 65 strain showed nine protein bands and there are 3 protein bands did not appear, namely 132.182 kDa; 34.858 kDa and 30.623 kDa. The other reference strain *G. hansenii* NBRC 1420$^T$ has the least amount of protein bands among all tested strains because there are only 7 protein bands and there were 5 protein bands that were not owned by the 132.182 kDa; 120.573 kDa; 54.967 kDa; 34.858 kDa and 30.623 kDa.

Based on the dendrogram from numerical analysis of whole cellular protein profiles four cellulose-producing acetic acid bacteria strains and two reference strains (Figure 3), indicating that the strains separated into three distinct clusters that joined at the similarity value of 68.3%. The first cluster consists of three strains namely *G. xylinus*

**Table 1**. The molecular weight of protein from selected acetic acid bacteria and members of the genus *Gluconacetobacter* reference strains..

| No. | Molecular weight of protein bands (KDa) | *G. xylinus* BTCC 769 | ANG 29 | ANG 32 | SAL 53 | KRE 65 | *G. hanssenii* NBRC 14820 |
|---|---|---|---|---|---|---|---|
| 1. | 132,182 | - | - | + | - | - | - |
| 2. | 120,573 | + | + | + | + | + | - |
| 3. | 102,015 | + | + | + | + | + | + |
| 4. | 81,750 | + | + | + | + | + | + |
| 5. | 69,168 | + | + | + | + | + | + |
| 6. | 54,967 | + | + | + | + | + | - |
| 7. | 49,515 | + | + | + | + | + | + |
| 8. | 43,681 | + | + | + | + | + | + |
| 9. | 39,845 | + | - | + | + | + | + |
| 10. | 34,858 | + | - | + | + | - | - |
| 11. | 30,623 | + | + | + | + | - | - |
| 12. | 19,099 | + | + | + | + | + | + |

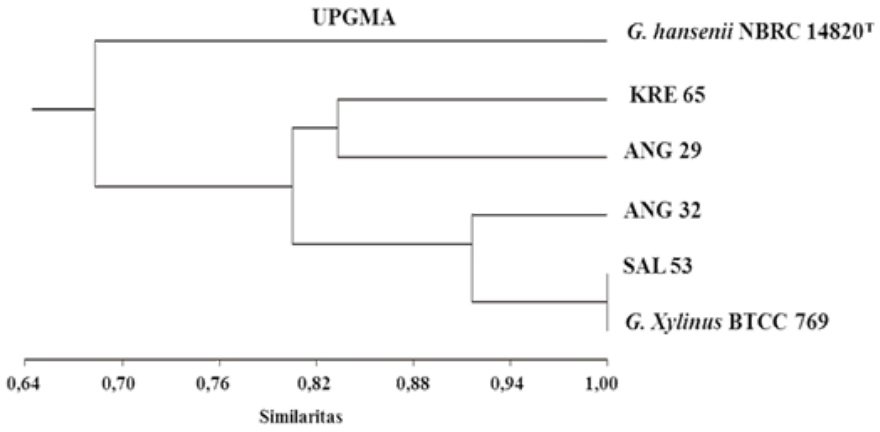


**Figure 3**. The molecular weight of protein from selected acetic acid bacteria and members of the genus *Gluconacetobacter* reference strains.

BTCC 769, SAL 53 and ANG 32 that joined in a cluster with a similarity value of 91.7%. Reference strain *G. xylinus* BTCC 769 and SAL 53 joined in a cluster with a similarity value of 100%. The third member of this first cluster different from each other because they do not have one specific band with the same molecular weight was132.182 kDa (Table 1). This protein band owned only by isolates ANG 32.

The second cluster consisting of two isolates namely ANG 29 and KRE 65 who joined with the similarity index of 83.3%. The first and second cluster joins with similarity index value of 80.6%. The second cluster members do not have two specific protein bands with the same molecular weight 132.182 and 34.858 kDa and a different specific protein with molecular weight of 39.845 kDa in the strain ANG 29 and 30.623 kDa in strain KRE 65. The third cluster consists of a reference strain *G. hansenii* NBRC 14820 were joined by the other cluster with similarity index of 68.3%.

Based on total soluble cellular protein profiles can be explained that the four cellulose-producing acetic acid bacteria showed higher similarity profile to the reference strain *G. xylinus* BTCC 769 than *G. hansenii* NBRC 14820$^T$. Strain SAL 53 have the highest level of similarity with the reference strain *G. xylinus* BTCC 769 with 100% similarity index. Strain ANG 32 has lower similarity with similarity index of 91.7%. Strains ANG 29 and KRE 65 have the lowest similarity to *G. xylinus* BTCC 769 with a similarity index of 80.6%. Fourth strains have low similarity with the reference strain *G. hansenii* NBRC 14820 with an index of similarity 68.3%. Dendrogram constructed based on the chemical character cellular protein profiles showed that the cellular protein profiles quite well used to differentiate strains of acetic acid bacteria based on an index of similarity. Higher similarity of strains ANG 29, ANG 32, SAL 53 and KRE 65 to the reference strain *G. xylinus* BTCC 769 in good agreement with numerical analysis based on phenotypic characters. This reinforces the notion that the four strains of indigenous cellulose producing acetic acid bacteria in this research were members of the species *G. xylinus*.

These results were consistent with previous research using SDS-PAGE techniques to distinguish the whole cellular protein profiles in the group of lactic acid bacteria (Ghazi *et al*., 2009). However, a different result was demonstrated when the technique is applied to a group of bacteria of the genus Salmonella (Aksakal, 2010). Ghazi *et al*. (2009) states that the result of analysis of whole cellular protein profiles can be used to strengthen, complement and confirm the results of the phenotypic identification of lactic acid bacteria group. SDS-PAGE method can generate a complex pattern of protein bands and stable so that it can be used to interpret and compare the protein profiles of samples tested strain with the reference strain. Meanwhile, Aksakal (2010) concluded that whole cellular protein profiles cannot be used to distinguish between strains in the genus *Salmonella*. This study showed that the whole cellular protein profiles by SDS-PAGE

technique can be used as one method to identify the new strains of acetic acid bacteria, although it is not necessarily suitable to the other group of bacteria. Congruence of differences between bacterial isolates between the total cellular protein profile and the phenotypic character of selected cellulose-producing acetic acid bacterial isolates with the reference strains indicate that at least, the protein profile can be used as a support for the identification of cellulose-producing acetic acid bacteria based on phenotypic characters.

**Conclusions**

Whole cell protein profiles can be used to differentiate cellulose-producing acetic acid bacteria strains based on index of similarity, thus can be used as one method for identifying cellulose-producing acetic acid bacteria strains to supplement the results of the phenotypically identification.

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