

Phylogenetic Molecular Ectorrhizosphere Bacteria from Medicinal Plant *Ageratum conyzoides* L. Employing Amplified Ribosomal DNA Restriction Analyses (ARDRA)

Any Fitriani^{1*}, Any Aryani², Wiedya Prieza³

¹ Program Study Biology, Department Biology Education, Universitas Pendidikan Indonesia
Dr. Setiabudhi 229 Bandung 40154

² Program Study Biology, Department Biology Education, Universitas Pendidikan Indonesia
Dr. Setiabudhi 229 Bandung 40154

³ Program Study Biology, Department Biology Education, Universitas Pendidikan Indonesia
Dr. Setiabudhi 229 Bandung 40154

*Corresponding author's email: anyfitriani [AT] upi.edu

ABSTRACT— The study on phylogenetic molecular employing Amplified Ribosomal DNA Restriction Analyses (ARDRA) of ectorrhizosphere bacteria from medicinal plant *Ageratum conyzoides* has been conducted. The aim of the research is firstly, to study restriction pattern of *Hha*1 or *Msp*1 in 16S rDNA resulting *in vitro* amplification from plasmid recombinant and secondly, to study phylogenetic molecular from ectorrhizosphere bacteria. The method was used chromosomal DNA isolation, *in vitro* amplification of 16S rDNA, insert verification applying Polymerase Chain Reaction (PCR), restriction of amplicon of 16S rDNA with *Hha*1 or *Msp*1, and phylogenetic construction employing MVSP 3.2. Restriction pattern showed genetic diversity of ectorrhizosphere bacteria. Phylogenetic analyses revealed endophyte bacteria has three big clusters. Shanon-Wiener diversity showed that ectorrhizosphere bacteria have high diversity. Phylogenetic revealed the genetic diversity of endophyte bacteria in *A. conyzoides*.

Keywords— *A. conyzoides*, ARDRA, ectorrhizosphere, phylogenetic

1. INTRODUCTION

Ageratum conyzoides is medicinal plant which was used as injury medicine, antiinflammatory and antitumour by Asia Pacific people. The role of medicinal plant close to role of ectorrhizosphere bacteria that living in surface and soil of root without plant injury. Interaction of ectorrhizosphere bacteria and host influenced physiological and ecological of one and the other [1]. Characterization of morphology and biochemistry of ectorrhizosphere bacteria has been done, and most of them include Gram negative bacteria, round shape, irregular shape of colony. Many of them have catalytic capability such as chitinase, protease, and amylase [2]. This capability related to genetic diversity of ectorrhizosphere community. The aim of the research is (1) to analyse restriction pattern of *Hha*1 or *Msp*1 of 16S rDNA resulting *in vitro* amplification from plasmid recombinant and (2) to characterize phylogenetic molecular from ectorrhizosphere bacteria.

2. MATERIALS AND METHODS

2.1 The Root and Soil of *A. conyzoides*

Root and soil of *A. conyzoides* is collected with cut the base of root. Root and soil are took from plant and went into plastic bag, then put them in the ice storage place. Plant picked from opened and shaded area.

2.2 DNA Chromosome Isolation

Bacterial DNA chromosomal from root is isolated as Fermentas procedure (Ukraina).

2.3 Amplification of 16S rDNA

Gen of 16S rDNA is amplified employing PCR with primer 63f and 1387r [3]. PCR worked in reaction condition as early denaturation 94 ° C, 5 min, denaturation 94 ° C, 30 sec, annealing 50 ° C, 30 sec, elongation 72 ° C, 1 min, end of PCR 72 ° C, 5 min. Reaction was done for 30 cycles.

2.4 Cloning of Amplicon to Vector

PCR product was cloned to vector cloning pGEM-T Easy (Promega) and transformed to competence *E. coli* DH 5 α [4]. Transformant that has plasmid recombinant is selected using ampicillin and screened by X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) as substrate in Luria Bertani Agar media.

2.5 Reaction of Restriction

White colony is taken for culture on LA agar addition ampicillin (100 mg/ml) and X-gal (40 mg/ml) as replica for further analyses. Ten transformants are taken randomly for plasmid recombinant isolation that has 16S rDNA. Plasmid isolated employing kit Biobasic. Plasmid recombinant amplified using primer 63F and 1387R. PCR product digested applying *Hha*I and *Msp*I.

