Diversity of Endophytic Bacteria Related to Antibacteria Activity Isolated from *Vetiveria zizanioides* L. (WT)

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ABSTRACT---- ***Diversity of endophytic bacteria isolated from Vetiveria zizanioides L. (WT) has been done. Endophytic bacteria is bacteria which living in the cell or intercellular space without cause desease to host. V. zizanioides (WT) is the medicinal plant as fragrance, antibacteria, antifungi. Endophytic bacteria lived in roots cell and produced secondary metabolites. The aim of the research is isolating bacteria from the roots, characterizing of morphology and biochemistry, evaluating antimicrobe activity, and identifying the microbes. Endophytic bacteria from the roots of V. zizanioides has been isolated and got 22 different isolates. Based on Gram staining, they have 20 baccil and 2 coccus, 17 Gram negative and 5 Gram positive. Biochemistry analysis was done and variability results. Antibacteria analysis showed that 7 isolates potentially produced secondary metabolites as antibacteria.***

Keywords---- endophytic bacteria, antibacteria, *Vetiveria zizanioides*

1. INTRODUCTION

Roots of *Vetiveria zizanioides* produced essential oils as frangrance, beauty materials, soap fragrance, medicines and insecticides. The ethanol extract of roots *V. zizanioides* has antibacteria activity to *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi*, *Staphyllococcus aureus* and *Vibrio cholerae* [1]. Vetiverin, alkaloid, was isolated succesfully from essential olis and showed antifungi activity to *Trichophyton mentagophytes* [2].

Higher plants have endophytic bacteria that could produced secondary metabolites [3]. Bioactive compounds extracted from endophytic bacteria have roled on antibiotics, antiparasites, antivirus, antioxidant, anticancer and insecticides [4]. Fitriani *et al*. [5] showed antibacteria activity from *Shewanella* and *Pseudomonas* isolated from *Ageratum conyzoides* L.

Antibiotics is bioactive compounds which the highest diversity structures and benefit in medicine in the world. Antibiotics was utilized over 40000 ton/year in food industry, fodder, agricultures, medicines, biochemistry, genetics, and molecular biology [6]. Endophytic bacteria was isolated and characterized from plant and cultured on synthetic media to produce secondary metabolites as plant host appropriately. Li *et al*. [7] revealed 41 isolates isolated from tropic forest in Yunnan, about 65.9% have antibacteria activity to *E. coli*, 24% isolates have antibacteria activity to *S. aureus*, 31.7% isolates have antibacteria activity to *S. epidermidis* and 12.2% isolates have antibacteria activity to *Candida albicans*.

The aim of the research is to isolate, characterize and identified endophytic bacteria from roots of *V. zizanioides*.

2. MATERIALS AND METHODS

2.1 Sample Treatment

Roots of *V. zizanioides* was washed and cut to small pieces. The pieces of roots was surface sterilized with 75% ethanol for 2 minute, 25% bayclin for 5 minutes, and 75% ethanol for 1 minutes. Finally, the pieces of roots was rinsed 3 times by aquadest. Pieces of roots was cultured on Luria Bertani Agar [8].

2.2 Bacteria identification

2.2.1 Morphology and Gram Staining

Morphology observation was done after 48 h incubation. Morphology observation refered to Cappuccino & Sherman [9]. Morphology characteristic was used in observation is shape of cell, color of colony, apperance of cell surface, elevated of surface, edge of cell, and darkness of colony.

2.2.2 Biochemistry Characteristic Observation

Biochemistry characterization was analysed including amylum hydrolysis, lipid hydrolysis, kasein hydrolysis, casein hydrolisis, Catalase test, Urease Test, Gelatine test, sucrose fermentation, lactose fermentation, dextrose fermentation, Citrate utilization, Indole production, Metil Red, and Voges-Proskauer.

2.2.3 Genus Identification

Genus of isolates was identified based on Bergeys Manual [10].

2.2.4 Antibacteria Bioassay

Antibacteria bioassay used disc difussion method [9]. One colony was culture in 25 mL medium in flask 100 mL for 16 h. Ten mL culture was centrifuged at 10000 g for 10 min. The paper disks was submerged in fifteen μL of supernatant. The treatment was done duplicate.

3. RESULTS AND DISCUSSION

Small pieces of roots of *V. zizanioides* was culture on LA media for 48 h. There are 35 colonies which growth on media. First colony analysis showed that 31 isolates were different, and the second colony analysis revealed 22 bacteria isolates were different. The yield last analysis went to further analysis.

All isolates were analysed morphology characteristic and showed that all isolates had differently combination characteristic. Actually, almost all of isolates are round and spheical (64%), color of colony are white (64%), shiny colony are very limited (23%), colony are thick and concentrated (73%), elevation of colony are flatted (82%), and edge of colony are smooth (36%).

Gram staining analysis indicated that 20 isolates have bacilli cell and 2 isolates have coccus cell. Only 5 isolates were as Gram positive and 17 isolates were as Gram negative. Analysis of biochemistry characteristic showed that all isolates had diversity character (Table 1).

