

## SHORT COMMUNICATION

# Physiological and Molecular Characteristics of Bacterial Isolates from Bandalit Coastal Area Jember, East Java, Indonesia

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Bacteria are the most dominant group of microorganisms in aquatic environments due to their role in organic matter decomposition. Decomposition activity is related to the type and dominance of bacteria in the communities. Therefore, study of bacterial diversity is an important step to understand their role in aquatic ecosystems. This study was to determine bacterial diversity and their physiological characters of bacteria from Bandalit Coast in Jember East Java Indonesia. The bacteria were confirmed by BOX-PCR profile for their genetic polymorphisms. Identification of potential isolate was conducted based on 16S rRNA gene sequence. The result showed that BA011109 isolate was able to utilize D-cellobiose as a sole substrate, indicating its ability to hydrolyse  $\beta$ -glucoside bond. This isolate was a potential decomposer in the area considering that most of organic pollutants were from plants that contain high cellulose. Based on its 16S rRNA gene sequence, this isolate was closely related to *Microbacterium esteraromaticum* with 100% homology. Further study on quantitative hydrolytic activities is needed to elucidate its role as an organic matter decomposer in aquatic environment.

Keywords: bacterial diversity, aquatic environments, *Microbacterium esteraromaticum*

## INTRODUCTION

Coastal area is the last place for disposal of waste such as household waste and agricultural waste that contained of organic matter. Accumulation of organic waste in a long period will cause the process of eutrophication that lead to reduction of dissolved oxygen. This can cause an imbalance of the ecosystem and endanger the lives of aquatic organisms. Therefore, waters bioremediation of organic waste is an important step to solve this environmental problem.

Bacteria have an important role in the waters bioremediation process through their decomposition activity (Pomeroy *et al.* 2007). Decomposition activity is highly related to the type of dominant bacteria in the communities (Pascoal & Cassio 2004). Therefore, a study of bacterial diversity is an important step to determine their role as decomposers in aquatic ecosystems. The confirmation of bacterial diversity from Bandalit Coast in Jember by BOX-PCR indicated that five bacteria isolates i.e. BA011109, BA041109, BA041109\*, BA091109, and

BA061109 had different patterns (Senjarini *et al.* 2010). Differences in the genetic profile of BOX-PCR showed genotype polymorphisms of bacterial isolates (Oda *et al.* 2002). Confirmation of bacterial diversity can also be determined by their metabolic activity. Diversity based on the metabolic activity is often referred to as the metabolic fingerprint. BIOLOG is one method to obtain the metabolic fingerprint of bacteria. BIOLOG is designed to identify bacteria based on the patterns of metabolic ability using different carbon sources in 95 microplate well. The patterns are specific for each bacterium.

Furthermore, determination of the bacterial species that make up the community of ecosystem is by identification. Identification could be done by molecular based on DNA sequence of 16S rRNA gene. This gene has conserved and variable regions, so that it can be used to differentiate bacteria in the genus and species (Woese 1987). Identification by using 16S rRNA gene sequence is faster, more accurate and objective than any other conventional methods (Petti *et al.* 2005).

This study aimed to determine molecular characteristics, physiological characteristics in utilizing carbon sources and species identification of bacterial isolates from the Bandalit coastal area in Jember

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Indonesia. Species Identification was determined based on DNA sequence of 16S rRNA gene.

## MATERIALS AND METHODS

**Determination of the Bacterial Diversity Based on the Metabolic Fingerprint.** Bacterial isolates used in this study were from a previous study that were obtained from a sea water sample of Bandetail coastal area-Jember (Senjarini *et al.* 2010). The determination of the bacterial diversity based on the metabolic fingerprint was done using the BIOLOG GN2 Microplate containing different organic substrates, i.e. carbohydrates, protein and lipid which consist of mono-di-polymer. The bacterial suspensions were added into the wells of BIOLOG GN2 Microplate and incubated at room temperature (30 °C) for 24-48 hours. The positive results of Microplate test were indicated by changing in the color of the culture into purplish indicating utilization of the carbon sources.

