

## Identification of Potential Bacteria on Several Lakes in East Java, Indonesia Based on 16S rRNA Sequence Analysis

Achmad Rodiansyah<sup>1</sup>, Ainul Fitria Mahmudah<sup>1</sup>, Mastika Marisahani Ulfah<sup>1</sup>, Uun Rohmawati<sup>1</sup>, Dwi Listyorini<sup>1</sup>, Eko Agus Suyono<sup>2</sup>, Sitoresmi Prabaningtyas<sup>1\*</sup>

<sup>1</sup>Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Malang, Malang, Indonesia

<sup>2</sup>Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

### ARTICLE INFO

#### Article history:

Received October 30, 2020

Received in revised form February 10, 2021

Accepted February 15, 2021

#### KEYWORDS:

Amylolytic bacteria,  
cellulolytic bacteria,  
indole acetic acid (IAA)-producing bacteria,  
phosphate solubilizing bacteria (PSB),  
16S rRNA

### ABSTRACT

Four bacterial isolates from Ranu Pani and Ranu Grati in east java had been revealed to be potentials to produce IAA (PIS isolate), phosphate solubilizer (GPS isolate), cellulose hydrolysis (PSS isolate) and, amylum hydrolysis (PAS), two dominant bacterial isolates from Rani Pani (PØD isolate) and Ranu Grati (GØD isolate) which were co-cultured with microalgae promoted microalgae growth, yet its taxonomical position has not been clearly known. The aim of this study was to identify those bacterial isolates using 16S rRNA barcode. This research conducted by gDNA isolation, the 16S rRNA sequence was amplified using 27F and 1492R primers. Reconstructed phylogenetic trees and genetic distance analysis showed that the isolate PIS and PSS identified as *Bacillus cereus* Group closely related to *Bacillus paramycooides*. PAS isolate identified as *Bacillus subtilis* Group closely related to *Bacillus amyloliquefaciens*, GPS isolate identified as novel species in genus *Enterobacter*, and two dominant isolates (PØD and GØD) identified as *Enterobacter cloacae* complex closely related to *Enterobacter cloacae*. The genomic approach and additional phenotypes-examination are required to clarify its taxonomical position.

## 1. Introduction

The previous research was found 96 bacterial isolates from Ranu Pani, Ranu Regulo, Ranu Grati, and Telaga Ngebel which have multiple potencies as follows, 40 isolates have the potency to produce IAA, 31 phosphate solubilizing bacterias, two dominant bacterias from Ranu Pani and Ranu Grati can promote microalgae growth (Prabaningtyas *et al.* 2018a, 2018b), 47 cellulolytic bacterias (Ardilla 2018), and 53 amylolytic bacterias (Basitoh 2018). Identification of bacteria that have the highest potential for producing IAA and solubilizing phosphate yet not been done. Moreover, the results of identification based on morphology and physiology of highest amylolytic bacteria identified as *Bacillus subtilis* (Basitoh 2018), highest cellulolytic bacteria identified as *Bacillus alvei* (Ardilla 2018), and identification of dominant bacteria from Ranu Grati identified as *Enterobacter gergoviae* and dominant bacteria from Ranu Pani

cannot be identified (Prabaningtyas and Witjoro 2017).

The identification of microorganisms important for microbiologists and scientists involved in many fields of research and applied industry (Bisen *et al.* 2012). This identification of potential bacteria has beneficial for biological conservation, regulation study, biological activity, and especially in a consortium of bacterial in co-culture microalgae. The consortium of bacteria in co-culture microalgae can increase the efficiency of biomass production from microalgae (Fuentes *et al.* 2016; Ramanan *et al.* 2016). This consortium can increase the growth of algae because bacteria provide inorganic essential mineral elements (Marañón *et al.* 2005; Thyssen 2005) like carbon that needed for metabolic algae (Jena *et al.* 2011; Kropat *et al.* 2011).

The identification of bacteria based on the phenotype has been carried out, but this identification method needs more time and energy (López-Campos *et al.* 2012), different environmental conditions may decrease the validity (Tshikhudo *et al.* 2013), some bacteria difficult to be cultured so that identification

\* Corresponding Author

E-mail Address: sitoresmi.prabaningtyas.fmipa@um.ac.id

cannot be done (Gugliandolo *et al.* 2011), and there are some morphological and physiological similarities that are difficult to be distinguished (Boivin-Jahns *et al.* 1995). A fast and reliable method is widely used to overcome this problem by analyzing the barcode 16S rRNA gene (Buszewski *et al.* 2017; Tshikhudo *et al.* 2013).

DNA barcoding is an efficient, accurate, cheap and standardized method for identifying organisms using short DNA sequences from whole-genome DNA in the sample (Cristescu 2014; Lebonah *et al.* 2014). This method can be used to deepen the understanding of ecology, biodiversity, evolution, taxonomy, and conservation (Hanner *et al.* 2011). The 16S rRNA gene is one of the barcode genes which is usually used to identify bacteria and its population. That gene was chosen because has an informative area and enough variation to distinguish between taxa in the taxon (Wang and Qian 2009). 16S rRNA sequences can be found in all prokaryotic cells with 1-15 copies (Werner *et al.* 2012; Rosselli *et al.* 2016), and have widely used in microbial community studies (Rosselli *et al.* 2016). The use of this gene depends on significant differences in base sequence between species and fewer differences in species (Woo *et al.* 2009) so it can be used to estimate the genetic distance between species.

