Screening of Quorum Quenching Activity of Bacteria Isolated from Ant Lion

BILLY CHRISTIANTO AND YOGIARA*

Faculty of Biotechnology, Universitas Katolik Indonesia Atma Jaya, Jalan Jenderal Sudirman 51, Jakarta 12930, Indonesia

Bacterial intercellular communication or quorum sensing controls the pathogenesis of many medically important organisms. Therefore, it is important to isolate bacteria that can disintegrate the communication, in a process called quorum quenching. Bacteria from ant lions (Myrmeleon sp.) were grown on Luria agar, and approximately 1.85 x 10^9 CFU mL was obtained. Seven morphologically different colonies were screened for quorum quenching activity using wild type Chromobacterium violaceum as an indicator. One isolate (Myr7) was found to possess quorum quenching activity, was later identified as Aeromonas by employing 16S rRNA.

Key words: ant lion, quorum sensing, quorum quenching

Antimicrobial agents, including antibiotics and related medicinal drugs, have long been used for treatments to reduce the threat posed by infectious diseases. Unfortunately, many pathogenic bacteria have become resistant to several antibiotics, for instance, penicillin-resistant Streptococcus pneumoniae (Bacquero 1995; Goldstein and Garau 1997), vancomycin-resistant enterococci (Dixon et al. 1985), methicillin-resistant Staphylococcus aureus (Sutherland and Rolinson 1964; Kareviene et al. 2006; Martins and Cunha 2007), multi-resistant salmonellae (Newell et al. 2010), and multi-resistant Mycobacterium tuberculosis (Espinal et al. 2001). The increasing resistance of pathogenic bacterial to antibiotics may lead to public health risk. To overcome this problem, innovative strategies were needed to discover novel antibiotic targets or antivirulent drugs as alternatives to classical antibiotics (Baron 2010).

It has been known that the nature of bacterial pathogenesis is controlled by quorum sensing mechanisms (Kievit et al. 2000; Williams et al. 2000). To be able to communicate with other cells, the bacteria produce, detect, and respond to a small signal molecule called autoinducer. The autoinducer is responsible to induce particular gene expression, including virulent genes. According to Finch et al. (1998), quorum sensing mechanism can be disrupted and has become a potential target for antiinfection therapy.

Antiquorum sensing activity is often called quorum quenching. This activity can be useful to prevent colonization of pathogenic bacteria that use quorum sensing to regulate virulent genes. In recent years, quorum quenching enzyme and inhibitor from various sources have been studied, both from prokaryotic and eukaryotic organisms. There are two types of prokaryotic quorum quenching enzymes such as AHL-lactonase and AHL-acylase. AHL-degrading enzymes from eukaryotic organisms can be found on pig kidney (acylase I) and on airway epithelial humans (lactonase) (Dong and Zhang 2005).

In this study we used ant lion (Myrmeleon sp.) whose bacterial community and potential activity are underexplored. Dunn and Stabb (2005) performed culture-independent 16S rRNA gene sequence analysis on the bacteria associated with the tissues of an ant lion, Myrmeleon mobilis. All 222 sequences obtained by Dunn and Stabb (2005) were identified as Proteobacteria. These sequences could be subdivided into two main groups, the α-Proteobacteria with 75 clones similar to Wolbachia spp. and the γ-Proteobacteria with 144 clones similar to the family

*Corresponding author, Phone: +62-21-5731740, Fax: +62-21-5719060, E-mail:yogiara@atmajaya.ac.id

ISSN 1978-3477, eISSN 2087-8575
Vol 5, No 1, March 2011, p 46-49
Enterobacteriaceae. They found that the Enterobacteriaceae-like 16S rRNA gene sequences were most commonly isolated from gut tissue, and Wolbachia-like sequences were predominant in the head and body tissues. Nishiwaki et al. (2007) has reported insecticidal activity of bacterial isolates of ant lion. Isolated Bacillus cereus, B. sphaericus, Morganella morganii, Serratia marcescens, and Klebsiella species killed 80% or more cutworms when injected at a dose of 5 x 10⁷ cells per insect. This study explored the possibility of finding antiquorum sensing molecule from ant lion-associated bacteria. The aim of this study is to obtain bacterial isolates that possess quorum quenching activity.