Table 1. Biochemistry identification of endophytic bacteria from roots of *Vetiveria zizanioides*

ISOLATE	Biochemistry characteristic											
	A	B	C	D	E	F	G	H	I	J	K	L
1	+	+	+	+	+	-	+	-	-	-	+	+
2	-	+	+	+	-	-	-	-	-	-	-	-
3	+	-	+	+	+	-	+/G	-	-	+	+	+
4	-	+	-	+	+	-	-	-	-	-	-	-
5	+	+	+	+	+	-	+/G	-	-	+	+	+
6	+	+	+	+	+	-	+	-	-	-	+	+
7	-	+	+	+	+	+	+	-	-	-	-	-
8	+	+	+	+	+	-	+/G	-	-	-	+	+
9	-	-	-	+	-	-	+/G	-	-	+	+	-
10	+	+	+	+	-	-	+/G	-	-	-	+	+
11	-	+	+	+	+	-	+/G	-	-	-	+	+
12	+	+	+	+	+	-	+/G	-	-	-	+	+
13	-	+	-	+	+	-	+	-	-	-	+	-
14	+	+	+	+	+	-	+	-	-	-	+	+
15	-	+	-	+	+	-	-	-	-	-	-	+
16	-	+	+	+	+	-	+	-	-	+	-	-
17	-	+	+	+	-	-	+	-	-	+	+	+
18	-	+	-	+	+	-	+	-	-	+	+	+
19	-	-	-	+	+	-	+	-	+	+	+	+
20	+	+	+	+	-	-	+	-	-	+	-	+
21	+	+	+	+	-	-	+	-	-	-	-	+
22	+	+	+	+	+	-	+	-	-	-	-	+

A : Amylum hydrolisis ; B : Lipid hydrolisis ; C : Kasein hydrolisis ; D : Catalase Test ; E : Sucrose fermentation ; F : Lactose fermentation ; G : Dextrose fermentation ; H : Citrate Utilization ; I : Indole production ; J : Methyl Red ; K : Voges-Proskauer ; L : Gelatine test

All isolates can not utilize citrate in metabolism, therefore the cell have no enzymes involved to citrate metabolism. Dextrose fermentation was occur in almost all isolates, showed dextrose as simple sugar could used as resource for catabolism in the cell. Only isolate 19 can produce indole compound. Indole is a structure that biosynthesis

through trypthophan pathway as precursor [11]. This isolate had potency as pharmacological activities. Dhani *et al*. [12], reported that indole is aromatic heterocyclic ring, consisting of a six-membered benzene ring fused to a five-membered nitrogen-containing pyrolle ring. They possess biological activities, such as anti-viral, antimicrobial, antitubercular, anti-inflamantory, anticancer, antioxidant.

Catalase was possessed by all isolates isolated from roots of *V. zizanioides*. Catalase test is a very simple method, thus easy applying to bacteria identification [13]. Sixteen isolates produced gelatinase which an exoenzyme could hydrolyzed gelatine in medium. Catalase and gelatine test are generally positive in enterobacteria [14].

Almost all isolates able to hydrolize lipid. Lipid are universally present in bacteria. Lipid in bacteria categorize to a polar component (fatty acids, alk-l-enyl ether, alkyl ether, more complex fatty acids), phospholipids, glycolipid, neutral lipid, and non extractable lipid [15].

Based on analysis and genus identification to Bergey's Manual, the isolates included to diverse genus. Morphology and biochemistry characteristic was applied to identify the genus isolate. Isolate 1 is *Acidiphyllum*, isolate 5 is *Beijerinckia*, isolate 13 is *Brochotrix*, isolate 15 is *Flavobacterium*, isolate 17 is *Pseudomonas*, isolate 19 is *Rhizomonas*, isolate 20 is *Gluconobacter*. *Beijerinckia*, *Brochotrix*, *Pseudomonas* and *Rhizomonas* are generally living in plant root [5].

Antibacteria bioassay revealed 7 isolates capable as antibacteria to *Pseudomonas aeruginosa, Escherichia coli* and/or *S. aureus* (Table 2). Extracelullar compound was produced to medium and could inhibited the growth of pathogen bacteria in the medium. Several kind of compound will synthesize in the bacteria cell through differently pathway.

Table 2. Antibacteria bioassay to pathogen bacteria

Isolates	**Inhibition zone to pathogen bacteria (mm)**								
	P. aeruginosa			***E. coli***			***S. aureus***		
	1	**2**	**X ± SD**	**1**	**2**	**X ± SD**	**1**	**2**	**X ± SD**
1	8,5	9,5	9,0 ± 0.671	0	0	0	0	0	0
5	8,1	7,6	7,85 ± 0,353	0	0	0	0	0	0
13	12,6	12,6	12,6 ± 0	7,8	6,4	7,1 ± 0,98	0	0	0
15	10,4	13	11,7 ± 1,83	6,6	7	6,8	0	0	0
17	0	0	0	8,3	6,1	7,2 ± 1,55	6,1	6,2	6,15 ± 0,07
19	0	0	0	6,2	6,5	6,35 ± 0,21	0	0	0
20	0	0	0	0	0	0	13,6	15,1	14,35 ± 0,06
Kontrol (+)	40	34,9	37,45 ± 2,54	10,8	9,0	9,9 ± 1,27	27,4	27,5	27,45 ± 0,07
Kontrol (-)	0	0	0	0	0	0	0	0	0

They can include to secondary metabolites or peptide [16]. Capability of inhibition of supernatant to pathogen bacteria is very various. Isolates 1 and 2 can inhibit *P aeruginosa*, meanwhile isolate 13 and 15 can inhibit *P. aeruginosa* and *E coli*. Isolates 17 obstructed *E coli* and *S. aureus*. Isolates 19 inhibited *E. coli* and isolates 20 inhibited *S. aureus*. Variability inhibition capability could showed variability of biosynthesis compound.

4. CONCLUSION

Roots of *V. zizanioides* have culturable endophytic bacteria. They are 22 isolates and have variability and diversity of morphology and biochemistry characteristic. Only 7 isolates can inhibit the growth of pathogen bacteria. Isolate 15 and 17 could inhibited 2 pathogen bacteria separately.

5. ACKNOWLEDGMENT

The research is done with fund provided by Hibah Bersaing, Ministry of Research Technology and Higher Education, Republic of Indonesia.

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