



## DNA isolation of potentially probiotic lactic acid bacteria from durian arilus fermentation (*jruek drien*)

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### ABSTRACT

*Jruek drien* is an Acehnese traditional fermented food containing lactic acid bacteria (LAB) from durian arilus fermentation. Lactic acid bacteria from *jruek drien* were considered as potential probiotics. The LAB from *jruek drien* were first identified by phenotyping and genotyping analysis. The aim of the research is to isolate DNA from lactic acid bacteria (JD-2 and JD-3) from *jruek drien* for identification based on 16S rDNA gene sequence analysis. The research steps consist of samples regeneration in MRSA medium with temperature of incubation at 37°C, samples culturing in TSB medium, DNA isolation, and measurement of the DNA concentration and DNA purity using nanophotometer. The result showed that the JD-2 and the JD-3 isolates grow well on selective media MRSA by incubation temperature at 37°C. JD-2 has a DNA concentration of 40.0 ng/μl with a purity level of 2.0, and JD-3 has a DNA concentration of 32.5 ng/μl with a purity level of 1.8 at the A260/A280 ratio. The 16S rRNA gene of JD-2 isolate was successfully amplified at 1426 bp and JD-3 isolate at 1435 bp. The JD-2 isolate was identified as *Bacillus subtilis* because it has the highest similarity with *Bacillus subtilis* strain WA3-4. The JD-3 isolate was identified as *Lactobacillus plantarum* because it has the highest similarity with *L. plantarum* strain CSI9 and strain CSI3.

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### 1. Introduction

*Jruek drien* is an Acehnese traditional fermented food made from durian arilus (Yulvizar et al., 2015). *Jruek drien* is usually yellow or white with a texture that resembles pasta and has a distinctive aroma (Widowati et al., 2013). It produces a sour aroma because it contains organic acids produced by lactic acid bacteria (LAB) (Yuliana et al., 2005). In addition, LAB is also capable of producing hydrogen peroxide, diacetyl and bacteriocins that can kill pathogenic bacteria, thereby potentially as probiotics (World Gastroenterology Organization, 2008; Utami, 2011). Probiotics are living microorganisms that if it is given in adequate amounts can be beneficial to the health of its host (World Gastroenterology Organization, 2008).

Genotypic identification of probiotic bacteria is carried out using the principle of Polymerase Chain Reaction (PCR) and sequence analysis of 16S rRNA gene (Nurhayati et al., 2011). 16S rRNA gene is a gene that is widely used to describe the kinship of a microorganism because it has sufficient genetic information and more easily analysed (Madigan et al., 2010).

Isolation of DNA is the first step to perform DNA analysis. In general, DNA isolation stages include the process of destruction of the cell wall, cell lyses, precipitation and purification of DNA (Fatchiyah et al., 2011). The resulting DNA concentration can be measured accurately by using ultraviolet absorbance spectrophotometry (Brown, 1995).

This study aims to isolate the 16S rRNA gene of *jruek drien* LAB isolates potentially as probiotic. Isolation of DNA containing the 16S rRNA gene is the first important stage to identify LAB genotypically. The results of DNA isolation will be followed by amplification and sequencing process to obtain the species of LAB isolates.

### 2. Results and Discussion

#### 2.1. LAB isolates regeneration

Potentially probiotic lactic acid bacteria (LAB) isolates regeneration was carried in the MRSA media with streak plate method. Regenerated isolates were from the stock cultures that are belonging to the Laboratory of Microbiology, Faculty of Science, Syiah Kuala University. Two LAB isolates (the JD-2 and JD-3) were isolated from Aceh traditionally fermented food, namely *jruek drien*. Regeneration of JD-2 and JD-3 isolates was successful and the colonies were grown in the MRSA media (Figure 1).