## 2. Materials and Methods

### 2.1. Isolates

Six bacterial isolates were obtained from the previous study has various highest potential (Listed in Table 1), those isolates were already in the Laboratory of Microbiology, Department of Biology, Universitas Negeri Malang. Each isolate was cultured on 5 ml nutrient broth and incubated in incubator for overnight at 37°C. These bacterial cultures were used for genome DNA (gDNA) isolation.

Table 1. Bacterial isolates in this study

Code	Source	Potential
PIS	Ranu Pani	IAA-Producer
GPS	Ranu Grati	Phosphate solubilizer
PSS	Ranu Pani	Cellulolytic
PAS	Ranu Pani	Amyolytic
PØD	Ranu Pani	Growth promoter
GØD	Ranu Grati	Growth promoter

### 2.2. gDNA Isolation and 16S rRNA Amplification

The gDNA from each sample was isolated using the QIAmp DNA Mini Kit (Qiagen, Germany). The result from gDNA isolation was measured using NanoDrop ND-2000 Spectrophotometer (ThermoScientific™) to determine the concentration and purity of gDNA. The results from gDNA isolation were used as a template in the Polymerase Chain Reaction (PCR) to amplify the 16S rRNA sequence. Top Taq Master Mix reagents from Qiagen were used for PCR. Primers used for amplification are 27F: 5'-GAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-ACGGYTACCTTGTTACGACTT-3' (Devereux and Willis 2004; Wang and Qian 2009). The PCR reaction for 30 cycles was performed as follows: initial denaturation 94°C/3 min, denaturation 94°C/1 min, annealing 50°C/30 s, extension 72°C/1 min 30 s, final extension 72°C/10 min, and hold 4°C. The PCR product was checked on 1% agarose gel electrophoresis.

### 2.3. DNA Sequencing, Phylogenetic Trees Reconstruction and Genetic Distance Analysis

The PCR products were determined by DNA sequencing in 1<sup>st</sup> BASE Laboratories, Malaysia. Sequencing results were analyzed using DNA baser software to determine sequence consensus and sequence alignment using BLAST program on NCBI (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic trees reconstruction and genetic distance were analyzed using MEGA6 software. The phylogenetic trees were constructed using Neighbor-Joining (NJ) method (Saitou and Nei 1987) and Minimum-Evolution (ME) method (Rzhetsky and Nei 1992). The evolutionary distances were calculated using Kimura 2-parameter method (Kimura 1980) with bootstrap test 1,000 replicates (Felsenstein, 1985).

## 3. Results

### 3.1. Genome DNA Isolation and PCR

Total gDNA obtained from isolation had the various purity and concentration from each isolate (Table 2). The PCR result showed that band DNA in gel electrophoresis has a target gene with length about 1500 bp (Figure 1) and then verified by DNA sequencing.

