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

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

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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 microbact™ 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 *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *Enterobacter aerogenes*; *Enterobact*8*agglomerans*; and *Nitrobacter* spp. The sequence analysis i7gene bank of NCBI indicated that these species had similarity (Accession _044978.); (Accession 1); *Bacillus marisflavi* strain TF-11 (Accession NR_118437.1); *Falsibacillus pallidus* strain CW 7 (Accession NR_116287.1); 5 (Accession ); and *Nitrobacter winogradskyi* Nb-255 (Accession _074324.). However, Test showed cultured bacterial were not in the same clade and also with (Accession _074910.); *Bacillus amyloliquefaciens* BCRC 11601; and *Escherichia coli* strain NBRC 102203 (Accession NR_114042.1) as in group species and *Micrococcus luteus* strain NCTC 2665 (Accession NR_075062.2); *Chloroflexus islandicus* strain isl-2 (Accession NR_148571.2); *Flavobacterium gondwanense* (Accession M92278.1); and *Cytophaga aurantiaca* strain JM110 (Accession MN758870.1) as their out group.

***Keywords***: bacterial, biochemistry, ex-tin mining lakes; molecular; 16S rRNA gene

**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 morfol23dan biokimia dan dikonfirmasimelalui pendekatan molekuler menggunakan sekuensing gen 16S ribosomal ribonucleic acid ( yang dapat dikultur danau pascatambang timah melalui analisis biokimiawi dan pendekatan molekuler. Sembilan bakteria berhasil dikultur dan diisolasi di media nutrient agar dan kemudian secara biokimiawi dikarakterisasi menggunakan microbact™ 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 *Bacillus amyloliquefaciens; Enterobacter gergoviae; Enterobacter aerogenes; Enterobacter agglomerans;* dan *Nitrobacter spp.* Analisis blasting pada *gene bank* di NCBI mengindikasikan bahwa spesi7spesies tersebut memiliki kemiripan atau similaritas dengan *Klebsiella variico*1*strain F2R9 (Accession NR_025635.* *(Accession _044978.); (Accession 1); Bacillus marisflavi strain TF-11 (Accession NR_118437.1); Falsibacillus pallidus strain CW 7 (Accession NR_116287.1); (Accession ); dan Nitrobacter winogradskyi Nb-255 (Accession _074324.).* Namun, pohon filogenetik yang dikonstruksi2n dengan *Neighbor-Joining Test* menunjukkan bahwa bakteri yang dikultur tersebut tidak berada pada *clade* dan juga dengan *(Accession _074910.); Bacillus amyloliquefaciens BCRC 11601; dan Escherichia coli strain NBRC 102203 (Accession NR_114042.1)* yang digunakan sebagai spesies *in group species* maupun *Micrococcus luteus strain NCTC 2665 (Accession NR_075062.2); Chloroflexus islandicus strain isl-2 (Accession NR_148571.2); Flavobacterium gondwanense (Accession M92278.1);* dan *Cytophaga aurantiaca strain JM110 (Accession MN758870.1)* sebagai *out group*nya.

***Kata kunci***: bakteri, biokimia, danau pascatambang timah, molekuler, gen 16S rRNA

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## 1. Introduction

Tin mining activity in Bangka Belitung Archipelago Province produces a water in a mined land looked like a lake, called kolong. The waters have poor characteristics such as low dissolved oxygen (DO) (Ashraf et al. 2011), cation exchange capacity (CEC), poor nutrient and organic component (Oktavia et al. 2014), acidic pH, and also heavy metals contamination (Kurniawan et al. 2019). Tin mining activities can also cause damage to the ecology, include distraction and alteration of microorganisms' ecology and functional stability of microbial community (Kurniawan et al. 2018; Kurniawan, 2016; Li et al. 2014). The ecological damage due to mining activities causes ecosystem imbalance and also changes in the diversity of micro anisms (Lad & Samant, 2015; Giri et al. 2014; Singh ; Ashraf ; Vyas and Pancholi, ; Fan . 2002).

