Whole genome analysis of Klebsiella: Unique genes associated with isolates from Indonesian tempeh

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ABSTRACT

Aims: Our previous study demonstrated that Klebsiella IIEMP-3 associated with tempeh was genetically different from those of medical isolates. In addition to the whole genome sequence of Klebsiella IIEMP-3, the draft genome sequence of another isolate, i.e. IWJB-6 was employed for comparison. In this study, the details of the virulence genes and unique gene in both Klebsiella isolates were compared employing in silico and in vitro analysis.

Methodology and results: Whole genome of Klebsiella IIEMP-3 and IWJB-6 were annotated to investigate the virulence factor. Klebsiella IIEMP-3 and IWJB-6 were obtained from tempeh producers in Bogor, West Java - Indonesia. Genome sequences were analyzed employing BLAST Ring Image Generator (BRIG) software. The results showed that all of the samples, including isolates IIEMP-3 and IWJB-6 did not harbor rmpA, i.e. DNA sequence for K. pneumoniae virulence factor.

Conclusion, significance and impact of study: Klebsiella could be found in almost all tempeh samples from Indonesia and could be harmless for human due to the absence of rmpA and other virulence-associated genes. The significance of this study showed that IIEMP-3 and IWJB-6 isolates were more closely related to K. variicola. However, K. variicola At-22 harbored sdsA gene which is lacking in those two tempeh isolates. Combined with PCR analysis for specific gene/s, our study suggested that isolates from Indonesian tempeh were closely related to K. variicola, and proposed to be designated as K. variicola subsp. tempehensis.

Keywords: Genome annotation, Klebsiella variicola subsp. tempehensis, sdsA, virulence factor

INTRODUCTION

Tempeh is an Indonesian indigenous soy fermented food which is also one of the most important protein source for most Indonesian. During the process of fermentation, in addition to Rhizopus spp., bacteria also play important roles in the formation of flavor and nutrient content. In tempeh, one of the bacteria that contributes to tempeh nutrient content is Klebsiella pneumoniae. Isolates of K. pneumoniae in tempeh are well known as vitamin B12 producers (Keuth and Bisping, 1994).

Klebsiella spp., especially K. pneumoniae, is found in the diverse environment, associated with plant and human, and is known as human pathogens that show the mucoid phenotype. Klebsiella pneumoniae of medical isolates are usually associated with human and cause infectious disease in human (Holt et al., 2015). Mucoviscosity associated gene A (maga) and regulator of mucoid phenotype (rmpA) are the genes that act in the production of mucoid phenotype in K. pneumoniae of medical isolates (Nasif et al., 1989; Fang et al., 2004).

The gene encoding rmpA is only 536 bp in length (Nadasy et al., 2007).

However, Klebsiella spp. have also been found in tempeh and these isolates usually did not indicate the presence of mucoid phenotype. Keuth and Bisping (1994) reported that Klebsiella from tempeh is safe for consumption because it did not contain enterotoxin genes. Genetically, based on 16S rRNA followed by Enterobacterial Repetitive Intergenic Consensus - Polymerase Chain Reaction (ERIC-PCR), K. pneumoniae from tempeh were shown to be different from those of medical isolates (Ayu et al., 2014).

Whole genome data of Klebsiella IIEMP-3 and IWJB-6 was used to scan for the presence of virulence genes and other genes that can distinguish the diversity of Klebsiella from tempeh. Genome annotation indicated that isolates from Indonesian tempeh were lack of virulence gene/s associated with pathogenic Klebsiella. Although the tempeh isolates were closely related to K. variicola At-22, they did not possess the unique genes available in K. variicola At-22, such as sdsA.

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Our previous study demonstrated that *Klebsiella* IIEMP-3 associated with tempeh was genetically different from those of medical isolate. In addition to the whole genome sequence of *Klebsiella* IIEMP-3, the draft genome sequence of another isolate, i.e. IWJB-6 was employed for comparison. In this study, the details of the virulence genes in both *Klebsiella* isolates were compared employing *in silico* and *in vitro* analysis.

