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SHORT COMMUNICATION

Screening of Quorum Quenching Activity of Bacteria Isolated from Ant Lion

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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 109 CFU mL^{-1} 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

Komunikasi intersel bakteri atau *quorum sensing* telah diketahui mengendalikan sifat patogen dari beberapa organisme yang penting secara medis. Oleh karena itu, isolasi bakteri yang dapat memutus rangkaian komunikasi intersel-yang disebut dengan *quorum quenching*-menjadi sangat penting. Bakteri dari undur-undur (*Myrmeleon* sp.) yang ditumbuhkan pada media agar-agar Luria, menghasilkan sejumlah 1.85 x 109 CFU mL^{-1}. Sebanyak tujuh koloni yang berbeda secara morfologi ditapis aktivitas *quorum quenching*-nya dengan *Chromobacterium violaceum* sebagai bakteri indikator. Penapisan tersebut menghasilkan satu isolat (Myr7) yang memiliki aktivitas *quorum quenching,* yang diidentifikasi sebagai *Aeromonas* menggunakan gen penyandi 16S rRNA.

Kata kunci: undur-undur, *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 (Dixson *et al*. 1985), methicillin-resistant *Staphylococcus aureus* (Sutherland and Rolinson 1964; Kareiviene *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 bacteria 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 epithelian 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

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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 10^5 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 quencing 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 ^oC 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) web site (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 10^9 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 wes submitted to Genbank database

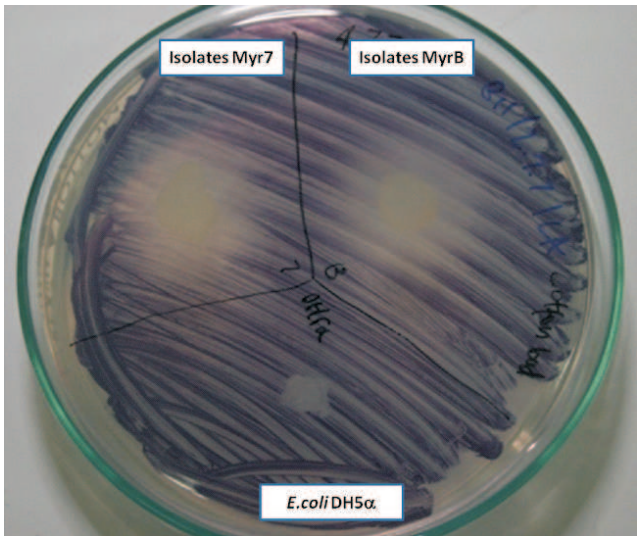

Fig 1 Screening of quorum quenching activity of isolates Myr7 and MyrB. *Escherichia coli* DH5 was used as a negative control.

(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^T) 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., *K. pneumoniae*, *Pseudomonas* strain PAI-A, *P. aeruginosa* PAO1, *Ralstonia* strain XJ12B (Dong and Zhang 2005). Not only prokaryotic organisms possess anti quorum