microorganisms' capability to responds the ecosystem changes quickly can be utilized as an indicator to understanding the changes of water quality (Lau & Lennon, 2012; scatelli et al., 2005; Niemi & McDonald, 2004; Paerl , 2003). link the changes to the microbial diversity because they have role in biogeochemical and biot sformation cycles in the biosphere (Gadd, 2010; for understanding the microbial community diversity, structures, dynamics, and functional. The existence and biochemical characteristics of microorganisms in an environment can known by growing them in synthetic medium and . This gene is a biological marker that is widely studied to explain the existence of microbe in an environment, molecular evolution for taxonomic classification, and microbial phylogenetic analysis. The 16S rRNA gene has a hypervariable region so that it can be used to identify microbes (Yang et al. 2016; Lozupone and Knight, 2008).

This research aimed to identify the diversity of microbes, especially the cultured bacterial from lakes . Identification bacterial by molecular approach showed name of species based on the gene, besides biochemical characteristics. This research showed the potential of bacterial as a bioindicator and their role in the ecosystem with their characteristics of biochemistry. Further, their capability as bioremediator can be elaborated to remediate and recover the waters quality of abandoned tin mining lakes.

## 2. Method

### 2.1. Study area

The research stations were located in areas were e oded as Station A (lake < 1 year), Station B (lake (lake > coordinates Station A were 01°59' S in points 36,0"; 36,2"; 36,4"; 36,5"; 36,6" and 106°06' E in points 36.5"; 36.9"; 37.3"; 37.4"; 37.5". The coordinates of Station B were 01°59' S in points 41.3"; 41.4"; 41.5"; 42.4"; 42.5" and 106°06' E in points 39.2"; 39.5"; 41.4"; 42.7"; 43.1". The coordinates of Station C were 01°55' S in points 40.9"; 58.9"; 59.1"; 59.2"; 59.5" and 106°06' E in points 19.5"; 19.7"; 19.9"; 22.4"; 29.2" (Figure 1) (Kurniawan et al. 2018).

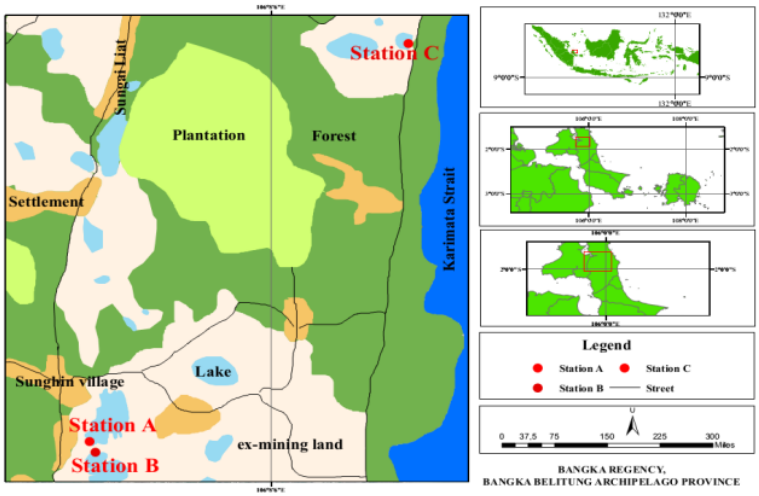


**Figure 1** Research location along with the research stations in Bangka Regency, Bangka Belitung Province Archipelago. Station (A) was ex-tin mining lake in < 1 year; Station (B) 5-10 years; Station (C) > 15 years

