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Research Article

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Identification and Antifungal Activity Test of Endophytic Bacterial Isolates from *Morinda citrifolia* L. Leaves against *Fusariumoxysporum*

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ABSTRACT

A research about identification and test of antifungal activity of endophytic bacteria isolates from the leaves of Morinda citrifolia L. against Fusarium oxysporum had been done. The purpose of this study was to identify and test the antifungal activity of endophytic bacteria isolates from the leaves of Morinda citrifolia L. against Fusariumoxysporum. Three isolates were obtained from the research, those were MC1 (2.0 × 10^2 CFU/g), MC2 (1.0 × 10^2 CFU/g), and MC3 (2.0 × 10^2 CFU/g). Results of identification by 16S rRNA analysis showed three consecutive isolate, which were Bacillus subtilis sub sp. Inaquosorum MER_77, Bacillus sp. SG3-2and Bacillus sp. CC-YY22. Bacillus subtilis sub sp. Inaquosorum MER_77 and Bacillus sp. CC-YY22 was showed antifungal activity against Fusarium oxysporum with inhibitory effect respectively 76.7% and 33.3% while Bacillus sp. SG3-2 did not have antifungal activity against Fusarium oxysporum.

Keywords: Identification; Antifungal; Endophytic; *Morinda citrifolia* L.; *Fusarium oxysporum*

INTRODUCTION

Fusarium is a genus of filamentous fungi, composed of several species that agronomically important because this species is plants pathogen, producing mycotoxins and opportunistic pathogens in humans [1]. Members of this genus which receiving much attention is *Fusarium oxysporum*. This species is a soil borne fungi and cause of vascular wilt disease that attacks many economically important plants in the world [2]. Generally, to reduce of the diseases that caused by fungi could be done by utilization of synthetic pesticides. Excessive use of synthetic pesticides will give bad impact on the environment, so the environmentally friendly counter measures such as using endophytic bacteria is really important.

Endophytic bacteria are cosmopolitan organisms, it can be found in all plants that have been studied. These bacteria can form mutualistic symbiotic relationship, including komensalis and tropobiotic [3-5]. In general, these bacteria come from the surrounding environment both from the rhizosphere of plants or filosfer [6]. The endophytic bacteria are able to produce compounds such as antibiotics and antimycotics [7]. The compounds produced by the endophytic bacteria has potential as biopesticide for controlling pests and plant diseases. Utilization of biopesticides is very beneficial for the environment. Content in biopesticides is more sustainable than synthetic pesticides. This is because biopesticides could be easily decomposed in nature, has specific employment targets, has the unique way of working, and non-toxic for humans [8].

The selection of plant sources for endophytic bacteria isolates generally done by the approach of ethnobotany and observation about the plants chemical compound. *Morinda citrifolia* L. was selected as isolates, because this plant is widely used for medicine. *Morinda citrifolia* L. is known for containing secondary metabolites such as tannins, flavonoids, saponins, steroids, alkaloids, and glycosides. This plant has antioxidant activity, antimicrobial [9] and anti helminitic [10]. One kind of fungi which the growth can be inhibited by this plant extract is *Fusarium*sp [11], thus enabling the plant tissue to contains endophytic bacteria that have antifungal

activity against the fungus. The purpose of this study was to characterize and test the antifungal activity of endophytic bacteria isolated from the leaves of *Morinda citrifolia* L. against *Fusariumoxysporum.*

MATERIALS AND METHODS

Material and Tools

Materials used in this research was distilled water, alcohol 70%, spritus, medium tryptic soy agar (TSA), medium tryptic soy broth (TSB), medium potato dextrose agar (PDA) and endophytic bacteria isolates. Tools used in this research were Petri disk, Erlenmeyer flask, measuring glass, pH meter, micropipete, shaker incubator, vial tube, paper dics, and sterilemembran filter 0.02 µm.

Sterilization of Material and Tools

All material and tools was sterilized using autoclave. Sterilization was done at 121°C temperature for 20 minutes [12].

