

**CHARACTERISATION OF QUORUM SENSING AND QUORUM
QUENCHING SOIL BACTERIA ISOLATED FROM MALAYSIAN
TROPICAL MONTANE FOREST**

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ABSTRACT

Quorum sensing (QS) describes bacterial cell density dependent signaling mechanisms through autoinducers that are involved in mediation of bioluminescence, biofilm development, antibiotic production and virulence factor expressions. Attenuation of quorum sensing signals termed as quorum quenching (QQ) have been identified in a range of living organisms, including bacteria and eukaryotes. The production and degradation of QS compound *N*-acyl homoserine lactones (AHL) by bacteria isolated from Malaysian montane forest soil were assessed in this study. Phylogenetic analysis indicated that these isolates were distinct members of genera of *Arthrobacter*, *Bacillus* and *Pseudomonas*. Biosensor screening and liquid chromatography mass spectrometry analysis have revealed the production of *N*-dodecanoyl-L-homoserine lactone (C12-HSL) by *Pseudomonas frederiksbergensis* (isolate BT9). Strong QQ activity with broad substrate specificity against various AHLs has been detected among *Arthrobacter*, *Bacillus* and *Pseudomonas* isolates using whole-cell AHL inactivation assay and rapid resolution liquid chromatography analysis (RRLC). In addition, degradation of *p*-coumaroyl-homoserine lactone by *Arthrobacter* and *Pseudomonas* spp. was documented first in this study. Further studies involving whole genome sequencing of these isolates for the purpose of assessing regulation of QS and QQ genes is currently being carried out.

ABSTRAK

Pengesanan kuorum (QS) merupakan mekanisme komunikasi bakteria melalui isyarat yang bergantung pada ketumpatan sel dan terlibat dalam aktiviti-aktiviti seperti biopendarkilau, pembangunan lapisan biofilm, sintesis antibiotik dan ungkapan faktor kebisaan. Pengurangan isyarat pengesanan kuorum yang dikenal sebagai penghambatan kuorum (QQ) telah dijumpai dalam pelbagai organisma hidup, termasuk bakteria dan eukariot. Pengeluaran dan degradasi isyarat QS dikenali *N*-acyl homoserine lactones (AHL) oleh bakteria yang diasingkan daripada tanah hutan gunung Malaysia telah dinilai dalam kajian ini. Analisis filogenetik menunjukkan bahawa bakteria-bakteria ini adalah antara ahli genera *Arthrobacter*, *Bacillus* dan *Pseudomonas*. Ujian pengesanan kuorum dan analisis kromatografi spektrometri jisim cecair (LCMS) telah mengenalpasti pengeluaran *N*-dodecanoyl-L-homoserine lactone (C12-HSL) oleh *Pseudomonas frederiksbergensis* (BT9). Aktiviti QQ yang ketara terhadap pelbagai substrat isyarat kuorum AHLs telah dikesan di kalangan bakteria *Arthrobacter*, *Bacillus* dan *Pseudomonas* dengan ujian degradasi isyarat AHL dan analisis kromatografi cecair (RRLC). Di samping itu, degradasi *p*-coumaroyl-homoserine lactone oleh *Arthrobacter* dan *Pseudomonas* spp. adalah pertama didokumenkan dalam kajian ini. Kajian lanjutan yang melibatkan penjujukan seluruh genom sedang dijalankan dengan tujuan untuk menilai perantaraan gen QS dan QQ.

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LIST OF ABBREVIATIONS

| | |
|---------------|--|
| °C | Degree Celsius |
| AHL | <i>N</i> -acyl homoserine lactone |
| ACP | Acyl carrier protein |
| α | Alpha |
| Amp | Ampicilin |
| β | Beta |
| BLAST | Basic Local Alignment Search Tool |
| bp | Basepair |
| DNA | Deoxyribonucleic acid |
| EDTA | Ethylenediaminetetraacetic acid |
| <i>et al.</i> | <i>et alia</i> (and others) |
| EtBr | Ethidium bromide |
| g | Gram |
| <i>g</i> | Gravity (relative centrifugal force) |
| h | Hour |
| HSL | Homoserine lactone |
| kb | Kilobase pair |
| γ | Lambda |
| LB | Luria-Bertani |
| M | Molar Concentration |
| MOPS | 3-(<i>N</i> -morpholino) propanesulfonic acid |
| min | Minute |
| mg | Milligram |

| | |
|--------------------|---|
| ml | Millilitre |
| mM | Millimolar Concentration |
| OD | Optical density |
| % | Percentage |
| PBS | Phosphate buffered saline |
| pC-HSL | <i>p</i> -coumaroyl-homoserine lactone |
| PCR | Polymerase chain reaction |
| QS | Quorum sensing |
| QQ | Quorum quenching |
| rDNA | Ribosomal deoxyribonucleic acid |
| rpm | Revolutions per minute |
| RRLC | Rapid Resolution Liquid Chromatography |
| SAM | <i>S</i> -adenosylmethionine |
| sdH ₂ O | Sterile distilled water |
| sp. | Species |
| TBE | Tris borate EDTA |
| UV | Ultra Violet |
| v/v | Volume per volume |
| w/v | Weight per volume |
| X-gal | 5-bromo-4-chloro-3-indoyl-beta-D-galacto-pyranoside |
| μg | Microgram |
| μl | Microlitre |