*2.2. Identification of the cultured bacterial*

The bacterial of ex-tin mining lakes were isolated by nutrient agar (NA) and showed nine bacterial isolates (Table 1) that were prepared for biochemistry analysis. The biochemical characteristics of cultured bacterial isolates were identified by microbact™ 12A and 24E (Oxoid) identification kits (Osuntokun et al. 2018). While, molecular analysis was done by 16S rRNA gene analysis with primer 1492R (5′GGTTACCTTGTTACGACTT3′) as reverse primer and primer 27F (5′GAGTTTGATCAT GGCTCAG3′) as forward primer for Polymerase Chain Reaction (PCR). The DNA template was prepared from an individual colony of each spe s of the cultured bacterial and then the amplification carried out denaturation process PCR w ccurred ), annealing process 94 ° ( ) with (3 ) (Senthilraj et al. 2016). The product quality of PCR was visualized by 0.80% agarose gel with amount of DNA ladder loaded per lane 0.1 µg, 1 kb DNA ladder (bp), and volume of sample loaded per lane was 1 µL.

*2.3. Sequence analysis*

*Sequence analysis was carried out by First Base Agent. Sequence alignments were analyzed by Program oEdit and then were compared with bacterial genes in )* (https:// *with Test in Program Mega 6.06. The phylogenetic tree for sequ nces of samples was constructed and compared* . (Accession _044978. ); (Accession ); *Bacillus marisflavi* strain TF-11 (Accession NR_118437.1); *Falsibacillus llidus* strain CW 7 (Accession NR_116287.1); (Accession ); *Nitroba r winogradskyi* Nb-255 (Accession _074324. (Accession _074910. ); *Bacillus amyloliquefaciens* BCRC 11601 (Accession _116022. ); and *Escherichia coli* strain NBRC 102203 (Accession NR_114042.1) as in group species of Phylum Proteobacteria and Firmicutes of Kingdom Bacteria. While, out group species for the phylogenetic tree were *Micrococcus luteus* strain NCTC 2665 (Accession NR_075062.2) from Phylum Actinobacteria; *Chloroflexus islandicus* strain isl-2 (Accession NR_148571.2) from Phylum Chloroflexi; *Flavobacterium gondwanense* (Accession M92278.1) from Phylum Bacteroidetes; and *Cytophaga aurantiaca* strain JM110 (Accession MN758870.1) from Phylum Cytophagia.

## 3. Result

Mustikasari The biochemistry analysis sh ed some characteristics of nine cultured bacterial ( , and fro tation A; from Station B; and , and from Station C). Kurniawan et al. (2018) have reported some biochemical characteristics of these cultured bacterial such as gram, oxidase, motility, ornithin, glucosa, indole, Voges-Proskauer (V-P), citrate, and catalase. The other properties of biochemistry was investigated (Table 1) and these characteristics indicated bacterial of *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *E. aerogenes*; *E. agglomerans*; and *Nitrobacter* spp.

These cultured bacterial was isolated and identified their DNA with PCR. The product of PCR showed e DNA quality and estimation of base pair (bp) of were ,400-1,500 bp (Figure 2).

The sequence analysis produce profile of sequences and then they were blasted analysis showed that the name of bacterial species did not represent the results of biochemistry analysis, there were differences species of these bacterial, although the blast of NCBI website showed that the cultured bacterial had high (90-100 %) similarity with strains were used as in group species. The research evidence revealed species name which analyzed by biochemistry approach to the cultured bacterial were different with blasting investigation in gene bank of NCBI.

The phylogenetic tree (Figure 3) was constructed by involving bacterial of in group species and out group species. All of the cultured bacterial were not in the same clade with in group species such as *K. variicola* strain F2R9; *E. cloacae subsp. dissolvens* strain LMG 2683; *S. marcescens* strain NBRC 102204; *B. marisflavi* strain TF-11; *F. pallidus* strain CW 7; *K. pneumoniae* strain DSM 30104; *N. winogradskyi* strain Nb-255; *S. enterica* subsp. enterica strain LT2; *B. amyloliquefaciens* strain BCRC 11601; and *E. coli* strain NBRC 102203. Further, the phylogenetic tree showed sequences of the cultured bacterial were also different form their outgroup species such as *M. luteus* strain NCTC 2665 from Phylum Actinobacteria; *C. islandicus* strain isl-2 from Phylum Chloroflexi; *F. gondwanense* from Phylum Bacteroidetes; and *C. aurantiaca* strain JM110 from Phylum Cytophagia.