**MATERIALS AND METHODS**

Tempeh samples

Tempeh samples were obtained from Jakarta (KA, KOP, KJA, RA, and CA); West Java (MWR and CIO); Central Java (BTG and CPU); Jogjakarta (SLM); East Java (SU, MA, PA, GW, and PCTN); Sulawesi (MKS and TRJ); and Kalimantan (BP). The tempeh samples were sent in fresh condition at 0 h. When it arrived at laboratory, tempeh was incubated until it reaches the fermentation time 48 h. After 48 h, 1 g tempeh samples was taken for total DNA extraction and the rest of tempeh samples put in freezer at 4°C.

**Isolates collection**

IIEMP-3, IWJB-6, and *K. pneumoniae* FK were in our collection and as described previously (Ayu et al., 2014).

**Genome annotation**

*Klebsiella* IIEMP-3 genome assembly was performed employing Velvet version 1.2.07 and evaluated by REAPR software (Yulandi et al., 2016a). *Klebsiella* IIEMP-3 genome annotation was conducted employing Rapid Annotation of Transfer Tool (RATT) software (Otto et al., 2011). *Klebsiella pneumoniae* subsp. *pneumoniae* NTUH-K2044 and *K. variicola* At-22 were used as genome reference. Meanwhile, *Klebsiella* IWJB-6 genome assembly and genome annotation process were done employing integrative bacterial genome analysis for Ion Torrent sequence data (IonGAP) (Baek-Ortega et al., 2015).

**BLAST Ring Image Generator (BRIG)**

The genome assembly of *Klebsiella* IIEMP-3 and IWJB-6 were visualized by BLAST Ring Image Generator (BRIG) software as described previously (Alikhan et al., 2011) employing *K. pneumoniae* subsp. *pneumoniae* NTUH-K2044 and *K. variicola* At-22 as reference strains. BRIG result was used as a reference to choose the genes that could be used for PCR primer design. The primers were used to group the diversity of *Klebsiella* isolates from tempeh.

**Polymerase Chain Reaction (PCR) primer design**

The gene(s) or specific sequences that could be used to distinguish the diversity of *Klebsiella* from tempeh were further analyzed to construct specific pairs of primers by primer3 (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi) (Rozen and Skaletsky, 2000). Primer pairs were verified by NetPrimer (http://www.premierbiosoft.com/netprimer/) and Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Specific pairs of new primers used to amplify the sdsA gene were sdsA-F 5′-AGCATCTCCTGCAGCTTACG-3′ and sdsA-R 5′-TCGCTGGAGAACATGGTGCT-3′. The other primers also used in this study, i.e. 16S rRNA, rmpA and cbiG (Table 1). The cbiG primer was used as a specific marker for *Klebsiella* (Alvin, 2014). The rmpA primer was used to confirm the presence of virulence gene responsible for the mucoid phenotype in *Klebsiella* (Nassif et al., 1989), while 16S RNA was used as a positive control for bacterial DNA amplification (Marchesi et al., 1998).

**Table 1: List PCR primers.**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer</th>
<th>Oligonucleotide primers</th>
<th>Size of amplicons (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>63f</td>
<td>5′-CGGGCCCTACACATGCAATGCTC-3′</td>
<td>1300</td>
<td>(Marchesi et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>1387r</td>
<td>5′-GAGGGCGGWGTGATACAAAGC-3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cbiG</td>
<td>cbiG-F</td>
<td>5′-TGCTGCCGCTACCTGCTAC-3′</td>
<td>755</td>
<td>(Alvin, 2014)</td>
</tr>
<tr>
<td></td>
<td>cbiG-R</td>
<td>5′-GGACAGCGCGGCTGTTGGC-3′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rmpA</td>
<td>rmpA-F</td>
<td>5′-ACTGCGGTACCTGCTTAACT-3′</td>
<td>536</td>
<td>(Nassif et al., 1989)</td>
</tr>
<tr>
<td></td>
<td>rmpA-R</td>
<td>5′-CCTGCATAGTCCATCTTTA-3′</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DNA preparation**

*Klebsiella pneumoniae* FK, *Klebsiella* IIEMP-3, and IWJB-6 were refreshed in Luria Broth (LB) at 37°C overnight (Ayu et al., 2014). Total DNA from the isolates were extracted employing Presto™ Mini gDNA Bacteria Kit protocols (Geneaid Biotech Ltd, Taiwan). *Klebsiella pneumoniae* FK was used as a positive control for virulence gene. Total DNA from tempeh samples were extracted employing Power Food® Microbial DNA Isolation Kit protocols (MOBIIO, Canada).

