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

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ABSTRACT— The study on phyllogenetic molecular employing Amplified Ribosomal DNA Restriction Analyses (ARDRA) of ectorhizosphere 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 phyllogenetic molecular from ectorhizosphere 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 phyllogenetic construction employing MVSP 3.2. Restriction pattern showed genetic diversity of ectorhizosphere bacteria. Phyllogenetic analyses revealed endophyte bacteria has three big clusters. Shanon-Wiener diversity showed that ectorhizosphere bacteria have high diversity. Phyllogenetic revealed the genetic diversity of endophyte bacteria in *A. conyzoides*.

Keywords— *A. conyzoides*, ARDRA, ectorhizosphere, phyllogenetic

1. INTRODUCTION

Ageratum conyzoides is medicinal plant which was used as injury medicine, antiinflamatory and antitumour by Asia Pacific people. The role of medicinal plant close to role of ectorhizosphere bacteria that living in surface and soil of root without plant injury. Interaction of ectorhizosphere bacteria and host influenced physiological and ecological of one and the other [1]. Characterization of morphology and biochemistry of ectorhyzosphere 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 ectorhizosphere 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 phyllogenetic molecular from ectorhizosphere 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 ^{o} C, 5 min, denaturation 94 ^{o} C, 30 sec, annealing 50 ^{o} C, 30 sec, elongation 72 ^{o} C, 1 min, end of PCR 72 ^{o} 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 5α [4]. Transforman that has plasmid recombinant is selected using amphicillin and screened by X-gal (5-bromo-4-kloro-3-indolil-β-D-galaktopiranosida) as substrate in Luria Bertani Agar media.

2.5 Reaction of Restriction

White colony is took for culture on LA agar addition amphicillin (100 mg/ml) and X-gal (40 mg/ml) as replica for further analyses. Ten transformans are took 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*1 and *Msp*1.

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 destilated water and let for 10 min. Gel agarose is put on UV transilluminator and documented with camera.

2.7 Construction of Phyllogenetic Tree

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

3. RESULTS AND DISCUSSION

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

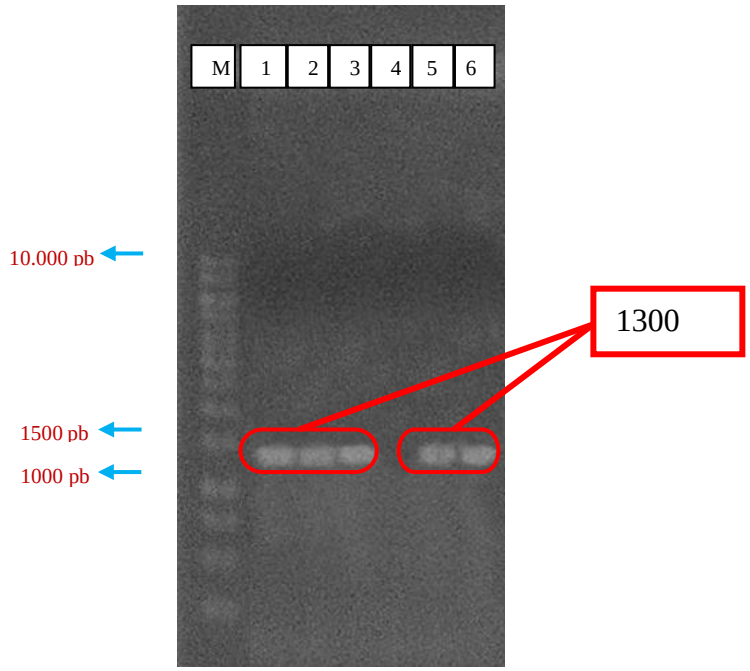
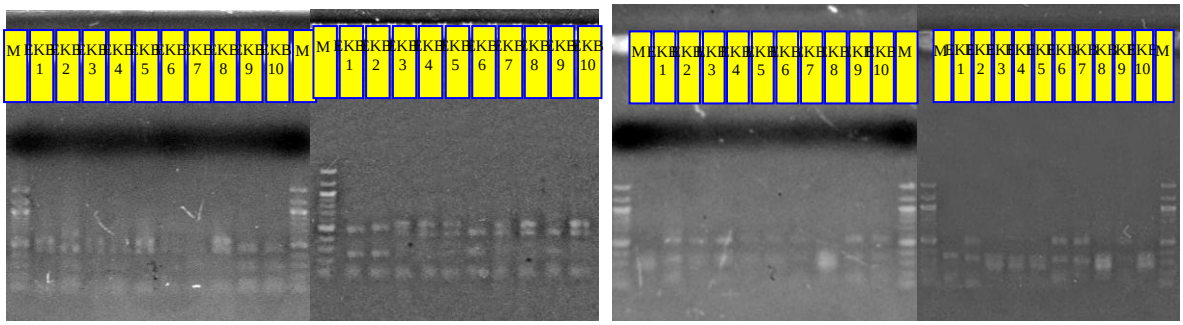