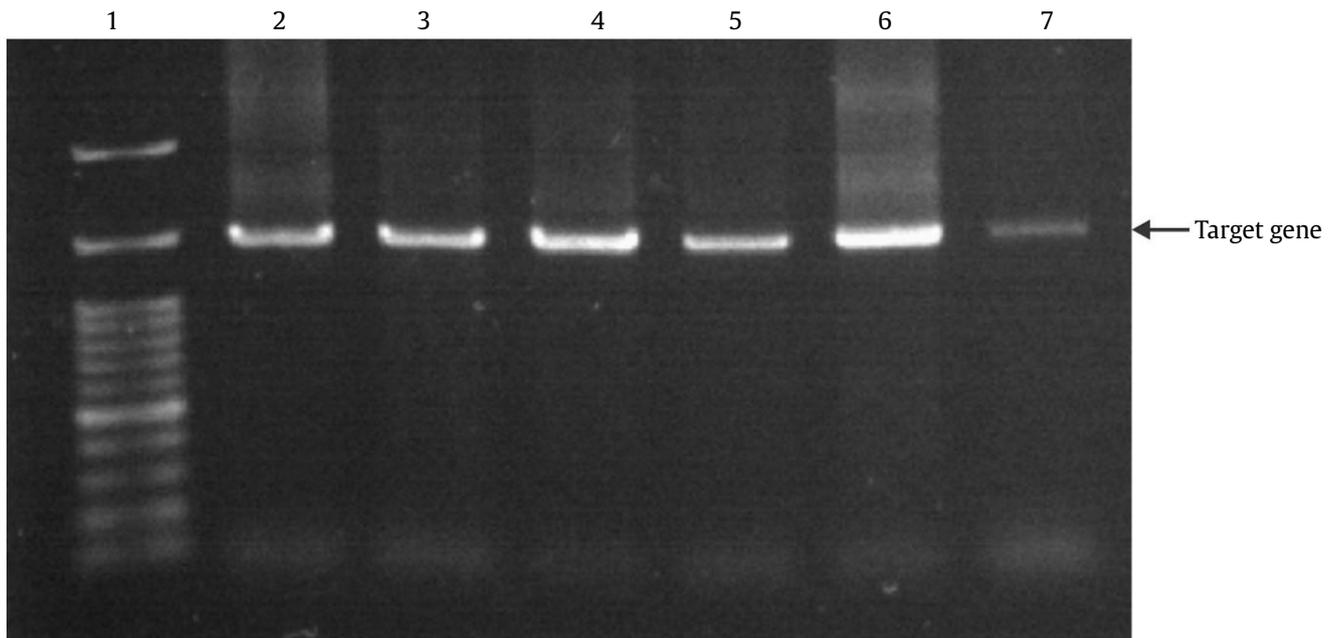


Figure 1. Electrophoregram of 16S rRNA gene amplification on gel electrophoresis 1 percent. Each sample was located from lane 1 to 6 (DNA marker 100 bp from Geneaid, PIS, GPS, PAS, PSS, PØD, GØD, respectively)

Table 2. Concentration and purity of gDNA

Code	Concentration (ng/ $\mu$ l)	$A_{260}/A_{280}$
PIS	231.5	1.98
GPS	65.7	1.97
PAS	46.5	1.94
PSS	253.8	2.01
PØD	234.3	1.84
GØD	291.0	2.08

Table 3. Consensus sequences length from DNA sequencing

Code	Sequence length (bp)
PIS	1431
GPS	1423
PAS	1424
PSS	1420
PØD	1333
GØD	1416

### 3.2. DNA Sequencing and Sequence Analysis

Consensus sequence length from DNA sequencing listed in Table 3. BLAST analysis from all consensus sequences showed that they have high similarity and high query coverage with 16S rRNA sequence on the NCBI database. Several similar sequences from BLAST were downloaded and used for phylogenetic trees reconstruction and genetic distance analysis.

### 3.3. Phylogenetic Trees Reconstruction and Genetic Distance Analysis

Phylogenetic trees reconstruction using NJ and ME showed that three main clades and one clade as out of the group, these three main clades are *Enterobacter* clade (red line), *Bacillus cereus* Group clade (blue line), and *Bacillus subtilis* Group clade (green line) (Figure 2a and b). The PIS and PSS isolates were located in *Bacillus cereus* Group clade, with bootstrap value NJ:100 and ME:100, the PAS isolate was located in *Bacillus subtilis* Group clade, with bootstrap value NJ:82 and ME:83, the GPS isolate located on *Enterobacter* clade with bootstrap value NJ:100 and ME:100, PØD and GØD

isolates identified as *Enterobacter cloacae* a member of *Enterobacter cloacae* Complex (ECC) with bootstrap value NJ:61 and ME:62.

Phylogenetic trees showed a correlation with genetic distance analysis. Three main clades in Figure 2 have a significant genetic distance value there are indicating a different genus. Group *Enterobacter cloacae* Complex has genetic distance value 0.270 with *Bacillus subtilis* Group and 0.258 with *Bacillus cereus* Group (black box in Figure 3). It means there's are 73.0-74.2% sequences similarity in the *Enterobacter* group with both genus *Bacillus* so that clearly defined as a different genus. *Bacillus cereus* Group has a genetic distance value 0.060 or 94% similarity with the *Bacillus subtilis* Group (red box in Figure 3), so as for them separate in sub-genus level.

Analysis of genetic distance in PIS and PSS samples identified in one species to members species of *Bacillus cereus* Group with similarity 99.9-100% (red square in Figure 4), and these samples located in the *Bacillus cereus* Group's clade. PAS sample is located in *Bacillus subtilis* Group's clade and this sample identified as *B. amyloliquefaciens* with 100% similarity but it has closely

