

Screening of Rhizosphere Bacteria from Rice Fields in The Coastal Area as Acc-Deaminase and Auxin Producer

Annisyia Zarina Putri[#], Djarot Sasongko[#], Dwi N. Susilowati^{*}

[#] Department of Biochemistry. Faculty of Mathematics And Natural Sciences, Bogor Agricultural University. Bogor, Indonesia
E-mail: annisyiaazp@gmail.com

^{*}Microbial Division, Indonesia Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD),
Jl. Tentara Pelajar 3A Bogor 16111, Indonesia
E-mail: d_nengsusi@yahoo.com

Abstract— Salinity and drought stress results in the production of ethylene. Rhizosphere bacterial activity suppresses the production of ethylene through the activity of 1-aminocyclopropane-1-carboxylate (ACC) deaminase. In this study, a sampel of rhizosphere bacteria from coastal rice plant area was tested. The method used was acc deaminase activity test performed on Dworkin - Foster (DF) media and PCR *acdS* gene using specific primers of ACC and a quantitative analysis of the production of auxin (IAA). Of 47 isolates obtained, 8 were positively have *acdS* activity. The positive result was indicated by the presence of 1080 bp amplicon. Quantitative analysis showed the highest yield of 10.6 ppm of auxin. The results prove that there are some bacteria originated from coastal rice plant area which have acc deaminase activity.

Keywords— ACC deaminase, auxin, drought, salinity, rhizosphere bacteria, rice

I. INTRODUCTION

Global warming has brought various impacts on environment. The sea level rises due either to the increasing of the volume of sea water as the result of the rising of the sea temperatures or the melting ice in the north and the south poles. Up to 2010, potential sea level rise varies from 60 cm to 100cm. Three main factors associated with global climate change that affects agricultural sector are : 1) changes in rainfall patterns ;2) increasing extreme climate events (floods and droughts); and 3) increasing air temperature and sea surface [1].

Rhizosphere is part of soil which is existed around plant roots and act as the outer defenses of plants against root pathogens. The population of microorganisms in the rhizosphere are usually more numerous and varied than on the ground instead of the rhizosphere [2].

According to Foster (1985) in [3] . Some rhizosphere microorganisms play an important role in nutrient cycling and soil formation processes, plant growth, affecting the activity of microorganisms as well as biological control against root pathogens. Rhizobacteria is a bacteria that lives in the root zone and colonize the root system of plants. Some bacteria are able to control the disease and plant growth promoters (Plant Growth Promoting Rhizobacteria) so it can

be used as an inoculant biofertilizer [4]. Directly, PGPR (Plant Growth Promoting Rhizobacteria) stimulates plant growth by producing growth hormones, vitamins and various organic acids and increase nutrient intake for plants. Several types of rhizobacteria are currently developed as a biofertilizer agent, such as species of *Pseudomonas*, *Bacillus*, *Streptomyces*, *Azospirillum*, *Agrobacterium*, *Rhizobium*, *Alkaligenes*, *Burkholderia*, *Beijerinckia*.

The use of microbe controlling extreme environment stress such as salinity, particularly bacteria producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase (E.C.4.1.99.4) enzyme has never yet been reported. ACC deaminase is sitoplasm enzyme which is produced by some soil bacteria to degradate ACC precursor of ethylene hormone on plants) to become ammonia and α -cetobutirate which are the source of Nitrogen and Carbon for bacteria [5]. Degradation of ACC by enzyme of ACC deaminase would then decrease ethylene in the plant and reduce the damage of the plant [6]. From the study of horticultures, wheat and nuts in research conducted in Canada, USA, India, dan Korea shows that the use of bacteria ACC deaminase could improve the freshness of the plants and improve the plants' resistance towards extreme environmental stress and pathogenic attack [7].

The encoding genetic diversity of *acdS* of rice plants in coastal area has also not yet been reported. Thus, this

research aimed to select some isolates of Rhizosphere bacteria in order to detect the variation of the *acdS* genes and to produce fitohormone auxin (indol-3-asetic acid). It was expected that the search of the potential of the isolates of these Rhizosphere bacteria could contribute to the agriculture along the coast land in anticipating the effect of the rise of the sea surface as the result of the climate change.

II. MATERIAL AND METHODS

A. Material and Equipment

Materials used for this research were 47 isolates of Rhizosphere taken from paddy field on the coast of Java island. The isolates are the collection of Research and Development Center for Biotechnology and Genetic Resources (BB-BIOGEN) Bogor-Indonesia. Other materials are PCR components: *GoTaq* DNA polimerase, *loading dye* 6x, *nuclease free water*, aquades, marker 1kb, DNA isolation kit, wizard DNA genom purification kit. Primer asds, agarose, buffer TAE, clonning kitt. Equipment used are PCR machine, incubator, fridge, *microwave*, *Air Flow* lamina, autoclave, *waterbath*, *shacker*, centrifuse, electroforesys agarosa, *UV-Translluminator*, and other equipment which are commonly used in a laboratory.