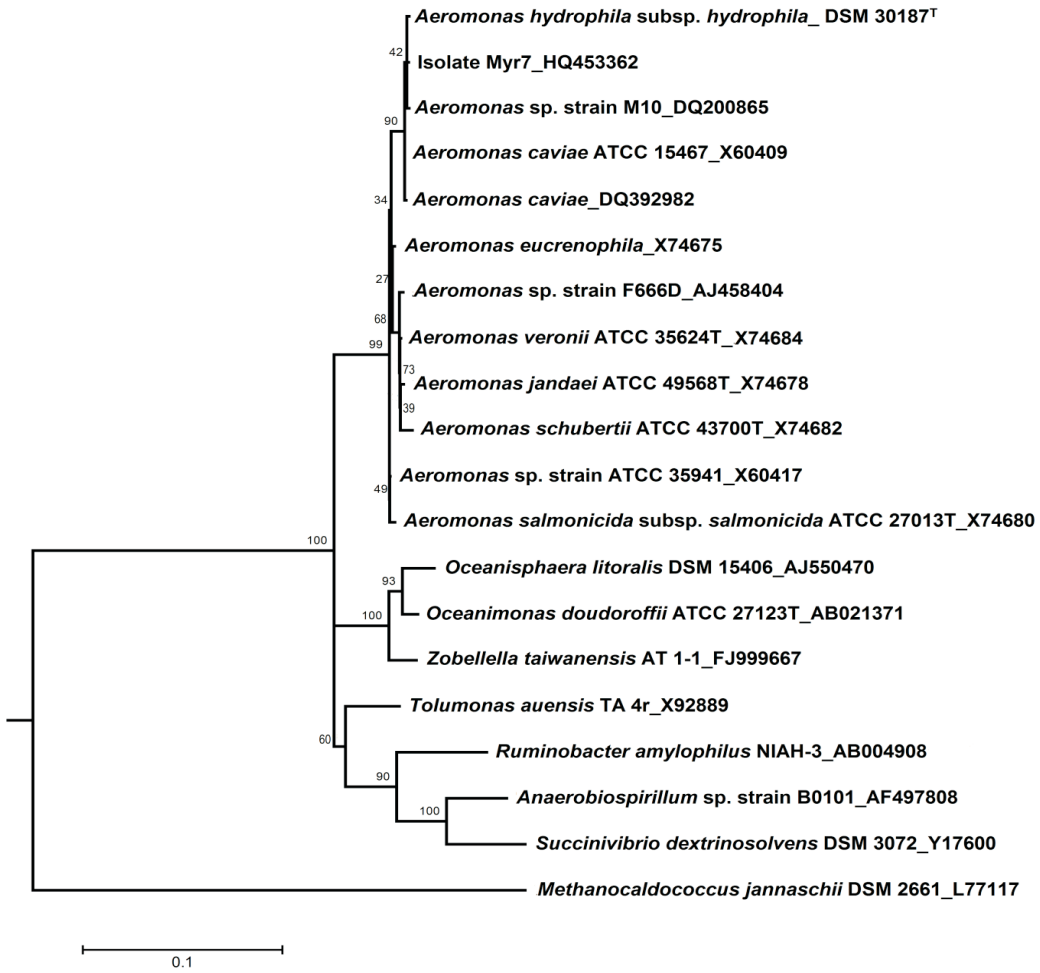

Fig 2 Phylogenetic tree of isolate Myr7 between the genus *Aeromonas* and the other genera within Aeromonadales order. The tree was constructed using weighted neighbor-joining tree building algorithm. The numbers in front of the branch point of the tree are bootstrap values with 100 replicates. The bar below the tree represents a distance scale. The code following underscore is genbank accession number.

sensing activity, eukaryotic organisms have also been known to have a quorum quenching activity. Extracts of pea plants (*Pisum sativum*) and crown vetch (*Coronilla variations*) (Teplitski *et al.* 2000), *C. erectus, Chamaecyce hypericifolia*, *Callistemon viminalis*, *Bucida burceras*, *Tetrazygia bicolor*, and *Quercus virgiana* (Adonizio *et al.* 2006) have been tested of their abilities to inhibit the expression of quorum sensing activity by AHL inactivation.

Aeromonas spp. has not been reported to possess quorum quenching ability. Therefore, this result was the first of such findings. Despite the ability to inhibit quorum sensing, member of the genus *Aeromonas* has been known to its quorum sensing machinery such as AhyRI and AsaRI that are homologous to LuxRI. *A. hydrophila* produces C4-HSL and N-hexanoylhomoserine lactone (C6-HSL) (Swift *et al*. 1997).

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

Adonizio AL, Downum K, Bennett BC, Mathee K. 2006. Anti-quorum sensing activity of medicinal plants in Southern Florida. J Ethnopharmacol. 105:427-435. doi:10.1016/j.jep.2005.11.025.

Baquero F. 1995. Pneumococcal resistance to -lactam antibiotics: a global geographic overview. Microb Drug Resist. 1(2):115-120. doi:10.1089/mdr.1995.1.115.

Baron C. 2010. Antivirulence drugs to target bacterial secretion systems. Curr Opin Microbiol. 13(1):100-105. doi:10.1016/j.mib.2009.12.003.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM. 2009. The Ribosomal database project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res. 37:D141-D145. doi: 10.1093/nar/gkn879.

Cole J R, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS,McGarrell DM, Bandela AM, Cardenas E, Garrity GM, Tiedje JM. 2007. The ribosomal database project (RDP-II): introducing *myRDP* space and quality controlled public data. Nucleic Acids Res. 35:D169-D172. doi: 10.1093/nar/gkl889.

Dixson S, Brumfitt W, Hamilton-Miller JM. 1985. In vitro activity of six antibiotics against multiresistant staphylococci and other gram-positive cocci. Eur J Clin Microbiol. 4(1):19-23.

Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH. 2001. Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. Nature. 411(6839):813-817. doi:10.1038/35081101.

