

## Short Communication: Biochemistry Analysis and Molecular Approach to Identify the Cultured Bacterial from Ex-Tin Mining Lakes

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<b>Author Order</b>	4 of 4
<b>Accreditation</b>	2
<b>Abstract</b>	<p>There are two methods to identify the bacterial characteristic, namely biochemical analysis and the 16S ribosomal ribonucleic acid gene (16S rRNA) sequencing analysis. The research aimed to identify the cultured bacterial from ex-tin mining lakes by biochemistry analysis and molecular approach. Nine bacterial were cultured and isolated in nutrient agar and then biochemically characterized by microbactÃ¢ÂÂ 12A and 24E (Oxoid) identification kits. In addition, molecular analysis by 16S rRNA gene was sequenced primer 1492R and primer 27F. Based on biochemistry analysis, these bacterial were identified as belonging to species of <i>Bacillus amyloliquefaciens</i>; <i>Enterobacter gergoviae</i>; <i>Enterobacter aerogenes</i>; <i>Enterobacter agglomerans</i>; and <i>Nitrobacter</i> spp. The sequence analysis in gene bank of NCBI indicated that these species had similarity with <i>Klebsiella variicola</i> strain F2R9 (Accession NR_025635.1); <i>Enterobacter cloacae</i> subsp. <i>dissolvens</i> strain LMG 2683 (Accession NR_044978.1); <i>Serratia marcescens</i> strain NBRC 102204 (Accession NR_114043.1); <i>Bacillus marisflavi</i> strain TF-11 (Accession NR_118437.1); <i>Falsibacillus pallidus</i> strain CW 7 (Accession NR_116287.1); <i>Klebsiella pneumoniae</i> strain DSM 30104 (Accession NR_117683.1); and <i>Nitrobacter winogradskyi</i> strain Nb-255 (Accession NR_074324.1). However, phylogenetic tree was constructed by Neighbor-Joining Test showed the cultured bacterial were not in the same clade and also with <i>Salmonella enterica</i> subsp. <i>enterica</i> strain LT2 (Accession NR_074910.1); <i>Bacillus amyloliquefaciens</i> strain BCRC 11601; and <i>Escherichia coli</i> strain NBRC 102203 (Accession NR_114042.1) as in group species and <i>Micrococcus luteus</i> strain NCTC 2665 (Accession NR_075062.2); <i>Chloroflexus islandicus</i> strain isl-2 (Accession NR_148571.2); <i>Flavobacterium gondwanense</i> (Accession M92278.1); and <i>Cytophaga aurantiaca</i> strain JM110 (Accession MN758870.1) as their out group.</p> <p>ABSTRAK Terdapat dua metode untuk mengidentifikasi karakteristik bakteri, yaitu analisis biokimia dan analisis sekuensing gen 16S ribosomal ribonucleic acid (16S rRNA). Karakterisasi bakteri telah dilakukan melalui analisis morfologi dan biokimia dan dikonfirmasi melalui pendekatan molekuler menggunakan sekuensing gen 16S ribosomal ribonucleic acid (16S rRNA). Penelitian ini bertujuan untuk mengidentifikasi bakteri yang dapat dikultur dari danau pascatambang timah melalui analisis biokimiawi dan pendekatan molekuler. Sembilan bakteri berhasil dikultur dan diisolasi di media nutrient agar dan kemudian secara biokimiawi dikarakterisasi menggunakan microbactÃ¢ÂÂ 12A and 24E (Oxoid) identification kits. Lebih lanjut, analisis molekuler menggunakan gen 16S rRNA dilakukan sekuensing dengan primer 1492R dan primer 27F. berdasarkan analisis biokimia, bakteri-bakteri tersebut termasuk ke dalam spesies <i>Bacillus amyloliquefaciens</i>; <i>Enterobacter gergoviae</i>; <i>Enterobacter aerogenes</i>; <i>Enterobacter agglomerans</i>; dan <i>Nitrobacter</i> spp. Analisis blasting pada gene bank di NCBI mengindikasikan bahwa spesies-spesies tersebut memiliki kemiripan atau similaritas dengan <i>Klebsiella variicola</i> strain F2R9 (Accession NR_025635.1); <i>Enterobacter cloacae</i> subsp. <i>dissolvens</i> strain LMG 2683 (Accession NR_044978.1); <i>Serratia marcescens</i> strain NBRC 102204 (Accession NR_114043.1); <i>Bacillus marisflavi</i> strain TF-11 (Accession NR_118437.1); <i>Falsibacillus pallidus</i> strain CW 7 (Accession NR_116287.1); <i>Klebsiella pneumoniae</i> strain DSM 30104 (Accession NR_117683.1); dan <i>Nitrobacter winogradskyi</i> strain Nb-255 (Accession NR_074324.1). Namun, pohon filogenetik yang dikonstruksikan dengan Neighbor-Joining Test menunjukkan bahwa bakteri yang dikultur tersebut tidak berada pada clade dan juga dengan <i>Salmonella enterica</i> subsp. <i>enterica</i> strain LT2 (Accession NR_074910.1); <i>Bacillus amyloliquefaciens</i> strain BCRC 11601; dan <i>Escherichia coli</i> strain NBRC 102203 (Accession NR_114042.1) yang digunakan sebagai spesies in group species maupun <i>Micrococcus luteus</i> strain NCTC 2665 (Accession NR_075062.2); <i>Chloroflexus islandicus</i> strain isl-2 (Accession NR_148571.2); <i>Flavobacterium gondwanense</i> (Accession M92278.1); dan <i>Cytophaga aurantiaca</i> strain JM110 (Accession MN758870.1) sebagai out groupnya.</p>
<b>Publisher Name</b>	School of Postgraduate Studies, Diponegoro Univer
<b>Publish Date</b>	2022-07-05
<b>Publish Year</b>	2022

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<b>Doi</b>	DOI: 10.14710/jil.20.3.563-569
<b>Citation</b>	
<b>Source</b>	Jurnal Ilmu Lingkungan
<b>Source Issue</b>	Vol 20, No 3 (2022): July 2022
<b>Source Page</b>	563-569
<b>Url</b>	<a href="https://ejournal.undip.ac.id/index.php/ilmulingkungan/article/view/44721/pdf">https://ejournal.undip.ac.id/index.php/ilmulingkungan/article/view/44721/pdf</a>
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