B. Research Methodology

1) Rejuvenate and puriry of bacteria isolates [8].

Rejuvenation and purification of bacterial isolates were done by preparing 1 ose colonies of bacteria taken from a stock culture and grown at SEA media (soil extract agar) with a solid streak method then incubated at room temperature for 7 days. *Isolation of DNA*. Isolation of bacterial genomic DNA is done by using the Promega Wizard genomic DNA purification KIT. Bacterial culture that had been grown in LB medium was placed to a micro tube about 1.5 mL, and then centrifuged for 2 minutes at a speed of 14000 rpm. Pellets that were formed were taken and the supernatant were discarded. To the pellet isolates with gram -negative bacteria cell lysis process was directly done meanwhile to the pellets with gram-positive bacteria resuspension with 50 mM EDTA as 480µl was done prior to a cell lysis process. Then, 120 mL lysozyme was added to the pellets before they were incubated at 37 ° C for 45 minutes. The samples were again centrifuged at a speed of 14000 rpm for 2 minutes. The pellet formed were taken and the supernatant was discarded. The next stage was a bacterial cell lysis which was done by adding 600 mL nuclei lysis solution to the sample which was then incubated at 80 ° C for 5 minutes. About 3 mL RNase was added into the sample and incubated at 37 ° C for 45 minutes.

2) Auxin Production Test.

Quantitative analysis of AIA was done using method by Gupta *et al.*[9].

3) Genetic Amplification of *acdS*.

Amplification was done using two primeries, they are DegACcf primary (5'-GGBGGVAAYAARMYVMGSAAGCTYGA-3') and DegACCr primery (5'TTDCCHYKRTANACBGGRTC-3'). PCR used was 25 µl.

4) Genetic Amplification Amplifikasi of 16S rRNA.

16S rRNA was amplified by PCR method using primary 63F (5'CAGGCCTAACACATGCAAGTC'3) dan 1387R (5'GGGCGGWTGTACAAGGC'3).

5) Genetic Sequensing of 16S rRNA.

III. RESULT

A. Activity *acdS* at DF Media

The results of examination of ACC deaminase activity is presented in Figure 1. The isolates were grown on three medias: media (1), DF (Dworkin-Foster), as negative control, which is shown by the absent of the bacteria; media (2) DF + AS (*amonium sulphate*), as positive control which is shown by the presence of isolate and media (3), DF + ACC (C), for isolates which can grow. This becomes an indication for the existence of enzim of ACC deaminase which is shown by the presence of a thick white circle. There were 4 isolates that have ACC deaminase activity.

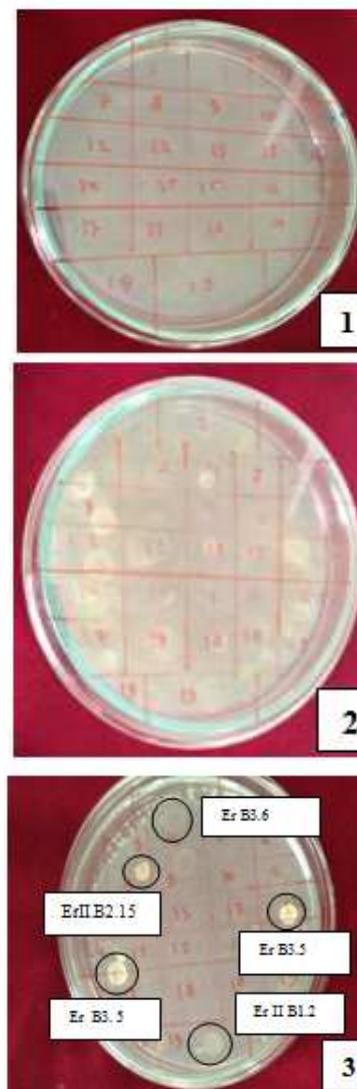


Fig. 1. ACC Deaminase Test Result
Remark : 1 : DF, 2: DF + amonium sulphate , 3:DF + ACC

B. Auxin Production

The auxin content which is produced by the isolate Rhizosphere taken from *paddy field* ranges from 3.6 to 10.6 ppm. Meanwhile the result of the analysis of auxin production presented in Table 1 shows that the highest activity was 10.6 ppm

TABLE I
QUANTITATIVE ANALYSIS RESULT OF ACTIVITIES OF AIA

Isolate	<i>AcdS</i>	AIA production (ppm)
Er II B2.5	+	3.6
Er I B1.8	+	47.3
Er I B3.6	+	4.1
Er II B2.13	+	10.6
Er II B2.10	+	2.3
Er II B2.4	+	10.35
Er II B2.15	+	3.4
Er II B1.2	+	6.05

The results of colorimetry test using spectrophotometer reveals that all bacteria on the supernatant culture tested produces AIA hormone with different concentration. Differences in the AIA production might be due to different species and strains tested, condition of the culture, the growth phase and the availability of the substrate [10]. Other factors that might result in the differences are genetic structure, growth speed, enzyme activity and its ability to convert tryptophan contained in AIA, and the length of time of incubating the culture [10].