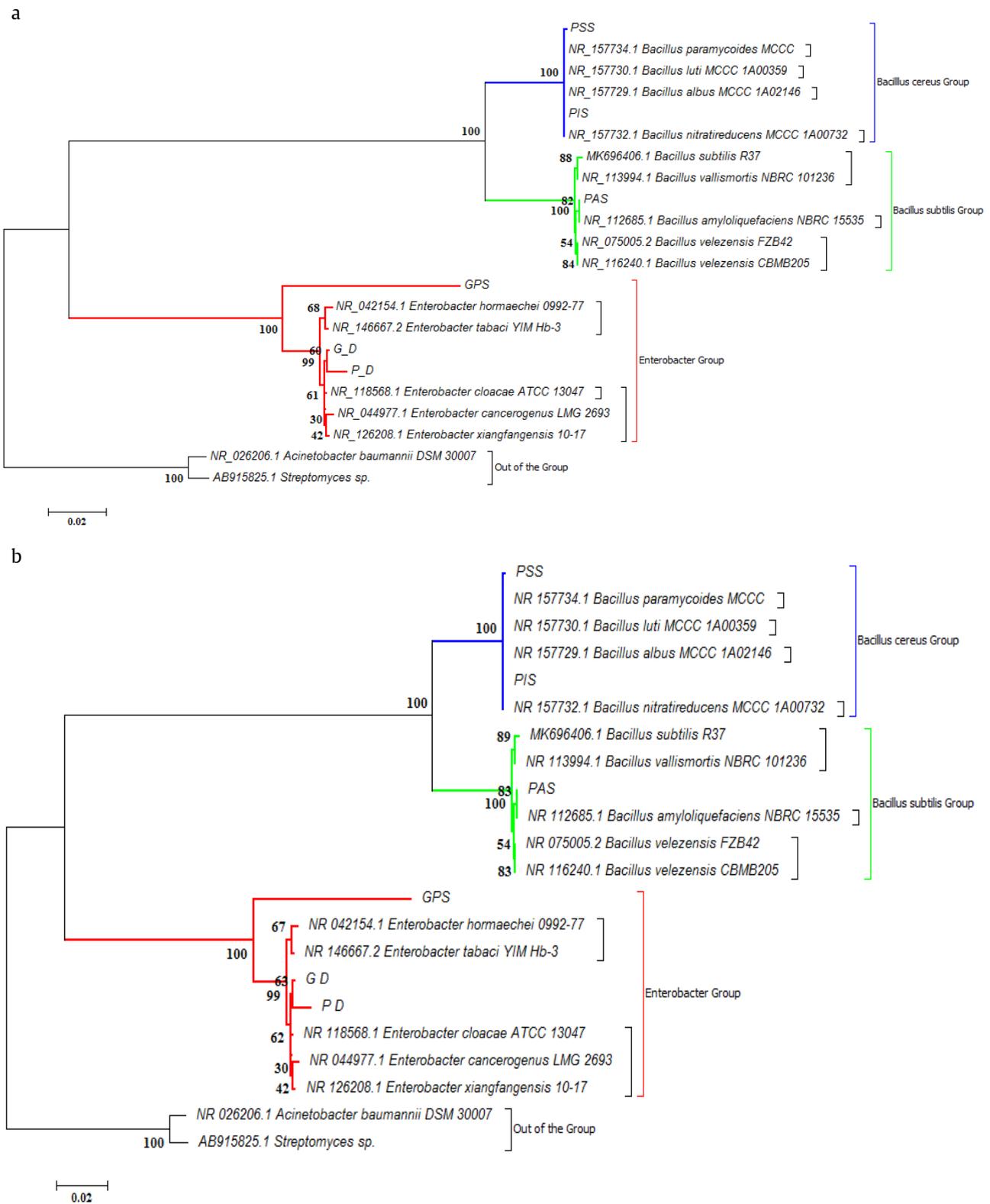


Figure 2. Phylogenetic trees based on 16S rRNA gene, (a) NJ tree, (b) ME tree. The trees were constructed with 1,000 replicates using Kimura 2-parameter model and *Acinetobacter baumannii* and *Streptomyces* sp. were used as an out of the group

related to with *B. subtilis*, *B. velezensis*, and *B. vallismortis* with similarity >99.8% (black square in Figure 4).

The genetic distance of the GPS sample has a similarity <95% with another species in genus *Enterobacter*, this result indicated that GPS sample cannot be defined as species but defined in the genus level (blue square in Figure 4). The genetic distance of PØD and GØD samples were defined as *Enterobacter cloacae* complex with similarity >99%, but these samples were closely related to *Enterobacter cloacae* with similarity values 99.8% on GØD and 99.2% on PØD (yellow box in Figure 4). Intraspecific genetic distance analysis showed that there's are no significant variation 16S rRNA sequences within groups, so the sequences variety each group are relatively homogeneous with similarity >99% (Figure 5).

#### 4. Discussion

Phylogenetic trees reconstruction revealed that PSS and PIS samples were located in clade with *Bacillus cereus* Group and PAS sample located in *Bacillus subtilis* Group closely to *Bacillus amyloliquefaciens* NBRC 15535, GØD and PØD located in *Enterobacter cloacae* Complex, while GPS is located in *Enterobacter* clade with form a new clade (can be defined as novel *Enterobacter*) (Figure 2). All of the branches from phylogenetic trees show a confident position with trusted bootstrap value of >50 (Gregory 2008). The position of each sample in the phylogenetic tree showed a correlation with genetic distance analysis. Almost all of them were separated in a new clade as a group supported by its significant genetic distance (Figure 3), and within-group genetic distance (>99%)

	1	2	3	4
1. Enterobacter Group		0.015	0.015	0.012
2. Bacillus subtilis Group	0.270		0.007	0.015
3. Bacillus cereus Group	0.258	0.060		0.015
4. Out of the Group	0.174	0.261	0.260	

Figure 3. Genetic distance between the group. The standard error(s) are shown in the cross of genetic distance calculation (blue text), these analyses were conducted using Kimura 2-parameter with 1,000 replicates

	d	S.E.
1. Enterobacter Group	0.008	0.002
2. Bacillus subtilis Group	0.002	0.001
3. Bacillus cereus Group	0.000	0.000
4. Out of the Group	0.018	0.004

