Short Communication:
Serratia rubidaea as contaminant in laboratory environment

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Abstract. Virgianti DP. 2021. Short Communication: Serratia rubidaea as contaminant in laboratory environment. Nusantara Bioscience 13: 47-51. There have been many cases of bacterial contamination in the laboratory. The bacterial genera identified as contaminants are Bacillus, Staphylococcus, Micrococcus, Pseudomonas, Shigella and Serratia. These bacteria are classified as non-pathogenic and pathogenic bacteria that can interfere with the test and potentially develop false-positive results. The present research has shown that red-colored contaminant bacteria develop in unused sterile media in our laboratory. Based on related information, Serratia marcescens is a red bacterial species that have been reported as a contaminant in the laboratory. The purpose of this study was to identify contaminant bacteria at the molecular level. Based on the phylogenetic characterization using the 16S rDNA gene region, this red contaminant bacterium was identified as Serratia rubidaea.

Keywords: Contamination, environment, gene barcoding, red bacteria, Serratia

INTRODUCTION

Contamination is an important concern in biological laboratories. They can be categorized into three major groups physical, chemical, and biological. Bacteria, mold, yeast, viruses, and mycoplasma are the most common biological contaminants (Abatenh et al. 2018). Bacterial contamination can occur in numerous laboratories, including animal and plant laboratories (Li et al. 2018), health faculty microbiology laboratories (Lutpiatina 2015), university microbiology laboratories (Ghayoor et al. 2015), as well as in hospital microbiology laboratories (Ng et al. 2011; Konar and Das 2013). Sources of contamination can come from a variety of sources, including air and surfaces (Konar and Das 2013). Sources of contaminated items include tables, floors, clothing, laboratory surfaces such as incubators, microscopes, computers, phones, and water taps (Ng et al. 2011; Ghayoor et al. 2015). In addition, contamination can derive from the body parts of laboratory staff, such as the hands (Konar and Das 2013; Ng et al. 2011).

According to Li et al. (2018), the number of microbial contaminants in animal laboratories and bacterial laboratories is found to be higher than in plant laboratories. These contaminant bacteria are pathogenic and non-pathogenic. Common bacterial genera as contaminants in the laboratory include Bacillus, Staphylococcus, and Micrococcus (Ghayoor et al. 2015; Konar and Das 2013). Pathogenic bacteria such as Methicillin-Resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Salmonella spp. and Enterobacteriaceae have also been identified as contaminants, especially in hospital microbiology laboratories, potentially giving false-positive test results (Ng et al. 2011). Environmental and human influences have a major impact on the diversity and dynamics of microbial contaminants (Li et al. 2018), as well as the usage of gloves and handwashing, which are very successful in minimizing pathogenic bacterial contamination in the hospital microbiology laboratory (Ng et al. 2011). In addition, proper disinfection and sterilization are needed to eliminate contaminant microorganisms and personal hygiene of laboratory staff is also required (Konar and Das 2013).

In a health education laboratory where microbiology is practiced, contaminant bacteria can interfere with the learning process. The genus Bacillus is the main contaminant of the laboratory (Lutpiatina 2015). In the present research, red bacterial isolates were collected from contaminated media that were kept in the washing area of the glassware in a medical school laboratory. In current reports, red bacteria were found as contaminated bacteria in clinical microbiology laboratory of hospital and identified as Serratia marcescens. The contamination caused a pseudo-outbreak as a result of diagnosis. These contaminant bacteria are found in saline solutions, soaps and disinfectants used in laboratories. This pseudo-outbreak emphasizes the importance of laboratory worker ability to perform specimen processing procedures (Dundar et al. 2009). Serratia typically causes neonatal nosocomial outbreaks. This outbreak is associated with personal hygiene, such as hand hygiene (Zingg et al. 2017) and environmental hygiene, such as the value of clean air conditioning, which can become a nosocomial reservoir (Uduman et al. 2002). The genus Serratia is a Enterobacteriaceae member that produces prodigiosin, a non-diffusible red pigment. The habitat of Serratia includes air, water, and soil. It can also be associated with plants, insects, and other animals (Grimont and Grimont 1978).
Information about the diversity of the contaminant bacteria in the laboratory and the origin of their spread is very essential. Therefore, this study emphasized the molecular characterization of contaminant red bacteria isolated from the culture media in the laboratory.

MATERIALS AND METHODS

Procedure

Isolation of red bacteria

Red bacteria were isolated from the contaminated culture medium of Sabouraud Dextrose Agar (SDA) to the Nutrient Agar (NA) medium. The red bacterium was called Bakteri Merah as the code.

Identification of red bacteria

Identification of red bacteria was based on morphological and molecular characterization. Morphological characterization was performed through observation of colony and Gram staining. Molecular characterization was performed by barcoding the 16S rRNA gene using 27F (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R (5’-TACGGYTACCTTGTTACGACTT-3’) as universal primer for bacteria. DNA genome extraction was carried out using the Fungal/Bacterial Miniprep Kit (Zymo Research, D6005). PCR amplification was conducted with MyTaq HS Red Mix (Bioline) with 1 x 25μL master mix PCR composition consisting of 9.5 μL ddH2O, 12.5 μL My Taq Red Mix, 2x, 1 μL 10 μmol/μL 27F primers, 1 μL 10 μmol/μL 1492R primer and 1μL DNA template. PCR was performed with the Agilent SureCycler 8800 Thermocycler. PCR was performed at an initial denaturation step of 95 °C for 1 min, followed by 35 cycles at 95 °C for 15 sec, 52 °C for 15 sec, and a final extension step at 72 °C for 72 sec. PCR products were purified with the Zymoclean Gel DNA Recovery Kit (Zymo Research). Sequencing was carried out in two ways, carried out commercially by Genetika Science Indonesia. Sequential data assembly was done with BLAST through http://www.ncbi.nlm.nih.gov/BLAST/.