C. Amplification of PCR *gen acdS*

Detection of the existence of ACC Deaminase gene can be done by using polymerase chain reaction (PCR). Of 47 samples tested, 8 is apparently positive. They are Er II B2, Er II B2. 13, Er B1 8, Er II B2. 10, Er II B2. 4, Er B3. 6, Er II B1. 2 and Er II B2. 15. The positive result was indicated by 1080 bp DNA with ACCf and ACCr specific primers. The production of ACC deaminase is very efficient and important for endophyte to stimulate the host plant.

The *Gen acdS* in this research was obtained on 1080 bp amplicon. This is similar with the result obtained by Yan Jun Jia *et al.*[11]. from a Rhizosphere bacteria, *Pseudomonas* sp. According to Jin Duan *et al* [12] of 27 isolates amplified with PCR using specific primers for ACC, 17 are ACC deaminase positive. This is marked by the existence of DNA ribbon on amplicon 1020 bp. The result of Jin Duan's research apparently different from that of this present research which is on 1080 bp.

Subrahmanyam [13] reports that agrose, soil bacteria in India, which is tested with ACC specific primers shows a result on ACC positive on 1080 bp amplicon after being PCR. The result of research by Mattos *et al.* [14] proves that endophyte bacteria has capability to improve the number of lateral roots and root hairs of paddy plants. The purpose of the amplification of *acdS* gene is to amplify the gene which positively brings gene with fragment of 1080 bp. The amplification of PCR with *gen acdS* specific primers indicates 8 positive isolates.

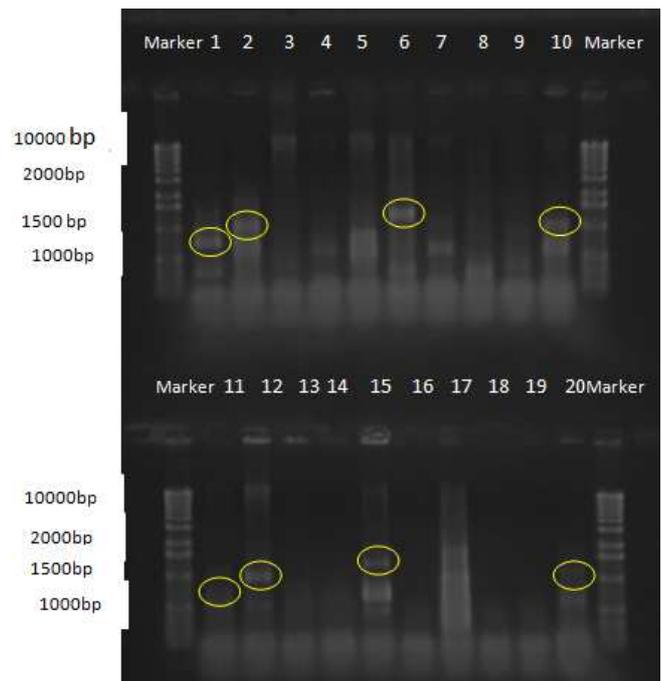


Figure 2 The Amplicon of PCR *gen acdS* with 1080 bp size

Notes: Marker: 1 kb, 1= Er II B2 5, 2= Er B1 8, 3= Er B2 2, 4= Er II B2 12, 5= Er II B1 1 6= Er B3 6 7= Er II B3 10 8= Er B2 3, 9= Er B1 7 10=Er II B2 13, 11= Er II B2 10, 12= Er II B2 4, 13= Er B3 10 14= Er II B3 10 15= Er II B2 15 16= Er B2 13 17=Er B2 1 18= Er B2 6 19=Er B3 1 20= Er II B1 2

At the last step, species identification of bacteria which is closer to the superior sample data taken from the GeneBank is done. The identification used blast N program taken from NCBI (National Center for Biotechnology Information) site. After the data was obtained, the genetic tree is made using Mega 5 program in order to get a closest family relation between the superior sample with the data from the Gene Bank. The amplification of the 16S rDNA test shows positive result (Figure 3). The result of phylogenetic analysis can be seen in Table 2. The blast result shows that the family relation was possessed by samples: ErII B2.13 (*Bacillus safensis*) and ErII B1.2 (*Bacillus Pumilus*).