**Confirmation of BA011109 Isolate.** Confirmation of the BA011109 isolate genetic profile was done by BOX-PCR method. Bacterial DNA was extracted using the modified Freeze and Thaw method of Tsai and Olson's (1991). The Primary BOX-AIR (5'-CTA CGG CAA GGC GAC GCT GAC G-3') (Oda *et al.* 2002) was used for amplifying the bacterial genome. Temperature gradient was set up with initial denaturation 95 °C for 6 minutes, 35 cycles at 94 °C for 1 minute, 54 °C for 1 minute, 65 °C for 8 minutes, and final extension at 65 °C for 16 minutes.

**Identification of the Isolate.** Identification of the isolate was based on DNA sequence of 16S rRNA gene using primers of 27F (5' AGA GTT TGA TCM

TGG CTC AG 3'), 533F (5' GTG CCA GCM GCC GCG GTA A 3'), 907R (5' CCG TCA ATT CMT TTG AGT TT 3'), and 1492R (5' GGT TAC CTT GTT ACG ACT T 3') (Lane 1991). The PCR for amplification of the gene was set up with initial denaturation at 94 °C for 5 minutes; denaturation at 94 °C for 1 minute, annealing at 56 °C for 1 minute, and extension at 72 °C for 1 minute for 24 cycles and final extension at 72 °C for 1 minute.

The PCR products were run on 1% agarose gel electrophoresis following by DNA clean-up using Gel Extraction NucleoSpin® Extract II procedure. Sequencing of extracted DNA was sent to a sequencing service company. The sequence was analyzed using BLAST-N and phylogenetic relationship tree was constructed using [MEGA 5.05 program with Neighbour Joining method (bootstrap 1000x)].

## RESULTS

Five isolates of bacteria were tested for their metabolic characters using BIOLOG GN2 Microplate. The results showed that all isolates could utilize dextrin except BA011109 isolate (Table 1). However, BA011109 isolate could utilize D-cellobiose as well as BA061109, and BA041109B isolates.

Confirmation of genome profile using BOX PCR indicated that the profile of BA011109 isolate was confirmed as BA011109 isolate. There were 8 bands on the BOX-PCR profile (Figure 1). The size of bands were 2000-2500, 1500-2000, 1500, 1000-1500, 750-1000, 750, 500, and 250-500 bp respectively.

Amplification of 16S rRNA gene resulted in a 900 bp PCR product (Figure 2). This PCR product was as expected due to the primers used were 533F and 1492R primers. The BLAST-N result of the PCR

Table 1. Diversity of physiological characters of the bacterial isolates based on BIOLOG GN2 Microplate assay

Substrate Group	Carbon source	BA011109	BA041109	BA061109	BA 091109	BA 041109*
<b>Carbohydrat:</b>						
Polimer/Dimer	D-Cellobiose	+	-	+	-	+
	D-Melibiose	-	-	-	-	+
	Lactulose	-	-	-	-	+
	Maltose	+	-	+	-	-
	Sucrose	-	-	-	+	-
	α-cyclodextrin	-	+	-	-	+
	Dextrin	-	+	+	+	+
Polimer/Dimer	Glycogen	-	+	-	-	+
	<b>Protein:</b>					
Polimer/Dimer	Glycyl-L-Aspartic Acid	-	-	-	-	-
	Glycyl-L-Glutamic Acid	-	-	-	-	-
	γ-Amino-Butyric-Acid	-	-	-	-	+
<b>Ester:</b>						
Polimer/Dimer	Tween 40	-	+	-	-	+
	Tween 80	-	+	-	-	+

product sequence indicated that BA011109 isolate was closely related to *Microbacterium esteraromaticum* with 100% homology (KC430861).