The JD-2 isolate have the same morphological characteristics as the JD-3 isolate of shapes, edges, and elevation. The JD-2 isolate colony is round with smooth edges, convex elevation and white colour, while the JD-3 isolate colony is round with smooth edges, convex elevation and whitish cream (Table 1).

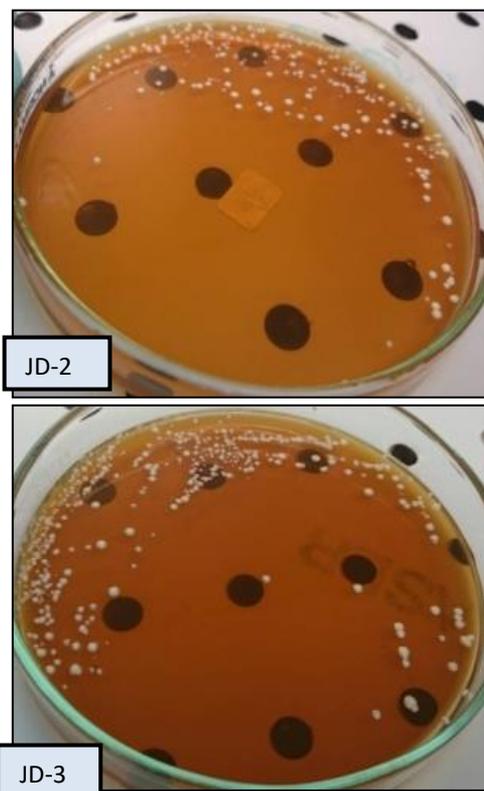


Fig. 1. LAB isolates, JD-2 and JD-3 colonies in the MRSA media.

Both LAB isolates, JD-2 and JD-3, experienced good growth in MRSA media with an incubation period of 24-48 hours at 37°C. The incubation temperature used is the optimum temperature for the growth of potentially probiotic lactic acid bacteria. Barman et al. (2011) suggests that probiotic bacteria can grow well at a temperature range of 30-37°C.

Colonies of the regenerated JD-2 and JD-3 isolates in the media MRSA then was grown in Trypticase Soy Broth (TSB) media to obtain more amount of bacteria. The process was carried in TSB culture media for 48 hours at 37°C in a water bath shaker. This condition produced good isolates growth in TSB media. The growth of bacteria in liquid media was proven by the change in media that became more turbid (Figure 2). Liquid culture of JD-3 and JD-2 isolates then would be used for the isolation of genomic DNA from the potentially probiotic lactic acid bacteria.

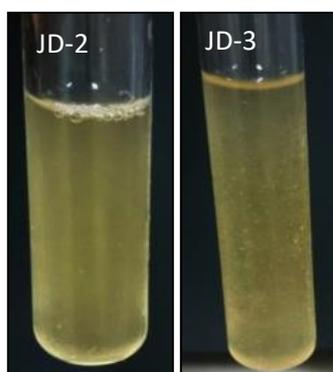


Fig. 2. Growth of LAB isolates JD-2 and JD-3 in the TSB media.

## 2.2. LAB genomic DNA isolation

Isolation of genomic DNA from the potentially probiotic lactic acid bacteria, the JD-2 and JD-3 isolates, was successfully performed that produced DNA with good purity level. Determining the success of DNA isolation process was done by calculating the concentration of DNA using nanophotometer.

Measurement of DNA concentration using nanophotometer showed a high degree of purity of DNA from JD-2 and JD-3 isolates. The JD-2 isolate had DNA purity level that reached 2,000 at A260/A280 with DNA concentration 40.0 ng/μl. The JD-3 isolate had DNA purity level that reached 1,852 at A260/A280 with DNA concentration 32.5 ng/μl (Table 2). DNA with high purity level can be used for the amplification of 16S rRNA gene. Cseke et al. (2011) states that the ratio A260/A280 is used to estimate the purity of nucleic acids. The ratio approaching 1.8 at the A260/A280 indicates the purity of DNA, while the ratio below 1.8 indicates contamination by protein, phenol or other substances.