Table 1. Biochemisty Characteristics of Bacterial by Microbact™ 12A and 24E

| Biochmistry Characteristics | Research Stations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Station A | | | Station B | | | Station C | | |
| Gram* | + | + | - | - | - | - | - | - | - |
| Spore | + | + | - | - | - | - | - | - | - |
| idase* | + | + | - | + | - | + | + | + | - |
| | - | - | + | - | + | - | - | - | + |
| | + | + | + | + | + | + | + | + | + |
| | - | - | + | - | + | - | - | - | + |
| Ornithin* | - | - | + | - | + | - | - | - | - |
| | - | - | + | - | - | - | - | - | - |
| Glucosa* | - | - | + | + | + | + | + | + | + |
| | - | - | + | - | + | - | - | - | + |
| | - | - | + | + | + | + | + | + | + |
| | + | + | + | - | + | - | - | - | + |
| | - | - | - | - | - | - | - | - | + |
| | - | - | + | - | - | - | - | - | + |
| * | + | + | + | + | + | + | + | + | + |
| Citrate* | - | - | + | - | + | - | - | - | + |
| | - | - | - | - | - | - | - | - | - |
| Gelatine | - | - | - | - | - | - | - | - | - |
| | - | - | - | - | - | - | - | - | - |
| | + | + | - | - | - | - | - | - | - |
| | - | - | - | - | - | - | - | - | - |
| Sucrose | - | - | - | - | - | - | - | - | - |
| Lactoss | - | - | - | - | - | - | - | - | - |
| | - | - | - | - | - | - | - | - | - |
| | - | - | - | - | - | - | - | - | - |
| | - | - | - | - | - | - | - | - | - |
| | - | - | - | - | - | - | - | - | - |
| | - | - | - | - | - | - | - | - | - |
| | + | + | - | - | - | - | - | - | - |
| | - | - | - | - | - | - | - | - | - |
| Haemolysis | Beta | Beta | Alpha | Alpha | Alpha | Alpha | Alpha | Alpha | Alpha |
| novobiosin | No | No | No | No | No | No | No | No | No |
| Starch | - | - | - | - | - | - | - | - | - |
| | + | + | - | - | - | - | - | - | - |

**Legend**: Station A (lake < 1 year), Station B (lake (lake > asterisk (*) biochemistry analysis indicated species *Bacillus amyloliquefaciens* (bac 1); *Bacillus amyloliquefaciens* (bac 2); *Enterobacter gergoviae* (bac 3); *Nitrobacter* spp. (bac 4); *Enterobacter aerogenes* (bac 5); *Nitrobater* spp. (bac 6); *Nitrobater* spp. (bac 7); *Nitrobater* spp. (bac 8); and *Enterobacter agglomerans* (bac 9) (Kurniawan *et al.*, 2018).

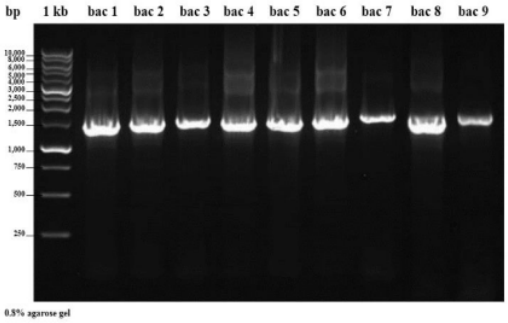


Figure 2 The PCR quality of nine cultured bacterial species for sequencing process