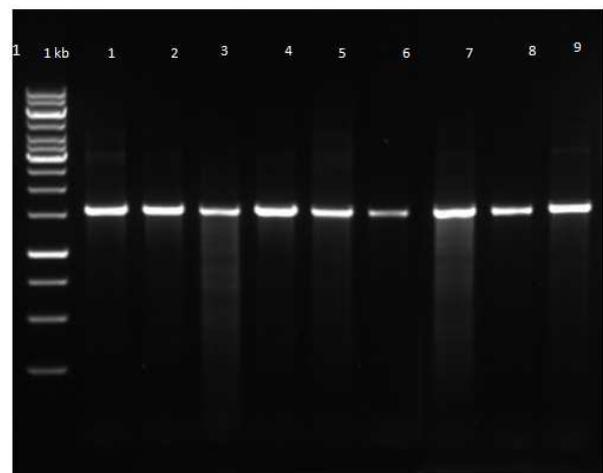


Figure 3 Product of DNA PCR 16S rRNA

TABLE II
SEQUENCING ANALYSIS RESULT OF DNA SAMPLE USING BLAST N
PROGRAM

Isolate	Sequence of homolog bacteria	% identity	Access No.
Er IIB2. 13	<i>Bacillus safensis</i> gene for 16S ribosomal RNA, partial sequence	99%	FO-036b.
Er II B2. 4	<i>Bacillus aerophilus</i> strain KUDC1727 16S ribosomal RNA gene, partial sequence	98%	KC414706
Er II B1.2	<i>Bacillus_pumilus</i> isolatee FM10 16S ribosomal RNA gene, partial sequence	99 %	DQ289077.1
Er II B2.5	<i>Microbacterium arborescens</i> . IARI-KPI 16S ribosomal RNA gene, partial sequence	99%	KF712889.1
ER I B1. 8	<i>Bacillus amyloliquefaciens</i> strain YCG37 16S ribosomal RNA gene, partial sequence	98%	JF775416.1
Er I B3.6	<i>Stenotrophomonas maltophilia</i> 16S ribosomal RNA gene, partial sequence	90 %	

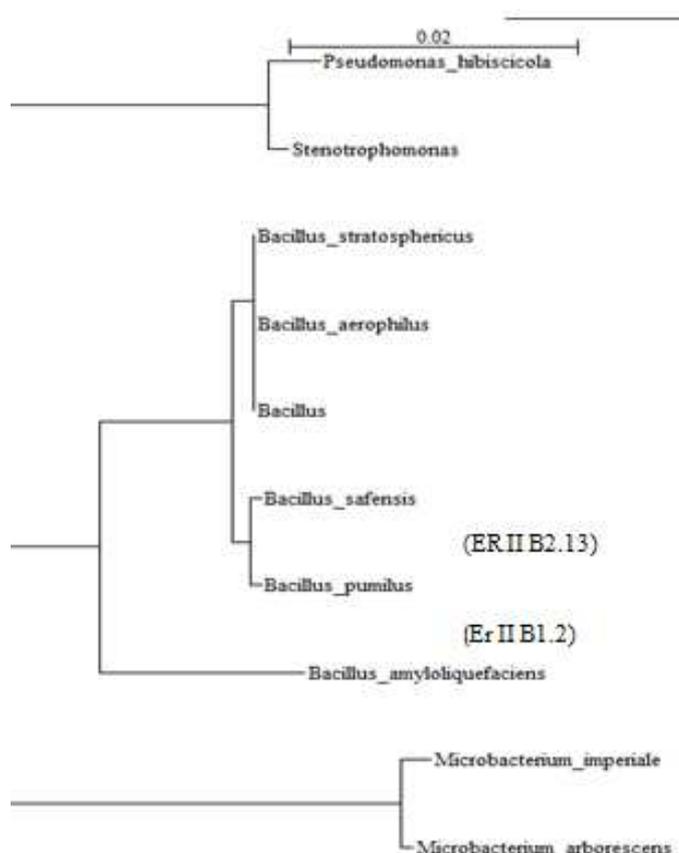


Fig 4. Phylogenetic tree of superior sample and GeneBank data using Neighbor Joining (NJ) with bootstrap 100x

IV. CONCLUSIONS

The result of the research reveals that 8 of 47 isolates of rhizosphere bacteria contains amplicon with 1080 bp and specific primary ACCf and ACCr. The bacteria produces auxin (AIA) with 3,5 ppm to 10.6 ppm concentration. The ACC deaminase activity test done on DF media shows a

positive result of Er B3.5 and Er II B2.5. Blast result reveals that the closest family relationship was shown by ErII B2.13 (*Bacillus safensis*) dengan ErII B1.2 (*Bacillus pumilus*). Further research to inoculate plant in a direct way needs to be done to observe the effect of ACC deaminase gene resulted by the isolates of superior bacteria towards the growth of plants. In addition, pathogenicity test to superior bacteria is also needed.

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