**PCR**

Total PCR volume was 10 µL containing 5 µL KAPA2G Robust HotStart (KAPABiosystems, MA, USA), 10 pmol of forward and reverse primers, 100 ng of a DNA template, and nuclelease-free water (Promega, WI, USA). The amplifications were performed using PCR (Applied Biosystems 2720 Thermal Cycler; Life Technologies, CA, 2015).
USA: GeneAmp PCR System 2400; PerkinElmer, MA, USA) with a predetermined PCR condition (Table 2). After polymerization process was completed, the samples were verified by electrophoresis (Mini-Sub Cell GT Cell; Bio-Rad Laboratories, CA, USA) in 1% (w/v) agarose in 1× TAE buffer for 35 minutes, 80 V. Agarose gel was visualized under UV light (Biometa T1; Biometa, Göttingen, DE).

Table 2: Predetermined PCR condition.

<table>
<thead>
<tr>
<th>Gene</th>
<th>PCR</th>
<th>Cycle</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>Pre-denaturation (94 °C, 5 min);</td>
<td>35 ×</td>
<td>(Marchesi et al., 1998)</td>
</tr>
<tr>
<td></td>
<td>denaturation (92 °C, 30 s);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>annealing (55 °C, 30 s);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>extension (72 °C, 1 min);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-extension (72 °C, 5 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cbiG</td>
<td>Pre-denaturation (94 °C, 5 min);</td>
<td>35 ×</td>
<td>(Alvin, 2014)</td>
</tr>
<tr>
<td></td>
<td>denaturation (94 °C, 30 s);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>annealing (60 °C, 30 s);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>extension (72 °C, 1 min);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-extension (72 °C, 20 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rmpA</td>
<td>Pre-denaturation (95 °C, 10 min);</td>
<td>35 ×</td>
<td>(Nassif et al., 1989)</td>
</tr>
<tr>
<td></td>
<td>denaturation (94 °C, 30 s);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>annealing (55 °C, 1 min);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>extension (72 °C, 1 min);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-extension (72 °C, 5 min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sdsA</td>
<td>Pre-denaturation (94 °C, 5 min);</td>
<td>35 ×</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>denaturation (94 °C, 30 s);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>annealing (53 °C, 30 s);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>extension (72 °C, 1 min);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-extension (72 °C, 10 min)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Klebsiella isolates from tempeh did not contain virulence gene and are different from K. pneumoniae

The whole genome of IIEMP-3 and IWJB-6 isolates was analyzed employing RATT and IonGAP software to identify the virulence gene/s (Table 3). Comparative genomic sequence analysis indicated that the virulence genes were absence in either IIEMP-3 or IWJB-6. Unlike the virulence genes, multidrug resistance protein was present in both IIEMP-3 and IWJB-6 isolates. Multidrug resistance proteins might be one form of defense to a number of antibacterial secreted by Rhizopus spp. in tempeh.

Table 3: Genome annotation data employing RATT and IonGAP program.

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Gene or protein*</th>
<th>virB1-virB11</th>
<th>magA</th>
<th>rmpA</th>
<th>Multidrug resistance protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae subsp.</td>
<td></td>
<td>P</td>
<td>P</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>pneumoniae NTUH-K2044</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. variicola At-22</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
</tr>
<tr>
<td>Klebsiella IIEMP-3</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
</tr>
<tr>
<td>Klebsiella IWJB-6</td>
<td></td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>P</td>
</tr>
</tbody>
</table>

* P denotes presence of the gene/s or protein.
A denotes absence of the gene/s or protein.

The BRIG profile showed that there was no magA, rmpA, irp1, irp2 genes on Klebsiella IIEMP-3, IWJB-6, and K. variicola At-22 (Figure 1). PCR results showed that all of DNA samples from tempeh Jakarta, Bogor, Central Java, Jogjakarta, East Java, Sulawesi, Kalimantan, IIEMP-3, and IWJB-6 did not contain rmpA gene when compared to K. pneumoniae FK used as a positive control for virulent isolate (Table 4). The BRIG profile based on Yulandi et al. (2016b) showed that IIEMP-3 isolates did not show virB1-virB11, which encodes type IV system secretion (T4SS). This study showed that T4SS was absence in K. variicola At-22 and IWJB-6 isolates (Figure 1). General features from K. pneumoniae subsp. pneumoniae NTUH-K2044, K. variicola At-22, IIEMP-3 and IWJB-6 isolates showed that all of the parameters were similar (Table 5).