**Figure** The phylogenetic tree showed that cultured bacterial were different from some species sequences database of NCBI. The biochemistry analysis indicated species *Bacillus amyloliquefaciens* (bac 1); *Bacillus amyloliquefaciens* (bac 2); *Enterobacter gergoviae* (bac 3); *Nitrobacter* spp. (bac 4); *Enterobacter aerogenes* (bac 5); *Nitrobater* spp. (bac 6); *Nitrobater* spp. (bac 7); *Nitrobater* spp. (bac 8); and *Enterobacter agglomerans* (bac 9)

## 4. Discussion

The biochemistry analysis served as preliminary characterization of bacterial and this identification test gives some information about morphology, physiology, chemistry, and what these microorganisms were able to do with their specific biochemical functions. While, molecular methods are always useful to identify microbes to the species or strain (Franco-Duarte et al. 2019; Bochner, 2009). The biochemical properties of nine cultured bacterial were included as species of *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *E. aerogenes*; *E. agglomerans*; and *Nitrobacter* spp. However, they can't be justified enough as those species because the molecular analysis by 16S rRNA gene did not indicate them. Approximately, more than 1400 bp in length of the 16S rRNA genes of nine cultured bacterial were sequenced. Analysis of these sequences confirmed that species were most similar to biochemistry identification species. For examples bac 1 and bac 2 namely species of *Bacillus amyloliquefaciens* by biochemistry analysis showed the different clade with *B.* (Accession ) in gene bank of NCBI.

The results of all 16S rRNA gene sequencing presented different group with the blasted species. They were not in the same group or clade, although they had similarity blasting percentage > 90-100% with species of Phylum Proteobacteria and Firmicutes and also so different with the other bacterial from out group species of Phylum Actinobacteria, Chloroflexi, Bacteroidetes, and Cytophagia. has in length this gene has differentiation at the genus level of bacterial. This gene usually related to more than one individual which the similar sequences (Clarridge, 2004).

The 16S rRNA gene as genetic marker identification process bacterial, discovery novel species, taxonomy, and also to construct the bacterial phylogeny (Al Kaabi & Al Yassari, 2019; Manjul & Shirkot, 2018; Woo et al., 2008). Whatever the explanation of this discordance, the discrepancy between these two methods gave important information. Their biochemical characteristics can be explored and elaborated to be used as biological profile of cultured bacterial from ex-tin mining lake. They can be used for various purposes such as bioremediation of ex-tin mining wate ecosystem. It due to their capacity as bioremeditor , wastewater, pollution (Li . 2019; Badiefar ; Sonia

. 2015; Cardak & Altug, 2014; Raja ; Amin . 2013; Ogot . 2013; Naggar ). In spite of their biochemical characteristics indicated their roles, howe the further researches are needed to verify them. analysis has proven similarity and relationship of cultured bacterial gene with the other bacterial from gene bank. The results of this study have convinced that the cultured bacterial were different with blasting results. Nevertheless, the advanced researches are also needed to prove them as novel species of bacterial.

## 5. Conclusion

Thirty-three of biochemical properties from the cultured bacterial were used for identification of them include carbohydrate, amino acid, and lipid utilization or degradation, gram characteristic, motility, sulphuric activity, etc. In this study, species of bacterial from ex-tin mining lakes in Bangka Regency were isolated in NA and identified with microbact test kits. There were nine cultured species from ex-tin mining waters in this study. The biochemistry analysis showed bacterial were identified as belonging to species of *Bacillus amyloliquefaciens*; *Enterobacter gergoviae*; *E. aerogenes*; . *agglomerans*; and *Nitrobacter* spp. However, Test showed cultured bacterial were not in the same clade with the blasted species from gene bank of NCBI. Those bacterial were not similar with some bacterial of gene bank such as species from Genus *Bacillus*, *Enterobacter*, *Nitrobacter*, *Klebsiella*, *Serratia*, *Falsibacillus*, *Salmonella*, and *Escherichia*. They indicated the different clade with them and also with species of Genus *Micrococcus*, *Chloroflexus*, *Flavobacterium*, and *Cytophaga*. In this study, we indicate a new species bacterial were found, although this claim must be proven by further research.