Evolution analysis was conducted with MEGA7 (Kumar et al. 2016) and using the Neighbor-Joining method to obtain a percentage of the tree repetition in which the taxa cluster was associated with the bootstrap test (1000 repetitions). The evolutionary distance was calculated using the Maximum Composite Likelihood method.

RESULTS AND DISCUSSION

Purification of red bacteria

Contaminated SDA medium was sterilized unused medium, but was previously cut into laminar air flow with aseptic tools for other purposes. Contamination occurs when the medium was placed in washing room of glasswares located inside the laboratory. The pure culture of red bacterial colonies showed characteristics of round, medium-sized colonies of 2-3 mm, flat edges, convex elevations, and pink to red color (Figure 1). Gram staining results exhibited that the bacterium was cocobacilli Gram-negative.

Phylogenetic analysis

Molecular identification was performed to get a confirmed identification of the bacteria. The 16S rRNA sequence was successfully amplified. The PCR product was shown by DNA fragment at 1500 bp (Figure 2). Evolutionary analysis conducted by MEGA7 showed the red bacteria were clustered into S. rubidaea (Figure 3). The clade of S. rubidaea was distinctly distinguished from the other species of Serratia. According to the phylogenetic tree as described in figure 3, the most closely related strains were S. rubidaea NR_024644 strain JCM 1240 and S. rubidaea NR_114232 strain NBRC 103169. A similar result was obtained from BLASTN result that the similarity of the strain reached 99.65% identity with S. rubidaea NR_024644 strain JCM 1240 and 99.58% with S. rubidaea NR_114232 strain NBRC 103169. Meanwhile, other clades include other Serratia species namely S. ficara, S. odorifera, S. marcescens, S. ureilytica and the other genus of Cedecea. Based on NCBI information, S. rubidaea NR_024644 strain JCM 1240 is Japan’s bacterial strain used as comparative reference in the journal of investigation of the origin of intracellular aerobic gut bacteria symbiont of the Buchnera aphid (Harada et al. 1995).

Figure 1. Contaminants of red bacteria grown in unused culture medium (A), pure colonies of red bacteria (B) Cocobacilli Gram-negative bacteria (C)
The genus *Serratia* belongs to the Enterobacteriaceae family and the Gammaproteobacteria class. The genus consists of 15 species, namely *S. entomophila*, *S. ficaria*, *S. fonticola*, *S. glossinae*, *S. grimesii*, *S. liquefaciens*, *S. marcescens*, *S. nematophilica*, *S. odorifera*, *S. plymuthica*, *S. proteamaculans*, *S. quinivorans*, *S. rubidaea*, *S. symbiotica*, and *S. ureilytica* (http://www.catalogueoflife.org). *S. rubidaea* was first described in 1940 as *Bacterium rubidae* and also as *Serratia marinaruba*, but was reclassified as *S. rubidaea* (Ewing et al. 1973). There are three subspecies in it: *S. rubidaea* subsp. *burdigalensis*, *S. rubidaea* subsp. *rubidaea*, and *S. rubidaea* subsp. *colindalensis* (Grimont and Grimont 2006).

The habitat of *S. rubidaea* is not known for certain, but it has reported to have been isolated from foods such as coconuts (Siva et al. 2012), tomato salad (Abd-Alla et al. 2011), green chillies, and milk (Al-Mijali et al. 2008). It has also been identified as phytopathogen in tulips (Stoyanova and Bogatzevska 2011), epiphytic of *tulips* (Stoyanova and Bogatzevska 2011), epiphytic of *Nilaparvata lugens* Stål (Priyatno et al. 2011), *Plutella xylostella* (Indiragandhi et al. 2011), *Myrmica* ants, and *Maculinea* (Salvo et al. 2019). *S. entomophila* also plays a role in the chemical cross-talk in larval stage between of *Maculinea* and also in the *Myrmica* ants (Salvo et al. 2019).

Some *Serratia* species have been associated with insects. *S. marcescens*, has been associated with a number of insects such as *Nilaparvata lugens* Stål (Priyatno et al. 2011), *Plutella xylostella* (Indiragandhi et al. 2011), *Myrmica* ants, and *Maculinea* (Salvo et al. 2019). *S. entomophila* also plays a role in the chemical cross-talk in larval stage between of *Maculinea* and also in the *Myrmica* ants (Salvo et al. 2019).

In conclusion, *S. rubidaea* has been identified as contaminant bacteria in laboratories. This result provided further knowledge of the variety of contaminant bacteria obtained from the laboratory environment, such as insects as vectors.
Figure 3. Neighbor-joining dendrograms based on 16S rRNA sequences show a phylogenetic relationship between red bacteria (Bakteri Merah) and a sequence of bacteria obtained from BLAST results. Bootstrap values based on 1000 repetitions are displayed on branch nodes. Phylogenetic trees recognize red bacterial isolates in one clade as *Serratia rubidaea*

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