## 2.3. 16S rRNA gene amplification

The amplification process was carried out to increase the number of DNA strands that had been isolated from lactic acid bacteria. This process was carried out using a pair of DNA primer Bact 27F - UniB 1492R which is a common primer pair for amplifying the 16S rRNA gene. The successful of the 16S rRNA gene amplification process is known based on the results of visualization of the electrophoresis process using 1% agarose gel. The results of electrophoresis on both DNA samples from JD-2 and JD-3 isolates showed the presence of DNA bands that were parallel to the 1500 bp marker range (Figure 3). This result is in accordance with Bushell and Burns (2012) which states that the 16S rRNA gene is part of ribosomal RNA which has a size of 1542 bp.

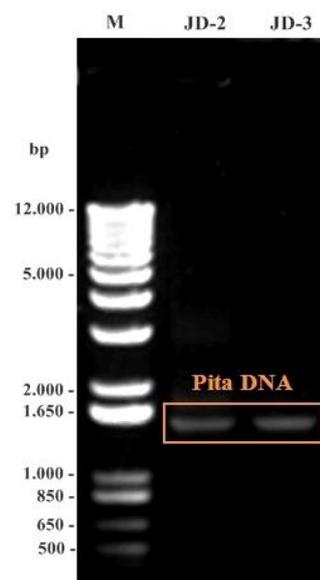


Fig. 3. The visualization of 1% agarose gel electrophoresis on the results of 16S rRNA gene amplification of JD-2 and JD3 isolates using Bact 27F-UniB 1492R primer pair and marker 1 Kb Plus DNA ladder (Invitrogen)

## 2.4. Sequencing of the 16S rRNA gene

Sequencing of the 16S rRNA gene was carried out by the Sanger method. An important factor in gene sequencing is the purity level of the DNA template. In addition to the purity of DNA template, the primer used also affects the sequencing process. Sequencing can be done using the same primer pair as the amplification primer, those are Bact 27F and UniB 1492 R. According to Hill et al. (2014), sequencing with the Sanger method can be done using a specially designed primer, or primer that is the same as the gene amplification process.

Sequencing results were in the form of an ABI format. The ABI file was processed using the Baser Assembly DNA program so that it can be seen as a chromatogram. Chromatograms are data produced in the process of DNA sequencing. Chromatograms display peaks of different colours, according to nucleotide bases. Chromatograms of JD-2 and JD-3 isolates 16S rRNA gene using Bact 27F and UniB 1492 R primers were very good with an average quality range of 45-51.

The chromatograms of JD-2 and JD-3 isolates 16S rRNA gene were then combined using the Baser Assembly DNA program. The results of the sequences collection of the two isolates showed that JD-2 had a nucleotides of 1426 bp with a percentage of GC of 54.9% and AT of 45.02%, while JD-3 had a nucleotides of 1435 bp with a percentage of GC of 51.3% and AT of 48.64%. Based on the results of sequences collection, DNA sequences of the JD-2 and JD-3 isolates can be aligned with data on Genebank. The base sequences of Genebank data can be accessed on the National Centre for Biotechnology Information website ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) using the Basic Local Alignment Search Tool Nucleotide (BLAST-N) program.

## 2.5. BLAST-N analysis

BLAST-N analysis shows the distribution graph of 100 organisms that have the highest kinship level with JD-2 and JD-3 isolates. The distribution graph of 100 organisms on JD-2 and JD-3 isolates shows red lines which means a high homology level with an alignment value of more than 200 (Figure 4).

In addition to the distribution graph, there are also data on 100 organisms that have high homology levels with JD-2 and JD-3 isolates. The data is stored in FASTA format. Data in FASTA format can be used to process phylogenetic tree reconstruction using MEGA 7.0 software.