Figure 5. Genetic distance analysis within the group. The standard error(s) are shown in the second column, these analyses were conducted using Kimura 2-parameter with 1,000 replicates

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1. G_D		0.002	0.008	0.017	0.017	0.018	0.002	0.002	0.001	0.002	0.002	0.018	0.018	0.018	0.018	0.018	0.017	0.017	0.017	0.017	0.013	0.013
2. P_D	0.007		0.008	0.018	0.018	0.018	0.003	0.003	0.003	0.003	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.013	0.013
3. GPS	0.076	0.081		0.020	0.020	0.020	0.008	0.008	0.008	0.008	0.008	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.015	0.016
4. PIS	0.258	0.264	0.305		0.001	0.007	0.017	0.018	0.017	0.017	0.017	0.007	0.007	0.007	0.007	0.007	0.000	0.000	0.000	0.000	0.017	0.017
5. PSS	0.259	0.265	0.306	0.001		0.007	0.017	0.018	0.017	0.017	0.017	0.007	0.007	0.007	0.007	0.007	0.001	0.001	0.001	0.001	0.017	0.017
6. PAS	0.270	0.276	0.312	0.060	0.061		0.018	0.018	0.018	0.018	0.017	0.001	0.000	0.002	0.001	0.001	0.007	0.007	0.007	0.007	0.017	0.017
7. NR_042154.1 Enterobacter hormaechei 0992-77	0.006	0.013	0.078	0.259	0.260	0.271		0.002	0.002	0.002	0.002	0.018	0.018	0.018	0.018	0.018	0.017	0.017	0.017	0.017	0.013	0.013
8. NR_044977.1 Enterobacter cancerogenus LMG 2693	0.005	0.011	0.077	0.261	0.262	0.270	0.008		0.002	0.002	0.003	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.013	0.013
9. NR_118568.1 Enterobacter cloacae ATCC 13047	0.002	0.008	0.076	0.257	0.258	0.269	0.007	0.004		0.001	0.002	0.018	0.018	0.017	0.018	0.018	0.017	0.017	0.017	0.017	0.013	0.013
10. NR_126208.1 Enterobacter xiangfangensis 10-17	0.004	0.010	0.075	0.258	0.259	0.270	0.005	0.003	0.002		0.003	0.018	0.018	0.018	0.018	0.018	0.017	0.017	0.017	0.017	0.013	0.013
11. NR_146667.2 Enterobacter tabaci YIM Hb-3	0.006	0.013	0.080	0.258	0.259	0.267	0.003	0.009	0.007	0.008		0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.017	0.013	0.013
12. NR_075005.2 Bacillus velezensis FZB42	0.270	0.276	0.310	0.059	0.060	0.002	0.271	0.270	0.269	0.270	0.267		0.001	0.002	0.001	0.000	0.007	0.007	0.007	0.007	0.017	0.017
13. NR_112685.1 Bacillus amyloliquefaciens NBRC 15535	0.270	0.276	0.312	0.060	0.061	0.000	0.271	0.270	0.269	0.270	0.267	0.002		0.002	0.001	0.001	0.007	0.007	0.007	0.007	0.017	0.017
14. MK696406.1 Bacillus subtilis R37	0.270	0.276	0.313	0.061	0.062	0.004	0.273	0.270	0.268	0.270	0.270	0.004	0.004		0.001	0.002	0.007	0.007	0.007	0.007	0.017	0.017
15. NR_113994.1 Bacillus vallismortis NBRC 101236	0.272	0.279	0.312	0.059	0.060	0.002	0.273	0.272	0.271	0.272	0.270	0.002	0.002	0.002		0.001	0.007	0.007	0.007	0.007	0.017	0.017
16. NR_116240.1 Bacillus velezensis CBMB205	0.270	0.276	0.310	0.059	0.060	0.002	0.271	0.270	0.269	0.270	0.267	0.000	0.002	0.004	0.002		0.007	0.007	0.007	0.007	0.017	0.017
17. NR_157729.1 Bacillus albus MCCC 1A02146	0.258	0.264	0.305	0.000	0.001	0.050	0.259	0.261	0.257	0.258	0.258	0.059	0.060	0.061	0.059	0.059		0.000	0.000	0.000	0.017	0.017
18. NR_157730.1 Bacillus luti MCCC 1A00359	0.258	0.264	0.305	0.000	0.001	0.060	0.259	0.261	0.257	0.258	0.258	0.059	0.060	0.061	0.059	0.059	0.000		0.000	0.000	0.017	0.017
19. NR_157732.1 Bacillus nitratireducens MCCC 1A00732	0.258	0.264	0.305	0.000	0.001	0.060	0.259	0.261	0.257	0.258	0.258	0.059	0.060	0.061	0.059	0.059	0.000	0.000		0.000	0.017	0.017
20. NR_157734.1 Bacillus paramyoides MCCC	0.258	0.264	0.305	0.000	0.001	0.060	0.259	0.261	0.257	0.258	0.258	0.059	0.060	0.061	0.059	0.059	0.000	0.000	0.000		0.017	0.017
21. NR_026206.1 Acinetobacter baumannii DSM 30007	0.175	0.178	0.234	0.266	0.268	0.266	0.176	0.176	0.174	0.175	0.175	0.267	0.266	0.267	0.267	0.267	0.266	0.266	0.266	0.266	0.003	
22. AB915825.1 Streptomyces sp.	0.175	0.181	0.237	0.266	0.267	0.267	0.177	0.178	0.176	0.177	0.176	0.268	0.267	0.268	0.268	0.268	0.266	0.266	0.266	0.266	0.013	0.013