2.6 Electrophoresis and DNA visualization

Electrophoresis was done using gel agarose matrix with concentration 1%. Gel agarose soaked in ethidium bromide for 5-10 min, after that solvent replaced with distilled water and left for 10 min. Gel agarose is put on UV transilluminator and documented with camera.

2.7 Construction of Phylogenetic Tree

Pattern of DNA fragment resulting digest reaction translated to binary digit. The data processed using MVSP program V.3.2 for phylogenetic tree construction.

3. RESULTS AND DISCUSSION

Chromosomal DNA amplified employing PCR and amplicon was runned employing electrophoresis (Fig 1).

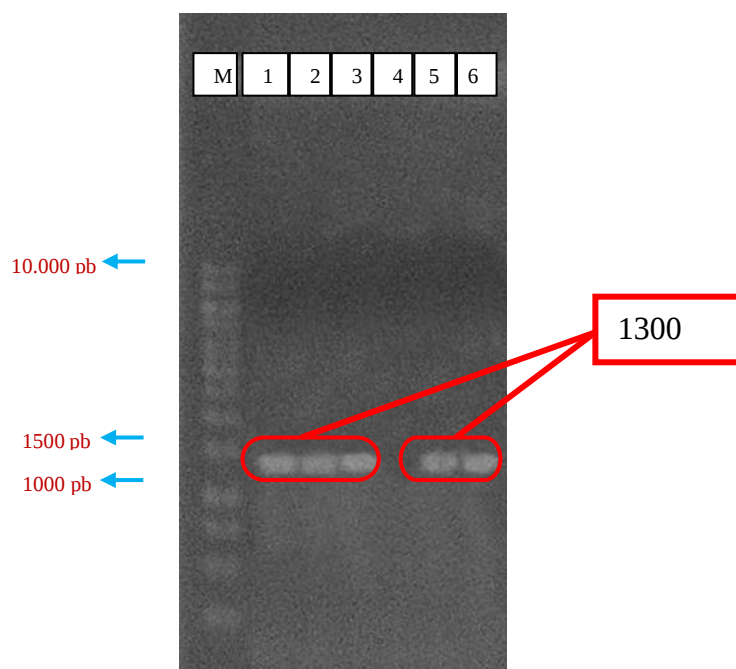


Fig 1. Electrophoregram of amplicon 16S rDNA ectorrhizosphere bacteria from *A. conyzoides*. M : 1 Kb DNA Ladder (NEB); 1&2) amplicon of shaded ectorrhizosphere bacteria; 3) Positive control; 4) Negative control; 5&6) amplicon of opened ectorrhizosphere bacteria

Cloning of 16S rDNA to vector produced clone that has recombinant plasmid. Recombinant plasmid is amplified employing 67F and 1387R and then amplicon restricted with *Hha*I and *Msp*I. Reaction restriction showed pattern of diversity of DNA fragment length (Fig. 2).

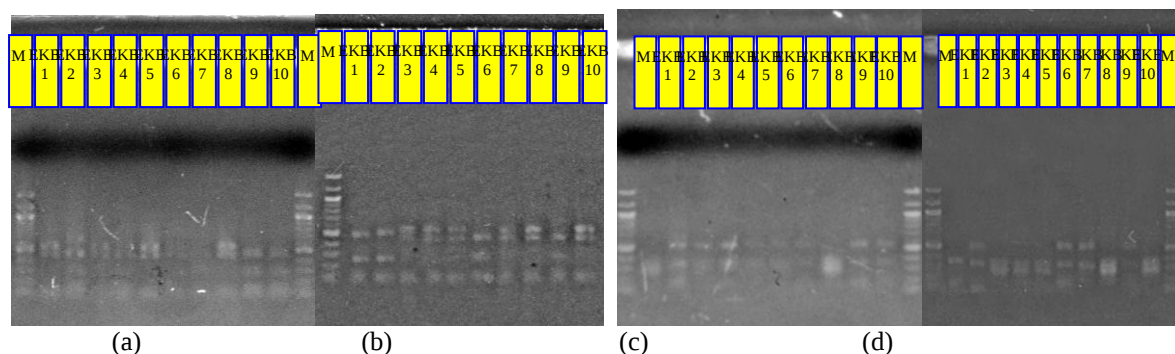


Fig 2. Electrophoregram of fragment DNA from restriction reaction of DNA amplicon ectorhizosphere bacteria *A. conyzoides*. (a) opened-*Msp1*; (b) shaded-*Msp1*; (c) opened-*Hha1*; (d) shaded-*Hha1*. M : 1 Kb DNA Ladder (NEB); EKB : Opened area, EKN : shaded area