Phylogenetic analysis using the BLAST-N program showed that JD-2 isolates had the highest similarity with the genus *Bacillus*. The JD-2 isolate has a value of expectation value (e-value) of 00.00 which indicates that the JD-2 sequence has a high similarity to the database sequence. In addition to e-value, a high homology level is also determined by the percentage value of identity. JD-2 isolates have a percentage of identity reaching 99-100% with a query coverage value of 99-100%. While JD-3 isolates have the highest similarity with the genus *Lactobacillus*. The sequence of JD-3 isolates has an e-value of 00.00, the percentage of identity reaches 99% and the query coverage value is 100%.

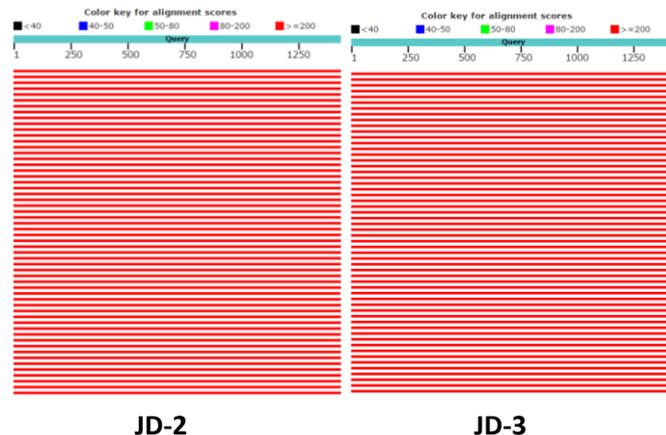


Fig. 4. Distribution graph of 100 organisms on JD-2 and JD-3 isolates (BLAST-N analysis).

## 2.6. Analysis of phylogenetic trees

The phylogenetic tree construction was carried out using the MEGA 7.0 program with the Neighbour Joining method. JD-2 isolate has a bootstrap percentage in the node of 98% (Figure 5). A large percentage of bootstrap indicates that the node has a high level of confidence. Ryan et al. (2013), stated that phylogenetic trees that have a bootstrap percentage of more than 50% are reliable phylogenetic trees. JD-2 isolates formed a monophyletic group with one strain of the *Bacillus* genus, which is *Bacillus subtilis* strain WA3-4. JD-2 isolates had the closest kinship with the *Bacillus subtilis* strain WA3-4 with a bootstrap percentage reaching 98%. Based on this, it can be said that JD-2 isolate is belong to *Bacillus subtilis* species.

The JD-3 isolate has a bootstrap percentage in the node of 82% (Figure 6). JD-3 isolates form a monophyletic group with two species of the genus *Lactobacillus*, they are *Lactobacillus plantarum* strain CSI9 and *L. plantarum* strain CSI3. JD-3 isolates have the highest kinship with *L. plantarum* strain CSI9 and CSI3 species with a bootstrap percentage of 82%. Based on this, it can be said that the JD-3 isolate is belong to *L. plantarum* species. According to the World Gastroenterology Organization (2008), species of *Lactobacillus* are the most widely used bacteria as probiotics.

## 3. Conclusion

Based on the research that has been done, it can be concluded that the JD-2 and JD-3 isolates grew well on MRSA media with the incubation temperature at 37°C, and the DNA isolated from JD-2 and JD-3 isolates showed a high degree of DNA purity at A260 / A280. The 16S rRNA gene of JD-2 isolate was successfully amplified at 1426 bp and JD-3 isolate at 1435 bp. The JD-2 isolate was identified as *Bacillus subtilis* because it has the highest similarity with *Bacillus subtilis* strain WA3-4. The JD-3 isolate was identified as *Lactobacillus plantarum* because it has the highest similarity with *Lactobacillus plantarum* strain CSI9 and strain CSI3. *Bacillus subtilis* and *Lactobacillus plantarum* are probiotic bacteria.