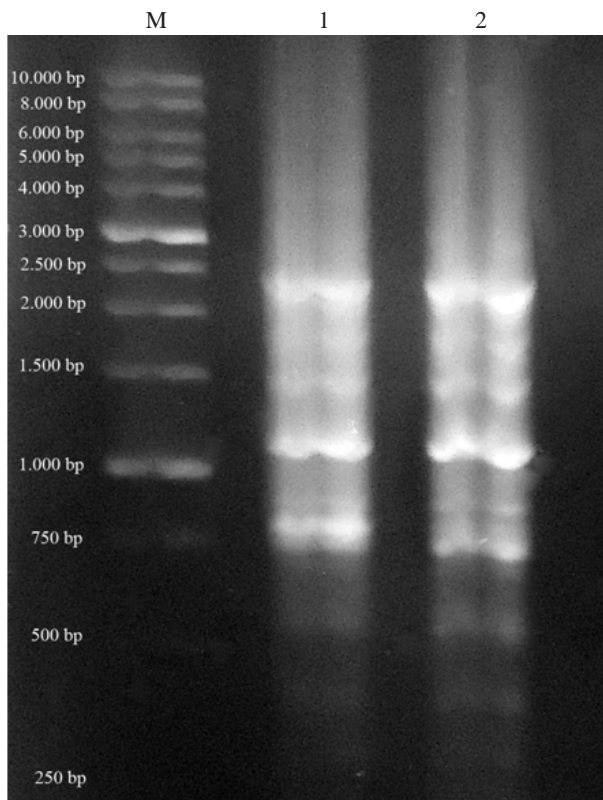


Figure 1. BOX-PCR profile of BA011109 isolate from Bandalit Coastal area Jember, East Java, Indonesia. M = Marker 1 kb, 1 = Reference isolate (Senjarini *et al.* 2010), and 2 = BA011109 isolate used in this study.

## DISCUSSION

**Bacterial Diversity Based on Metabolic Fingerprint.** BIOLOG GN2 Microplate applications were first developed to classify bacteria based on the ability of isolates to oxidize 95 different carbon sources (Smalla *et al.* 1998). By this method, 95 different tests can be done and could produce metabolic profiles, known as “metabolic fingerprint”. The reaction of BIOLOG GN2 Microplate based on the reduction of tetrazolium as a response to the respiration process of bacteria. The positive result of this test is marked by the color change of culture be purplish in microplate wells that indicated the use of carbon sources.

Bacterial isolates from Bandalit coastal area had metabolic diversity of bacteria in these waters (Table 1). The isolate that could utilize Tween 40 and 80 indicated that the bacteria were able to produce esterase/lipase that hydrolysed them become a fatty acid (monopalmitat) and monooleat respectively. While the isolates (BA 011109, BA 061109, dan BA 041109 isolates) that could utilize D-cellobiose indicated that the bacterial isolates were able to produce cellulase. Cellulase is an enzyme that can degrade  $\beta$ -1,4- glycosidic bonds. The ability to produce cellulase is important for the decomposition of agricultural waste derived from plants (Barman *et al.* 2011).

**Confirmation of BA011109 Isolate.** The similarity of the BOX-PCR profile of reference isolate of previous study and this study isolate