Dong YH, Zhang LH. 2005. Quorum sensing and quorum quenching enzymes. J Microbiol 43(1):101-109.

Dunn AK, Stabb EV. 2005. Culture-independent characterization of the microbiota of the ant lion *Myrmeleon mobilis* (Neuroptera: Myrmeleontidae). Appl Environ Microbiol. 71(12):8784-8794. doi:10.1128/AEM.71.12.8784-8794.2005.

Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim SJ, Reniero A, Hoffner S, Rieder HL, Binkin N, Dye C, Williams R, Raviglione MC. 2001. Global trends in resistance to antituberculosis drugs. N Engl J Med. 344(17):1294-1303.

Finch RG, Pritchard DI, Bycroft BW, Williams P, Stewart GSAB. 1998. Quorum sensing: a novel target for anti-infective therapy. J Antimicrob Chemother. 42(5):569-571.

Goldstein FW, Garau J. 1997. 30 years of penicillin-resistant *S. pneumoniae*: myth or reality? Lancet. 350(9073):233-234. doi:10.1016/S0140-6736(05)62222-2.

Kareiviene V, Pavilonis, A, Sinkute, G, Liegiute S, Gailiene G. 2006. *Staphylococcus aureus* resistance to antibiotics and spread of phage types. Medicina. 42(4):332-339.

Kievit TR de, Iglewski BH. 2000. Bacterial quorum sensing in pathogenic relationships. Infect Immun. 68(9):4839-4849.

Leadbetter JR, Greenberg EP. 2000. Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*. J Bacteriol. 182(24):6921-6926.

Martins A, Cunha M de L. 2007. Methicillin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci: epidemiology and molecular aspects. Microbiol Immunol. 51(19):787-795.

Morohoshi T, Someya N, Ikeda T. 2009. Novel N-acylhomoserine lactone-degrading bacteria isolated from the leaf surface of *Solanum tuberosum* and their quorum-quenching properties. Biosci Biotechnol Biochem. 73(9):2124-2127. doi:10.1271/bbb.90283.

Newell DG, Koopmans M, Verhoef L, Duizer E, Aidara-Kane A, Sprong H, Opsteegh M, Langelaar M, Threfall J, Scheutz F, van der Giessen J, Kruse H. 2010. Food-borne diseases-the challenges of 20 years ago still persist while new ones continue to emerge. Int J Food Microbiol. 139:S3-S15. doi:10.1016/j.ijfoodmicro.2010.01.021.

Nishiwaki H, Ito K, Shimomura M, Nakashima K, Matsuda K. 2007. Insecticidal bacteria isolated from predatory larvae of the antlion species Myrmeleon bore (Neuroptera: Myrmeleontidae). J Invertebr Pathol 96:80-88. doi:10.1016/j.jip.2007.02.007.

Sutherland R, Rolinson, GN. 1964. Characteristics of methicillin-resistant *Staphylococci*. J Bacteriol. 87(4):887-899.

Swift S, Karlyshev AV, Fish L, Durant EL, Winson MK, Chhabra SR, Williams P, Macintrye S, Stewart GS. 1997. Quorum sensing in *Aeromonas hydrophila* and *Aeromonas salmonicida*: identification of the LuxRI homologs AhyRI and AsaRI and their cognate N-acylhomoserine lactone signal molecules. J Bacteriol. 179(17):5271-5281.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 24(8):1596-1599. doi:10.1093/molbev/msm092.

Teplitski M, Robinson JB, Bauer WD. 2000. Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. Mol Plant Microbe Interact. 13(6):637-648. doi:10.1094/MPMI.2000.13.6.637.

Uroz S, Chhabra SR, Cámara M, Williams P, Oger P, Dessaux Y. 2005. N-Acylhomoserine lactone quorum sensing molecules are modified and degraded by *Rhodococcus erythropolis* W2 by both amidolytic and novel oxidoreductase activities. Microbiology. 151:3313-3322. doi:10.1099/mic.0.27961-0.

Waters CM, Bassler BL. 2005. Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol. 21:319-346. doi: 10.1146/annurev.cellbio.21.012704.131001.

Williams P, Camara M, Hardman A, Swift S, Milton D, Hope VJ, Winzer K, Middleton B, Pritchard DI, Bycroft BW. 2000. Quorum sensing and the population dependent control of virulence. Philos Trans R Soc London Ser B. 355(1397):667-680. doi:10.1098/rstb.2000.0607.