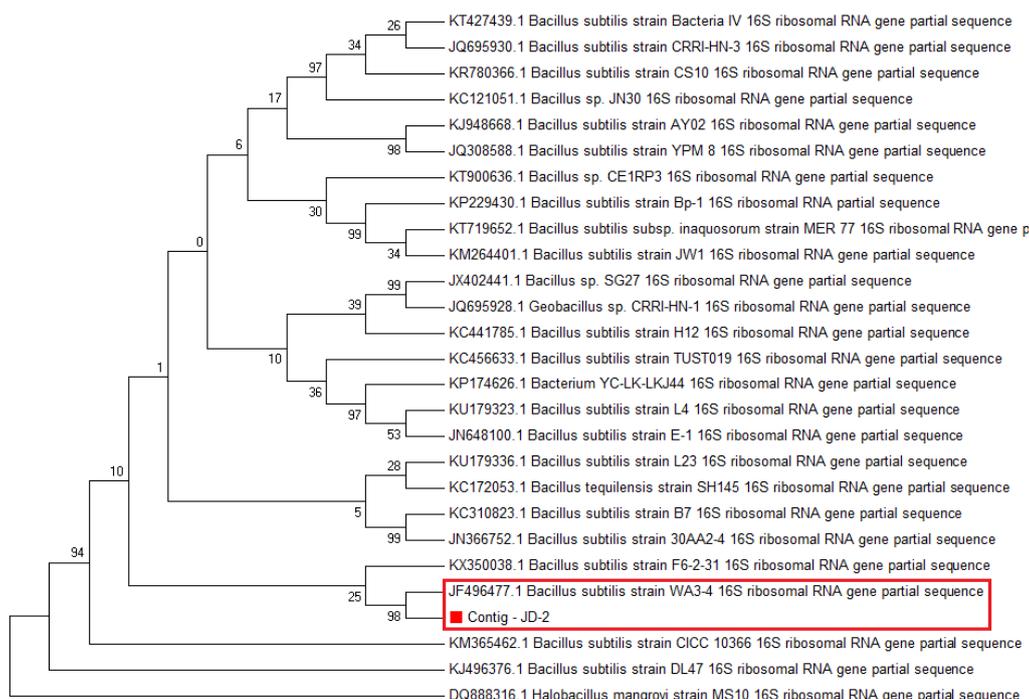


Fig. 5. The results of the phylogenetic tree reconstruction of JD-2 isolates.