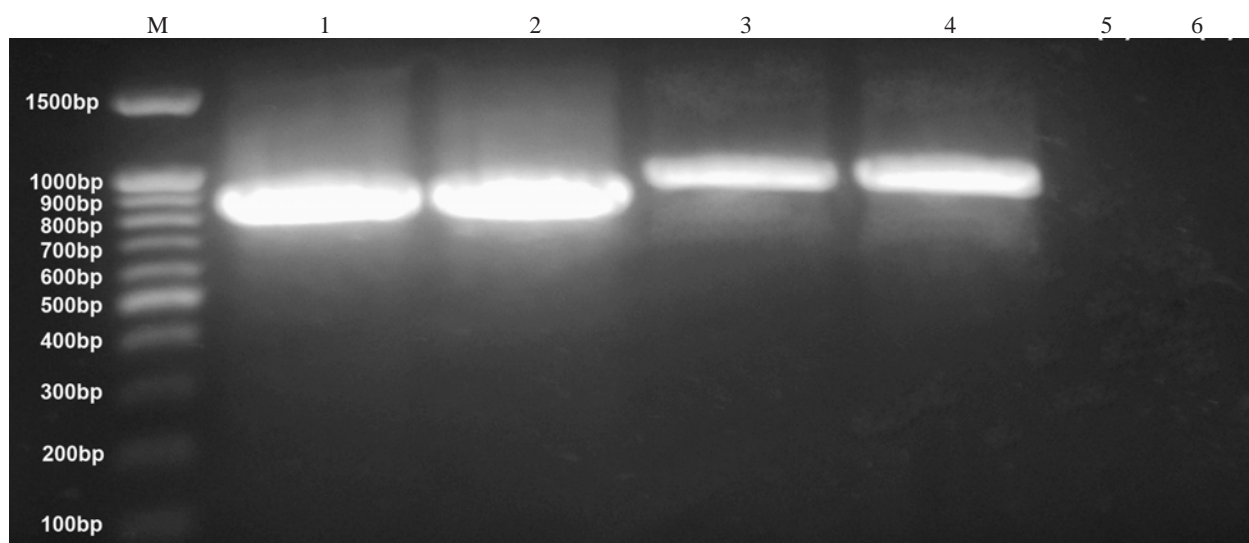


Figure 2. The Result of agarose gel electrophoresis of 16S rRNA gene PCR products of BA01109 isolate. M = Marker 100 bp; 1,2 = PCR product using 27F and 907R primers; 3,4 = PCR product using 533F and 1492R primers; 5 = Negative control of 27F and 907R primers; 6 = Negative control of 533F-1492 primers.

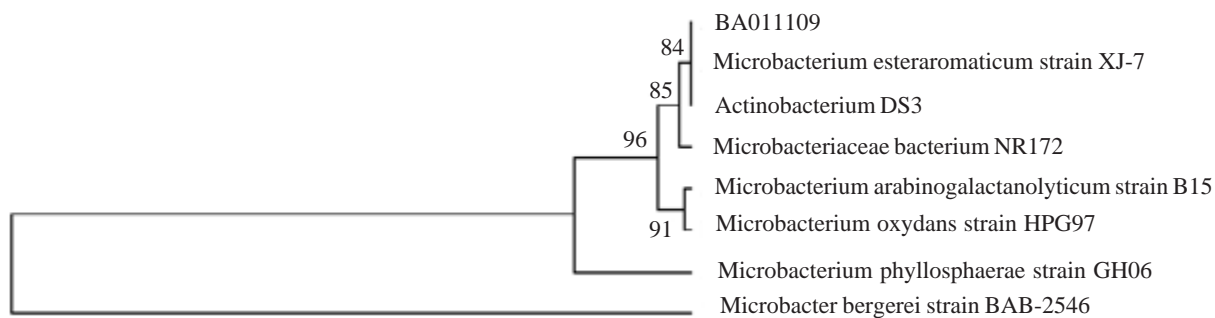


Figure 3. Phylogeny tree showing relationships of BA011109 isolates with related bacteria from Gene Bank.

confirmed the stability of the genetic profile and the purity of the isolate. BOX-PCR using repetitive extragenic palindromic-PCR is BOXA1R. This single primer binds to repetitive sequences in bacterial genomes, therefore this sequences will be polymerized. Repetitive sequences of the bacterial genome are conserved and specific for each species. Sensitivity of this genetic profile method had revealed the diversity of *Rhizobium leguminosarum* (Adiguzel *et al.* 2010).

**Identification of the Bacterium.** Nucleotide sequence of 16S rRNA gene can be used to identify bacterial isolate. Sequence of 16S rRNA gene contain conserved and variable regions. Variable sequence can be used to determine the diversity and placing strains within the same species. Nedashkovskaya *et al.* (2005) identified marine bacteria using 16S rRNA gene sequence and reported that *Cyclobacterium amurskyense* was a new species of the genus *Cyclobacterium*. The sequence is also useful to construct a universal phylogenetic tree due to their relatively slow changes.

The analysis of 16S rRNA gene sequence from BA011109 isolate using the BLAST-N program showed that the isolate was closely related to *Microbacterium esteraromaticum* with 100% similarity. BA01109 isolate also showed similar physiological characteristics to *M. esteraromaticum*. Phylogenetic tree (Figure 3) showed that BA011109 isolate was also located in *M. esteraromaticum* group. This result indicated that BA011109 isolates was *M. esteraromaticum*. Genus of *Microbacterium* was found in various environments, including soil; water; crops; and dairy products. For example, *Microbacterium koreense* was isolated from sea water in the South Sea of Korea (Lee *et al.* 2006). The capability of BA011109 isolate to utilize D-cellobiose as a sole substrate in BIOLOG GN2 Microplate indicated its ability to produce cellulase. This enzyme plays an important role in the decomposition of agricultural or organic waste from

plants. This indication lead to suggestion that BA011109 isolate can be used as a bioremediation agent in coastal area.

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