Plant Collection

Plants were collected from the area of Simpang Haru, Lakuk Regency of Padang City, West Sumatra, Indonesia. Collected plants was confirmed as healthy plant. The methods of collecting plants refers to de Melo et al. [13] method with some modification. The leaves was cut using a sterile blade and then washed with sterile distilled water, then placed in a plastic bag and put in a cooler (temperature maintained + 10°C).

Sterilization of Plant's Organ Surface

Leaves pieces that have been collected usually over approximately 1 cm^2. Pieces of leaves then disinfected with 70% ethanol for 1 min, sodium hypochlorite 2% for 6 minutes, 70% ethanol for 30 seconds to remove the last of sodium hypochlorite and washed with sterile distilled water [14].

Isolation and Characterization of Endophytic Bacterial Isolates

Isolation methods used in this study was refer to Zam et al. [15]. Sterile plant leaves was crushed using a sterile mortar and pestle then put in physiological saline solution 0.85% and homogenized. After it homogeneous, the bacteria was inoculated in Petri dish containing TSA medium with spread plate method, and then incubated at 27°C for 1-3 × 24 hours. Characterization of endophytic bacteria isolates carried through morphological observation of growing colonies (colony shape, color colonies, edge shape, and elevation), Gram staining and biochemical tests, which refers to Capucino and Sherman [16] and Harley and Prescott [17].

16S rRNA Analysis of Endophytic Bacteria

16S rRNA analysis for all bacteria isolates had done in Microbiology Industry Laboratories, Biotechnology Research Centre, LIPI. Base pair sequence was checked and edited by using *BioEdit Sequence Alignment Editor*. Similarity analysis was done by using *Basic Local Alignment Tool*on National Center for Biotechnology Information. Evolution analysis was done using *ClustalW2 Phylogenetic Tree.*

Antifungal Activity Test Against*Fusarium oxysporum*

Antagonistic test was done according to method by Melliawati et al. [18]. *Fusarium oxysporum* was obtained from Phytopathology Laboratory, Faculty of Agriculture, Andalas University. Each of endophytic bacterias was inoculated in*tryptic soy broth*medium for 24 hours at 27°C temperature and agitation for 120 rpm. *Fusarium oxysporum* was inoculated in petri dish which was filled with PDA medium. Then, pieces of sterile filter paper with 0.5 cm diameter that has been soaked in10 mL suspension of endophytic bacteria was pasted. Petri dishes were incubated at 30°C for 1-3 × 24 hours until the visible growth or a clear circle around the pieces of paper can be observed. The clear circles is a sign of bioactive compounds produced by the endophytic bacteria to protect themselves against attack or the growth of *Fusarium oxysporum.*

Inhibitory Effect Test of *Fusarium oxysporum* Growth

Inhibition test was done throughpoisoned food technique [19]. The results from the fermentation of endophytic bacteria that have antifungal activity was filtered using 0.02 µm membrane sterilized filter and aseptically collected in a sterile vial bottle. As much as 1 ml of the filtrate was poured using a micropipette into a sterile Petri dish, then PDA medium was added and homogenized. Once the medium is hardened then *Fusarium oxysporum* was inoculated with a diameter of 10 mm. After that, the medium and inoculation was incubated at 30°C for 3 × 24 hours. The addition of diameter was measured by using a ruler. Inhibition of growth was calculated using the formula:

$$\text{Inhibition of growth (\%)} = (C\text{-}T/C) \times 100\%,$$

Where, 'C' is increase of control colony diameter and 'T' is increase of treatment colony diameter [20].

RESULTS AND DISCUSSION

The result was showed 5 endophytic bacteria colonies by the number of cells 5.0×10^2 CFU / gr. Result of characterization showed the differences from three isolates in colony morphology and biochemical activity, while the results of microscopic observations showed the three isolates is a member of Gram-positive bacteria and has bacil cell form (Table 1 and Figure 1). Therefore, it may be stated that isolates obtained in this research were from 3 different species. The number of cells each MC1 2.0×10^2 CFU/g, MC2 1.0×10^2 CFU/g, and MC3 2.0×10^2 CFU/g (Table 1 and Figure 2). Results of research by Yuliar et al. showed that the number of isolates obtained from plant sources ranged between 1-6 isolates and isolates obtained from *Morinda citrifolia* L. was 3 isolates [21]. Shu-Mei et al. reported that endophytic bacteria cells isolated from soybean plants was ranged for 1.4×10^2 - 3.4×10^3 [22].