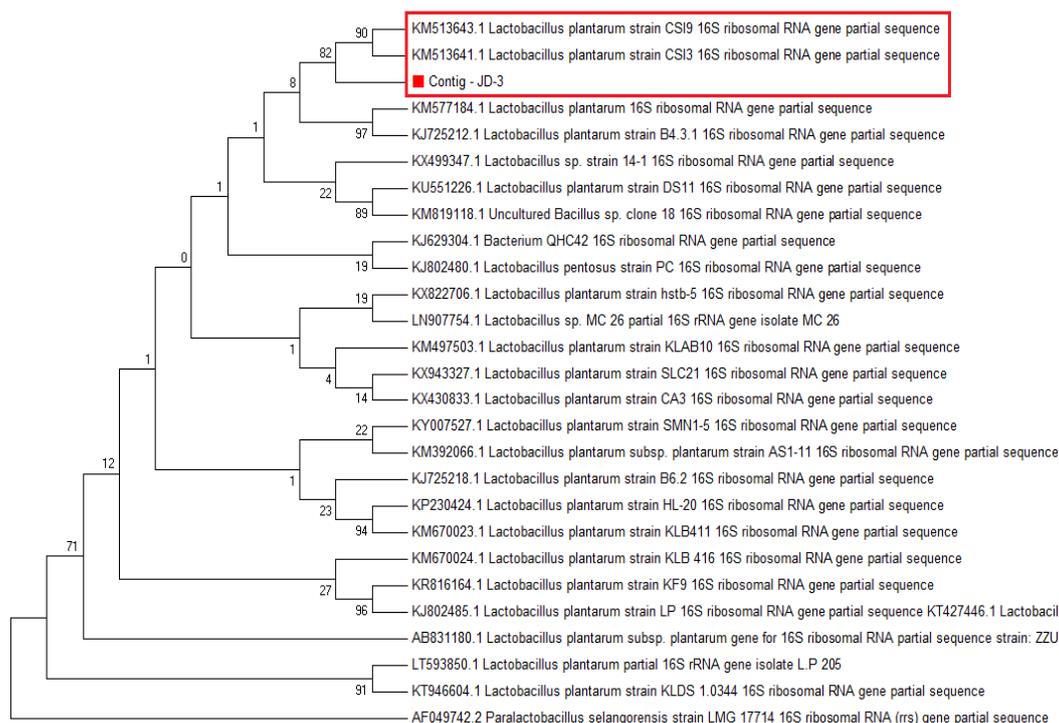


Fig. 6. The results of phylogenetic tree reconstruction of JD-3 isolates.

## 4. Materials and methods

### 4.1. Time and location

This study began with the regeneration of LAB isolates that were conducted at the Laboratory of Microbiology, Faculty of Science, Syiah Kuala University, and followed by DNA isolation in the Laboratory of Biomolecular, *Dinas Kesehatan Hewan dan Peternakan Aceh*, and calculating the isolated DNA concentration using nanophotometer in the Laboratory of LOKA *Litbang Biomedis Aceh*.

### 4.2. Tools and materials

The equipment used in the study were autoclave, Laminar Air Flow Cabinet (LAFC), Petri dishes, test tubes, incubators, shaker water bath, micropipettes, vortex, centrifuge, and nanophotometer.

The sample used was LAB isolates which were isolated from *jruek drien* obtained from Aceh Barat, Aceh Barat Daya, Aceh Jaya, Aceh Selatan and Nagan Raya district. Growth media used were Man Rogosa Sharpe Agar (MRSA) and

Trypticase Soy Broth (TSB). DNA isolation used QIAamp DNA Mini Kit (Qiagen).

#### 4.3. LAB isolates regeneration

JD-2 and JD-3 isolates from the stock cultures were inoculated on MRSA with streak plate method. The isolates were incubated for 24 hours at 37°C. The LAB growing colonies were inoculated into a liquid TSB medium and incubated in a shaker water bath for 48 hours at 37°C.

#### 4.4. LAB genomic DNA isolation

LAB genomic DNA isolation is done by using QIAamp DNA Mini Kit (Qiagen). Liquid culture isolates of JD-2 and JD-3 were inserted into the microtube 1.5 mL. Then centrifuged for 10 min at 13,300 rpm to form pellets. ATL buffer was then added to a volume of 180 µL. Then 20 µL proteinase K was added and incubated for 30 min at 56°C. Then 200 µL of buffer AL added to the tubes and incubated for 10 min at 70°C. Then 200 µL of absolute ethanol (96-100%) added and homogenized with a vortex to produce a lysate.

Lysates of samples 3, 6, and 8 put into QIAamp Mini Spin Column (in a 2 ml collection tube). Then they were centrifuged at 8000 rpm for 1 minute. The QIAamp Mini Spin Column was transferred into a new 2 mL collection tube. Then added 500 µL of buffer AW1 and centrifuged at 8000 rpm for 1 minute. The QIAamp Mini Spin Column was transferred into a new 2 mL collection tube. Then added 500 µL of buffer AW2 and centrifuged at 13.3000 rpm for 3 minutes. The QIAamp Mini Spin Column transferred into a 1.5 mL microtube, added 200 µL buffer AE, and centrifuged at 8000 rpm for 1 minute. The QIAamp Mini Spin Column removed and DNA stored in a 1.5 mL tube microtube.

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#### Conflicts of interests

The authors declare that this research has no conflict of interests.

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