Figure 4. Genetic distance analysis for each sample. The standard error(s) are shown in the cross of genetic distance calculation (blue text), these analyses were conducted using Kimura 2-parameter with 1,000 replicates

(Figure 5). The Genetic distance value between  $\leq 0.03$  defined in one species, genetic distance  $\leq 0.05$  defined in one genus, defined in one class if  $\leq 0.15$ , and 0.20-0.40 defined in one phylum (Bukin *et al.* 2019; Schloss and Handelsman 2004; Truong *et al.* 2017). Species are organisms with similarity  $>97\%$ , the definition of species “match” if similarity  $>99\%$  (Drancourt *et al.* 2000; Janda and Abbott 2007), and if the similarity has less than 97% can be defined as novel species (Stackebrandt and Goebel 1994).

Molecular signatures for *Bacillus* spp. were categorized on several groups based on 16S rRNA, whereas *Bacillus subtilis* Group in clade I and *Bacillus cereus* Group in clade II (Rooney *et al.* 2009; Bhandari *et al.* 2013; Fan *et al.* 2017). *B. amyloliquefaciens*, *B. siamensis*, *B. velezensis*, *B. methylotrophicus* are a member of the new phylogenetic branch of “operational group *Bacillus amyloliquefaciens*” from *Bacillus subtilis* Species Complex (Fan *et al.* 2017). Identification based on morphology and physiology in the previous study PAS sample identified as *B. subtilis* (Basitoh 2018) but with 16S rRNA, we can identify more deeply to *B. amyloliquefaciens* NBRC 15535. This species can hydrolyze amylum using amylase enzymes (Deb *et al.* 2013). In the other group of *Bacillus* spp., *Bacillus cereus* Group or (*B. cereus* sensu lato) is sub-division in genus *Bacillus*, this group include *B. anthracis*, *B. cereus*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis*, *B. thuringiensis*, *B. paranthracis*, *B. pacificus*, *B. tropicus*, *B. albus*, *B. mobilis*, *B. luti*, *B. proteolyticus*, *B. nitratreducens*, *B. paramycoides* (Nakamura and Jackson 1995; Vilas-Bôas *et al.* 2007; Liu *et al.* 2017). Most species in genus *Bacillus* can produce IAA (Patten *et al.* 2013; Liu *et al.* 2017; Ozdal *et al.* 2017; Susilowati *et al.* 2018) via dependent pathways with tryptophan as a precursor (Spaepen *et al.* 2007; Shao *et al.* 2015) and also cellulose hydrolysis (Balasubramanian *et al.* 2012; Gupta *et al.* 2012; Lin *et al.* 2012; Chantarasiri 2015) with celluloses enzyme group such as endoglucanase, exoglucanase, and  $\beta$ -glucosidase (Bayer *et al.* 2007). The little genetic diversity on those group *Bacillus*, identification on species-level mostly cannot be defined with 16S rRNA (Rasko *et al.* 2005; Rooney *et al.* 2009).

In the *Enterobacter* clade, sample GPS identified as novel species in genus *Enterobacter* with similarity below 95%. In the research progress with that isolate, confirmed that Pho regulon includes *phoA*, *phoB*, and *phoR* genes are probably act on solubilizing of

complex phosphates (Ongoing research, data not published). Two dominant isolates (GØD and PØD) are located one clade with *Enterobacter cloacae* ATCC 13047 and they have  $>99\%$  similarity within group ECC, so it makes identification on species-level probably bias. Some species in genus *Enterobacter*, especially ECC has multiple potentials such as solubilizing phosphate, produce IAA, and produce ammonia (Nhu and Diep 2017).

This research has shown the PIS and PSS isolates were identified as *Bacillus cereus* Group closely related with *Bacillus paramycoides*, PAS isolate identified as *Bacillus amyloliquefaciens* which is a member of *Bacillus subtilis* Group, GPS isolate identified as novel species in *Enterobacter*, and GØD and PØD isolates identified as *Enterobacter cloacae* which is a member of ECC. The 16S rRNA sequence can be defining samples in sub-genus or group within a genus level. Species can be defined but not trusted. Other barcodes/marker genes are required to identification species-level in this group. Identification based on whole-genome sequencing, plasmid characterization, phenotypes characterization, and their combination are required to determining the further classification of species (Konstantinidis and Tiedje 2005; Woo *et al.* 2009; Tindall *et al.* 2010; Brady *et al.* 2013).

## Acknowledgements

The authors would like to extend our grateful to the Direktorat Riset dan Pengabdian Masyarakat (DRPM), Direktorat Jenderal Penguatan Riset dan Pengembangan from Ministry of Research and Technology Republic Indonesia for funding this research.