Restriction pattern showed difference of diversity 16S rDNA, it means that the species is very diverse. Ectorhizosphere bacteria of *A. conyzoides* in shaded habitat more diverse than opened habitat. This phenomena caused by differences abiotics condition especially sunlight intensity. Sunlight intencity related to soil water capacity and soil humidity. Water content will influence total water that can use by plant and total water that enter will influence survival of ectorrhizosphere bacteria that lives in surface of root organ [5]. Analysis of diversity index of Shannon-Wiener revealed high diversity of ectorrhizosphere bacteria ($H = 3,515$).

Pattern of restriction translate to phylogenetic tree (Fig 3). Ectorhizosphere bacteria from *A. conyzoides* has 3 big clusters and 1 small cluster. Bacteria from opened and shaded area separated one from each other. Uniquely, strain EKB20 separated from other strain in all opened bacteria strain.

Ectorhizosphere bacteria of *A. conyzoides* in shaded habitat more diverse than opened habitat. This phenomena caused by differences abiotics condition. Analysis of diversity index of Shannon-Wiener revealed high diversity of ectorrhizosphere bacteria. Ectorhizosphere bacteria from *A. conyzoides* has 3 big clusters and 1 small cluster.

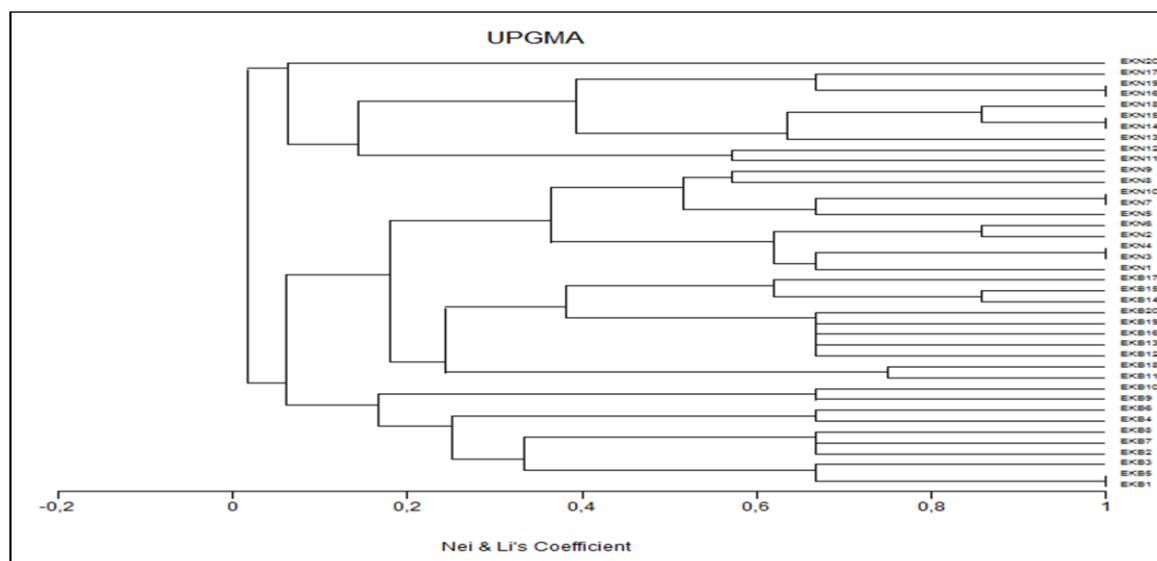


Fig 3. Phylogenetic tree of ectorrhizosphere bacteria from *Ageratum conyzoides*

4. ACKNOWLEDGMENT

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