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## References

Al Kaabi, H., Al-Yassari, A. 2019. 16SrRNA sequencing as tool for identification of *Salmonella* spp isolated from human diarrhea cases. Journal of Physics: Conference Series 1294(6): 062041. IOP Publishing

Amin, A., Naik, A., Azhar, M., Nayak H. 2013. Bioremediation of different waste waters-a review. Continental J. Fisheries and Aquatic Science 7(2): 7-17

Ashraf, M., Maah, M., Yusoff, I. 2010. Study of water quality and heavy metals in soil & water of ex-mining area Bestari Jaya, Peninsular Malaysia. International Journal of Basic & Applied Sciences 10(3): 7-12

Ashraf, M., Maah, M., Yusoff, I. 2011. Analysis of physio-chemical parameters and distribution of heavy metals in soil and water of ex-mining area of Bestari Jaya, Peninsular Malaysia. Asian Journal of Chemistry 23(8): 3493-3499

Badiefar, L., Yakhchali, B., Rodriguez-Couto, S., Veloso, A., Garcia-Arenzana, J., Matsumura, Y., Khodabandeh M. 2015. Biodegradation of bisphenol a by the newly-isolated *Enterobacter gergoviae* strain BYK-7 enhanced using genetic manipulation. RSC Advance 5(37): 29563-29572

Bochner, B. 2009. Global phenotypic characterization of bacteria. FEMS Microbiology Reviews 33(1): 191-205.

Çardak, M., Altug, G. 2014. Species distribution and heavy metal resistance of *Enterobacteriaceae* members isolated from Istanbul Strait. Fresenius Environmental Bulletin 23(10a): 2620-2626

Clarridge, J. 2004. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinical Microbiology Reviews 17(4): 840-862.

Fan, Y., Lu, Z., Chen, J., Zhou, Z., Wu, G. 2002. Major ecological and environmental problems and the ecological reconstruction technologies of the coal mining areas in China. Acta Ecologica Sinica 23(10): 2144-2152

Franco-Duarte, R., Cernakova, L., Kadam, S., Kaushik, K., Salehi, B., Bevilacqua, A., ... & Rodrigues, C. 2019. Advances in chemical and biological methods to identify microorganisms-from past to present. Microorganisms 7(5): 1-32

Gadd, G. 2010. Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156(3): 609-643

Giri, K., Mishra, G., Pandey, S., Verma, P., Kumar, R., Bisht N. 2014. Ecological degradation in northeastern coal fields: Margherita Assam. International Journal of Science, Environment and Technology 3(3): 881-884

Kurniawan, A. (2016). Microorganism communities response of ecological changes in post tin mining ponds. Research & Reviews: A Journal of Microbiology *and Virology 6(1): 17-26*

Kurniawan, A., Oedjijono., Tamad., Sulaeman, U. 2018. The phosphorus and sulphur distribution and culturable bacterial in time chronosequence of ex-tin mining ponds. Omni-Akuatika 14(3): 26-33

Kurniawan, A., Oedjijono., Tamad., Sulaeman, U. 2019. The pattern of heavy metals distribution in time chronosequence of ex-tin mining ponds in Bangka Regency, Indonesia. Indonesian Journal of Chemistry 19(1): 254-261.

Lad, R., Samant, J. 2015. Impact of bauxite mining on soil: a case study of bauxite mines at Udgiri, Dist-Kolhapur, Maharashtra State, India. International Research Journal of Environment Sciences 4(2): 77-83

Lau J., Lennon J. 2012. Rapid responses of soil microorganisms improve plant fitness in novel environments. Proceedings of the National Academy of Sciences 109(35): 14058-14062

Li, Y., Chi, M., Ge, X. 2019. Identification of a novel hydrolase encoded by *hy-1* from *Bacillus amyloliquefaciens* for bioremediation of carbendazim contaminated soil and

food. International Journal of Agricultural and Biological Engineering 12(2): 218-224.