## References

- Ardilla R. 2018. Isolasi bakteri selulolitik potensial dari Ranu Pani, Ranu Regulo, Ranu Grati, dan Telaga Ngebel [Thesis]. Malang, Indonesia: Universitas Negeri Malang.
- Balasubramanian N *et al.* 2012. Purification and biochemical characterization of a novel thermo-stable carboxymethyl cellulase from *Azorean* isolate *Bacillus mycoides* S122C. *Appl Biochem Biotechnol* 168:2191–2204.
- Basitoh YK. 2018. Eksplorasi bakteri amilolitik potensial dari Ranu Pani, Ranu Regulo, Ranu Grati, dan Telaga Ngebel [Thesis]. Malang, Indonesia: Universitas Negeri Malang.
- Bayer EA *et al.* 2007. The potential of cellulases and cellulosomes for cellulosic waste management. *Curr Opin Biotechnol* 18:237–245.

- Bhandari V *et al.* 2013. Molecular signatures for *Bacillus* species: demarcation of the *Bacillus subtilis* and *Bacillus cereus* clades in molecular terms and proposal to limit the placement of new species into the genus *Bacillus*. *Int J Syst Evol Microbiol* 63:2712–2726.
- Bisen PS *et al.* 2012. Identification and classification of microbes. In: *Microbes*. New Jersey: John Wiley and Sons Ltd. pp. 275–337.
- Boivin-Jahns V *et al.* 1995. Comparison of phenotypical and molecular methods for the identification of bacterial strains isolated from a deep subsurface environment. *Appl Environ Microbiol* 61:3400–3406.
- Brady C *et al.* 2013. Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radincitans*, *E. oryzae* and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. *Syst Appl Microbiol* 36:309–319.
- Bukin Yu S *et al.* 2019. The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Sci Data* 6:190007. DOI:10.1038/sdata.2019.7
- Buszewski B *et al.* 2017. Identification of microorganisms by modern analytical techniques. *J AOAC Int* 100:1607–1623.
- Chantarasiri A. 2015. Aquatic *Bacillus cereus* JD0404 isolated from the muddy sediments of mangrove swamps in Thailand and characterization of its cellulolytic activity. *Egypt J Aquat Res* 41:257–264.
- Cristescu ME. 2014. From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. *Trends Ecol Evol* 29:566–571.
- Deb P *et al.* 2013. Production and partial characterization of extracellular amylase enzyme from *Bacillus amyloliquefaciens* P-001. *Springerplus* 2:154. DOI:10.1186/2193-1801-2-154
- Devereux R, Willis SG. 2004. Amplification of ribosomal RNA sequences. In: Akkermans ADL, Van Elsland JD, De Bruijn FJ (Eds.). *Molecular Microbial Ecology Manual*. Dordrecht: Springer Netherlands. pp. 277–287.
- Drancourt M *et al.* 2000. 16S Ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J Clin Microbiol* 38:3623–3630.
- Fan B *et al.* 2017. *Bacillus amyloliquefaciens*, *Bacillus velezensis*, and *Bacillus siamensis* Form an “Operational Group *B. amyloliquefaciens*” within the *B. subtilis* Species Complex. *Front Microbiol* 8:22. DOI:10.3389/fmicb.2017.00022
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39:783–791.
- Fuentes JL *et al.* 2016. Impact of microalgae-Bacteria interactions on the production of algal biomass and associated compounds. *Mar Drugs* 14:100. DOI:10.3390/md14050100
- Gregory TR. 2008. Understanding evolutionary trees. *Evolution: Education and Outreach* 1:121–137.
- Gugliandolo C *et al.* 2011. Conventional and molecular methods to detect bacterial pathogens in mussels. *Lett Appl Microbiol* 52:15–21.
- Gupta P *et al.* 2012. Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *Int J Microbiol* 2012:1–5.
- Hanner R *et al.* 2011. DNA barcoding of billfishes. *Mitochondrial DNA* 1:27–36.
- Janda JM, Abbott SL. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J Clin Microbiol* 45:2761–2764.
- Jena U *et al.* 2011. Evaluation of microalgae cultivation using recovered aqueous co-product from thermochemical liquefaction of algal biomass. *Bioresour Technol* 102:3380–3387.
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120.
- Konstantinidis KT, Tiedje JM. 2005. Towards a genome-based taxonomy for prokaryotes. *J Bacteriol* 187:6258–6264.
- Kropat J *et al.* 2011. A revised mineral nutrient supplement increases biomass and growth rate in *Chlamydomonas reinhardtii*. *Plant J* 66:770–780.
- Lebonah DE *et al.* 2014. DNA barcoding on bacteria: a review. *Adv Biol* 2014:1–9.
- Lin L *et al.* 2012. Characterization of extracellular cellulose-degrading enzymes from *Bacillus thuringiensis* strains. *Electron J Biotechnol* 15:1–7.
- Liu Y *et al.* 2017. Proposal of nine novel species of the *Bacillus cereus* group. *Int J Syst Evol Microbiol* 67:2499–2508.
- López-Campos G *et al.* 2012. Microarray detection and characterization of bacterial foodborne pathogens. New York City: SpringerBriefs in Food, Health, and Nutrition. Springer.
- Marañón E *et al.* 2005. Continuity in the photosynthetic production of dissolved organic carbon from eutrophic to oligotrophic waters. *Mar Ecol Prog Ser* 299:7–17.
- Nakamura LK, Jackson MA. 1995. Clarification of the taxonomy of *Bacillus mycoides*. *Int J Syst Bacteriol* 45:46–49.
- Nhu VTP, Diep CN. 2017. Isolation and characterization of endophytic bacteria in soybean (*Glycine max* L. (Merrill)) cultivated on alluvial soil of Can Tho city, Vietnam. *IJJET* 8:208–221.
- Ozdam M *et al.* 2017. Continuous production of indole-3-acetic acid by immobilized cells of *Arthrobacter agilis*. *3 Biotech* 7:23–28.
- Patten CL *et al.* 2013. Activity, distribution and function of indole-3-acetic acid biosynthetic pathways in bacteria. *Crit Rev Microbiol* 39:395–415.
- Prabaningtyas S, Witjoro A. 2017. Eksplorasi bakteri sinergis dari beberapa danau di Jawa Timur untuk mempercepat pertumbuhan mikroalga renewable energy tahun ke 1 [Synergistic bacterial exploration of several lakes in East Java to accelerate the growth of microalgae renewable energy, 1st year]. Malang, Indonesia: Universitas Negeri Malang.
- Prabaningtyas S *et al.* 2018a. Eksplorasi bakteri sinergis dari beberapa danau di Jawa Timur untuk mempercepat pertumbuhan mikroalga renewable energy tahun ke 2 [Synergistic bacterial exploration of several lakes in East Java to accelerate the growth of microalgae renewable energy, 2nd year]. Malang, Indonesia: Universitas Negeri Malang.
- Prabaningtyas S *et al.* 2018b. Vertical distribution of bacteria in various lakes of East Java, Indonesia. *J Phys: Conf Ser* 1093:012017. DOI:10.1088/1742-6596/1093/1/012017
- Ramanan R *et al.* 2016. Algae-bacteria interactions: evolution, ecology and emerging applications. *Biotechnol Adv* 34:14–29.
- Rasko DA *et al.* 2005. Genomics of the *Bacillus cereus* group of organisms. *FEMS Microbiol Rev* 29:303–329.