About 20 ant lions were rinsed and surface-sterilized three times using sterile 0.85% of NaCl and vortexing. The specimen was put into a 50 mL conical tube and ground. About 1 mL of 0.85% of NaCl was added and serial dilutions were applied. Approximately 100 µL sample was spread onto modified Luria agar (0.25% (w/v) trypton, 0.125% (w/v) yeast extract, 0.25% (w/v) NaCl, and 1.5% (w/v) bacteriological agar), and then incubated at 30 °C for 2 - 3 d. Plate screening assay was used to evaluate quorum quenching activity with Chromobacterium violaceum as an indicator (Adonizio et al. 2006). In brief, Luria agar plates spread with Chromobacterium violaceum followed by spotting of tested bacteria. Plates were incubated for 2 d at 30 °C, and quorum sensing inhibition was detected by a ring of colorless, but viable, cells around the bacterial isolate colony (Adonizio et al. 2006). Molecular identification using 16S rRNA gene sequencing was carried out at Eijkman Molecular Biology Institute, Jakarta-Indonesia. DNA sequences were aligned to 16S-rRNA gene database provided by Ribosomal Database Project (RDP) website (http://rdp.cme.msu.edu/index.jsp) (Cole et al. 2007, 2009). Phylogenetic tree was constructed using Treebuilder software provided by RDP and viewed by MEGA4 software (Tamura et al. 2007).

A total of approximately 1.85 x 10⁸ CFU mL⁻¹ bacteria were observed and seven isolates were successfully isolated from ant lions. One out of seven isolate were detected to possess quorum quenching activity (Fig 1). Both isolate produced extracellular compound that may degrade the signal molecule required for quorum sensing activity. The degradation was indicated by colorless C. violaceum surround-tested isolates. These colorless bacteria were confirmed to produce purple pigment when streaked back onto another plate (data not shown).

Partial 16S-rRNA gene sequences of isolate Myr7 was submitted to Genbank database (www.ncbi.nlm.nih.gov) under accession number HQ453362. Molecular identification showed that isolate Myr7 had similarity to the genus Aeromonas. The isolate Myr7 had 98% similarity to Aeromonas strain M10 DQ200865. Phylogenetic tree analysis (Fig 2) showed phylogenetic position of isolate Myr7 among members of Aeromonadales order. The tree revealed that the isolate Myr7 was clustered in the genus Aeromonas cluster and shared the same branch with Aeromonas hydrophilla subsp. hydrophilla (DSM30187) and Aeromonas strain M10 (DQ200865). These results are different from the study conducted by Dunn and Stabb (2005), who successfully identified the Enterobacteriaceae family from the South American ant lion as stated above.

Quorum quenching activity was found in several bacteria, both Gram positive and Gram negative. It was reported that Variovorax paradoxus (Leadbetter and Greenberg 2000) and Rhodococcus erythropolis (Uroz et al. 2005) had the ability to use AHL molecules as nitrogen and carbon sources, respectively, as well as energy sources. Six bacteria isolated from the leaf surface of Solanum tuberosum, i.e. Agrobacterium larrymoorei, R. erythropolis, B. silvestris, Microbacterium testaceum, B. cereus, and Eschericia coli, were proved to actively degrade acylhomoserine lactone as a signal molecule in quorum sensing mechanism (Morohoshi 2009). The quorum quenching activity were also shown by other bacteria including Bacillus sp. strain 240B1, B. thuringiensis, B. cereus, B. mycoides, B. anthracis, A. tumefaciens, Arthrobacter sp. IBN110., Pseudomonas strain PAI-A, P. aeruginosa PAO1, Ralstonia strain XJ12B (Dong and Zhang 2005). Not only prokaryotic organisms possess anti quorum...
In conclusion, we have successfully isolated two bacterial isolates that possess antiquorum sensing activity against quorum sensing-regulated pigment production of *C. violaceum*. According to partial 16S-rRNA gene analysis, the bacterial isolate had similarity to *Aeromonas* spp. This finding was not expected because *Aeromonas* is one of the bacteria known to use quorum sensing mechanisms to regulate certain gene expression, but not for its antiquorum sensing activity.

REFERENCES


Volume 5, 2011 Microbiol Indones 49