Fig 1. Electrophoregram of amplicon 16S rDNA ectorhizosphere bacteria from *A. conyzoides.* M : 1 Kb DNA Ladder (NEB); 1&2) amplicon of shaded ectorhizosphere bacteria; 3) Positive control; 4) Negative control; 5&6) amplicon of opened ectorhizosphere bacteria

Cloning of 16S rDNA to vector produced clone that has recombinant plasmid. Recombinant plasmid is amplied employing 67F and 1387R and then amplicon restricted with *Hha*1 and *Msp*1. Reaction restricy showed pattern of diversity of DNA fragment length (Fig. 2).



(a) (b) (c) (d)

Fig 2. Electrophoregram of fragment DNA from restriction reaction of DNA amplicon ectorhizosphere bacteria *A. conyzoides*. (a) openned-*Msp*1; (b) shaded-*Msp*1; (c) opened-*Hha*1; (d) shaded-*Hha*1. 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. Ectorhyzosphere 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 ectorhyzosphere bacteria that lives in surface of root organ [5]. Analysis of diversity index of Shannon-Wiener revealed high diversity of ectorhizosphere bacteria (H= 3,515).

Pattern of restriction translate to phyllogenetic tree (Fig 3). Ectorhyzosphere 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.

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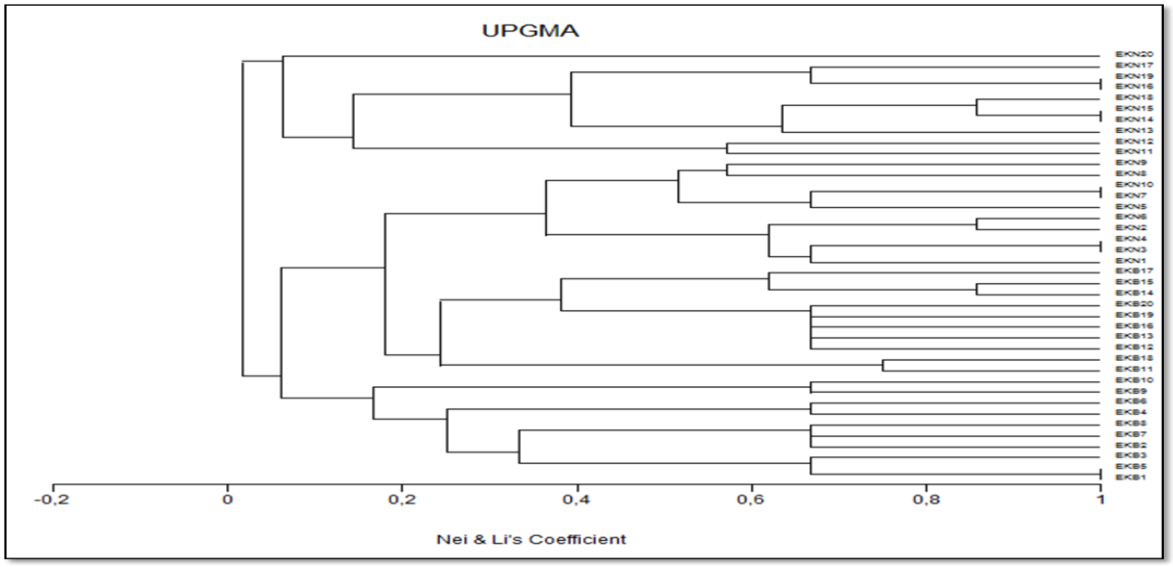

Fig 3. Phyllogenetic tree of ectorhyzosphere bacteria from *Ageratum conyzoides*

4. ACKNOWLEDGMENT

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5. REFERENCES

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