- Rooney AP *et al.* 2009. Phylogeny and molecular taxonomy of the *Bacillus subtilis* species complex and description of *Bacillus subtilis* subsp. *inaquosorum* subsp. nov. *Int J Syst Evol Microbiol* 59:2429–2436.
- Rosselli R *et al.* 2016. Direct 16S rRNA-seq from bacterial communities: a PCR-independent approach to simultaneously assess microbial diversity and functional activity potential of each taxon. *Sci Rep* 6:32165. DOI:10.1038/srep32165
- Rzhetsky A, Nei M. 1992. A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* 9:945–967.
- Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425.
- Schloss PD, Handelsman J. 2004. Status of the microbial census. *Microbiol Mol Biol Rev* 68:686–691.
- Shao J *et al.* 2015. Analysis and cloning of the synthetic pathway of the phytohormone indole-3-acetic acid in the plant-beneficial *Bacillus amyloliquefaciens* SQR9. *Microb Cell Fact* 14:130. DOI:10.1186/s12934-015-0323-4
- Spaepen S *et al.* 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS Microbiol Rev* 31:425–448.
- Stackebrandt E, Goebel BM. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Evol Microbiol* 44:846–849.
- Susilowati DN *et al.* 2018. Indole-3-acetic acid producing bacteria and its application on the growth of rice. *In: Proceedings of the 5th International Conference on Biological Science*. Yogyakarta: AIP Conference Proceedings. pp. 1–9.
- Thyssen M. 2005. Spatial distribution of heterotrophic bacteria in the northeast Atlantic (POMME study area) during spring 2001. *J Geophys Res* 110:1–16.
- Tindall BJ *et al.* 2010. Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 60:249–266.
- Truong DT *et al.* 2017. Microbial strain-level population structure and genetic diversity from metagenomes. *Genome Res*. 27:626–638.
- Tshikhudo P *et al.* 2013. Bacterial species identification getting easier. *Afr J Biotechnol* 12:5975–5982.
- Vilas-Bôas GT *et al.* 2007. Biology and taxonomy of *Bacillus cereus*, *Bacillus anthracis*, and *Bacillus thuringiensis*. *Can J Microbiol* 53:673–687.
- Wang Y, Qian PY. 2009. Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. *PLoS ONE* 4:e7401. DOI:10.1371/journal.pone.0007401
- Werner JJ *et al.* 2012. Impact of training sets on classification of high-throughput bacterial 16S rRNA gene surveys. *ISME J* 6:94–103.
- Woo PCY *et al.* 2009. Guidelines for interpretation of 16S rRNA gene sequence-based results for identification of medically important aerobic Gram-positive bacteria. *J Med Microbiol* 58:1030–1036.