Klebsiella isolates from tempeh possessed unique genes

Bioinformatics analysis indicated that cbiG gene was found in all of Klebsiella species in this study; i.e. K. pneumoniae subsp. pneumoniae NTUH-K2044, K. variicola At-22, Klebsiella IIEMP-3 and IWJB-6. PCR with our specific primers confirmed that all of the DNA samples derived from tempeh, contained sequences of cbiG gene. The DNA bands formation of cbiG gene in agarose gel was thick and the size of product from PCR analysis was 755 bp. MWR tempeh was the only tempeh that was failed to show cbiG (Table 4). The absence of cbiG gene in MWR might indicate the absence of Klebsiella or vitamin B12 in this tempeh (Alvin, 2014).

The BRIG analysis employing K. variicola At-22 as a reference showed that sdsA gene was found in K.
Figure 1: Whole genome comparison of *K. variicola* At-22, *Klebsiella* IIEMP-3, IWJB-6, and NTUH-K2044 based on BRIG analysis (Alikhan *et al.*, 2011). Please note the absence of virulence gene/s in IIEMP-3, IWJB-6, and At-22. Sequence in area A is only presence in NTUH-K2044, which contained hypothetical protein and conserved hypothetical protein compared to At-22, IIEMP-3, and IWJB-6.

Table 4: Presence of 16S rRNA, *cbiG*, *rmpA*, and *sdsA* genes in some *Klebsiella* isolates and tempeh samples.

<table>
<thead>
<tr>
<th>Bacterial isolates/tempeh samples</th>
<th>Source of isolates/tempeh samples</th>
<th>Gene*</th>
<th>16S rRNA</th>
<th><em>cbiG</em></th>
<th><em>rmpA</em></th>
<th><em>sdsA</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella</em> IIEMP-3</td>
<td>(Ayu <em>et al.</em>, 2014)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella</em> IWJB-6</td>
<td>(Ayu <em>et al.</em>, 2014)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> FK</td>
<td>(Ayu <em>et al.</em>, 2014)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tempeh KA</td>
<td>Karet – Central Jakarta</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh KOP</td>
<td>Kopro – West Jakarta</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh KJA</td>
<td>Koja – North Jakarta</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh RA</td>
<td>Ranco – South Jakarta</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh CA</td>
<td>Cakung – East Jakarta</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh GIO</td>
<td>Ciomas – Bogor</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh MWR</td>
<td>Mawar – Bogor</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tempeh BTG</td>
<td>Batang – Central Java</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh CPU</td>
<td>Cepu – Central Java</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh SLM</td>
<td>Sleman – Jogjakarta</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh SU</td>
<td>Sukorejo – East Java</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh MA</td>
<td>Malang – East Java</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh PA</td>
<td>Pasuruan – East Java</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh GW</td>
<td>Gedong Wetan – East Java</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh PCTN</td>
<td>Pacitan – East Java</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh MKS</td>
<td>Makassar – South Sulawesi</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh TRJ</td>
<td>Toraja – South Sulawesi</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tempeh BP</td>
<td>Balikpapan – East Kalimantan</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*a* : presence of PCR products. + : Presence of PCR products, but the DNA bands from PCR products were very thin. - : Absence of PCR products.
Table 5: General features of K. pneumoniae subsp. pneumoniae NTUH-K2044, variicola At-22, Klebsiella IIEMP-3 and IWJB-6 genome.

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Genome size (bp)</th>
<th>Coding sequence</th>
<th>DNA G+C content (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae subsp. pneumoniae NTUH-K2044</td>
<td>5248520</td>
<td>5006</td>
<td>57.7</td>
<td>(Wu et al., 2009)</td>
</tr>
<tr>
<td>K. variicola At-22</td>
<td>5458505</td>
<td>4979</td>
<td>57.6</td>
<td>(Pinto-Tomás et al., 2009)</td>
</tr>
<tr>
<td>Klebsiella IIEMP-3</td>
<td>5362779</td>
<td>5096</td>
<td>57.8</td>
<td>(Yulandi et al., 2016a)</td>
</tr>
<tr>
<td>Klebsiella IWJB-6</td>
<td>5159329</td>
<td>4911</td>
<td>57.5</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: $sdsA$ gene was absence in either IIEMP-3 or IWJB-6 but presence in At-22. $sdsA$ in NTUH-K2044 was only found as a truncated gene.