Li, Y., Wen, H., Chen, L., Yin, T. 2014. Succession of bacterial community structure and diversity in soil along a chronosequence of reclamation and re-vegetation on coal mine spoils in China. PLoS ONE 9(12): e115024

Lozupone, C., Knight, R. 2008. Species divergence and the measurement of microbial diversity. FEMS Microbiology Reviews 32(4): 557-578.

Manjul, A., Shirkot, P. 2018. 16S rRNA gene sequencing for bacterial identification of pullulanase synthesizing thermophilic bacteria contributing to big data. International Journal of Chemical Studies 6(2): 2769-2773

Moscatelli, M., Lagomarsino, A., Marinari, S., De Angelis, P., Grego, S. 2005. Soil microbial indices as bioindicators of environmental changes in a poplar plantation. Ecological Indicators 5(3): 171-179.

Naggar, A., Kamel, M., Aladly, A., Ismail, N. 2010. Bioremediation of paraffinic and polynuclear aromatic hydrocarbons using laser irradiated *Bacillus amyloliquefaciens*. Journal of American Science 6(10): 661-670

Niemi, G., McDonald, M. 2004. Application of ecological indicators. Annual Review of Ecology, Evolution, and Systematics 35(2004): 89-111

Ogot, H., Boga, H., Budambula, N., Tsanuo, M., Andika, D., Ogola H. 2013. Isolation, characterization and identification of roundup degrading bacteria from the soil and gut of *Macrotermes michaelseni*. International Journal of Microbiology and Mycology 1(1): 31-38

Oktavia, D., Setiadi, Y., Hilwan, I. 2014. Sifat fisika dan kimia tanah di hutan kerangas dan lahan pasca tambang timah Kabupaten Belitung Timur. Jurnal Silvikultur Tropika 5(3): 149-154

Osuntokun, O., Jemilaiye, T., Thonda, A. 2018. Phenotypic identification and antibiogram profile of citrobacter species. Journal of Clinical Research and Pharmacy 1(1): 1-6

Paerl, H. W., Dyble, J., Moisander, P., Noble, R., Piehler, M., Pinckney, J., ..., aldes, L. 2003. Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. FEMS Microbiology Ecology 46(3): 233-246

Prosser, J., Bohannan, B., Curtis, T., Ellis, R., Firestone, M., Freckleton, R., ..., Young, J. 2007. The role of ecological theory in microbial ecology. Nature Reviews Microbiology 5(5): 384-392

Raja, S., Dinesh, K., Kesavan, K., Kodungallur, T., Thrissur, K. 2014. Bioremediation by using of microbes and algae with special reference to coastline environment. International Journal of Biosciences and Nanosciences 1(6): 130-140

Senthilraj R., Prasad G., Janakiraman K. 2016. Sequence-based identification of microbial contaminants in non-parenteral products. Brazilian Journal of Pharmaceutical Sciences 52(2): 329-336

Singh, P. K., Afzal, I., Ravi, S., Dhanesh, S., Shivi, S. 2013. A Study about ecological imbalance in Surguja (India) coalfield area due to mining. International Research Journal of Environment Sciences 2(4): 10-14

Sonia, V., Rajagopalsamy, C., Ahilan, B., Francis, T. 2015. Microbial bioremediation: a potential tool for sustainable aquaculture. Journal of Industrial Pollution Control 31(1): 105-113

Vyas, A., Pancholi, A. 2009. Environmental degradation due to mining in South Rajasthan: a case study of Nimbahera, Chittorgarh (India). Journal of Environmental Research and Development 4(2): 405-412

Woo, P., Lau, S., Teng, J., Tse, H., Yuen, K. 2008. Then and now: use of 16s rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. Clinical Microbiology and Infection 14(10): 908-934

Yang, B., Wang, Y., Qian, P. 2016. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. BMC Bioinformatics 17(1): 135-143

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