$variicola$ At-22, but absence in Klebsiella IIEMP-3 and IWJB-6. A specific primer was designed to amplify this region in K. variicola At-22 chromosome and used them to explore similar gene/s in IIEMP-3 and IWJB-6, as well as a number of tempeh samples (Figure 2). The result of $sdsA$ gene amplification was tabulated in Table 4.

$sdsA$ gene was found in some samples of tempeh (Table 4). However, the DNA bands derived from PCR products of KA, KJA, SU, CPU, or BP samples were very thin, which might indicate only a small number K. variicola presence in tempeh. Although IIEMP-3 and IWJB-6 isolates were similar to K. variicola, these tempeh isolates and nine tempeh samples did not have $sdsA$ gene (Table 4), which indicated that Klebsiella commonly associated with tempeh was a different subspecies.

DISCUSSION

Vitamin B12 is not only complex structurally but also unique, because this vitamin only formed by bacteria or archaea, and tempeh is one of a few vegan diet known to contain vitamin B12 (Liem et al., 1977; Keuth and Bisping, 1994). There are genes that could indicate the presence of Klebsiella in tempeh, such as $cbiG$ gene (Alvin, 2014). $cbiG$ gene has an important role in tempeh fermentation to create vitamin B12 through an anaerobic pathway (Rodionov et al., 2003; Moore et al., 2013). Alvin (2014) reported that the thickness of $cbiG$ gene PCR bands
could be used to estimate semiquantitatively Klebsiella population in tempeh.

This study indicated that Klebsiella from tempeh is different from those of medical isolates. magA, rmpA, irp1, irp2 genes are known as virulence genes in pathogenic K. pneumoniae (Nassif and Sansonetti, 1986; Nassif et al., 1989; Carniel, 2001; Fang et al., 2004). rmpA, a Klebsiella virulence gene, was not found in all Klebsiella associated with tempeh and tempeh samples. The absence of virulence gene in Klebsiella from tempeh supported the previous study, which is showed genetically by ERIC-PCR analysis and the absence of mucoid phenotype on solid media in both IIEMP-3 and IWJB-6 isolates (Ayu et al., 2014). Yeh et al. (2007) reported that rmpA negative isolate is less resistant and less virulent than the rmpA positive isolate. T4SS may be important to cause diseases (Fodah et al., 2014). The absence of T4SS in IIEMP-3 and IWJB-6 isolates also suggested that the isolates might not be pathogenic to human.

Smillie et al. (2010) reported that T4SS, type IV coupling protein (T4CP), the origin of transfer locus (oriT), and relaxase were needed in a conjugative plasmid. T4SS was found in plasmid of K. variicola Bz19, one of clinical isolates strain from K. variicola; pKPC-NY79 from 258 K. pneumoniae strain that was isolated from a patient hospitalized in New York, United States; and plasmid from K. pneumoniae KpQ3 (Andrade et al., 2014; Ho et al., 2013; Tobes et al., 2013). However, K. variicola At-22 did not have both a plasmid and T4SS (Pinto-Tomás et al., 2009). Either IIEMP-3 or IWJB-6 might not harbor this plasmid because of the absence of T4SS in their genome.

The gene that could differentiate K. variicola At-22 from either IIEMP-3 or IWJB-6 isolates was sdsA. sdsA gene was first described as a member of the group III sulfatase which was distinguished by the existence of metallo-ß-lactams domain and have responsibility in alkyl sulfatase activity of sdsA protein. SdsA, involved in sodium dodesyl sulfate (SDS) degradation (Hageluken et al., 2006; Navais et al., 2014). Based on these data, we proposed that IIEMP-3 and IWJB-6, and some Klebsiella isolates from tempeh, were renamed as Klebsiella variicola subsp. tempehensis. Further research is needed on the role of these isolates in tempeh, especially on the microbial community during tempeh fermentation and the impacts on tempeh as a functional food.

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REFERENCES