The result obtained showed that recent research is still in range of result from the previous research. This condition showed that the number of isolates and endophytic bacteria cells that can be isolated is very low. The presence of endophytic bacteria in host plants can occur throughout or in part of their life cycle [23]. Based on this condition, endophytic bacteria can be grouped into two, those are obligate and facultative [24]. Facultative endophytic bacteria are able to survive in the ground, plant surface, inside the plants and synthetic nutrition. Endophytic which live inside plant tissues during its whole life is called as obligate endophytic [25]. In this study, all of the isolates obtained are the member of Gram-positive bacteria. This suggests that bacteria from this group are able to penetrate and colonize in the plant tissue. Bacterial colonization occurs through the roots as plant roots release its excudate, and the exudate acts as chemoatractan for bacteria which are located around the plant, so at first the bacteria will form the colony at the root surface [26]. Bacteria that were originally attached to the roots surface will penetrate through wounds in the roots, or through the cavity located at the base of lateral roots, or by degrading the root's cell walls using enzymes endoglucanase and endopoligalakturonase [27], then it will spread through the xylem tissue [26].In addition, the source of bacteria can be from the aerial and attached to the surface of the organ then penetrate through wounds, intercellular space, and stomata on leaves [28]. From isolation and amplification of 16S rRNA fragments using PCR in Microbiology Laboratory of Industrial Biotechnology Research Center, LIPI was obtained 1500 bp fragment size (Figure 3). The results of BLAST analysis towards sequences of fragments of 16S rRNA showed that isolateMC1 is *Bacillus subtilis* sub sp. *Inaquosorum* MER_77 (KT719652.1) with similarity 99%, MC2 is *Bacillus* sp. SG3-2 (KP992136.1)with similarity 99%, and MC3 is *Bacillus* sp. CC-YY22 (KU534259.1) with similarity 99% (Table 2). *Bacillus* is a dominant genera of endophytic bacteria found in plants that have been studied Y Liu et al. [29]. The composition of endophytic bacteria obtained in this study is different from the composition of endophytic bacteria results of research conducted by Liu et al. [30]. Differences in the composition of endophytic bacteria can be caused by environmental factors. It is because the isolates come from different source plants in different environments. XX Gao et al. [31] declared that environmental factors are one of the factors that affect the composition of endophytic bacteria in a plant. In addition, the number and composition of endophytic bacteria in a plant can be fluctuating. Therefore the number and composition of endophytic bacteria on the same plant can vary TM Madigan et al. [32]. Phylogenetic analysis performed using *ClustalW2 Phylogenetic Tree* (www.ebi.ac.uk), the result showed that *Bacillus subtilis* subsp. *inaquosorum* MER_77 and *Bacillus* sp. CC-YY22 has a very close genetic relationship. The results of the analysis *Pairwise Sequence Alignment*showed was showed that both bacteria have a similarity of 99.5%. In this analysis,the outgroup used was *Pantoea stewartii* M073. Very close genetic relationship allows both isolates have nearly the same physiological mechanisms [33], so the metabolites produced also have a similar structure.

From three isolates obtained, only two isolates that have antifungal activity against *Fusarium oxysporum*, those are *Bacillus subtilis* sub sp. *Inaquosorum*MER_77 and *Bacillus* sp. CC-YY22 with consecutive inhibitory effect 76.7% and 33.3%, whereas *Bacillus* sp. SG3-2 did not have antifungal activity against *Fusarium oxysporum* (Table 3 and Figures 4-6). Endophytic bacteria's ability to inhibit the growth of pathogens can be through the parasitic mechanism, lysis enzymes activity such as chitinase and protease [34], as well as produce antibiotic compounds [35,36]. Genus *Bacillus* are known to have this ability, therefore this genus is able to be a potential biocontrol agent [37]. Members of this genus, such as *Bacillus subtilis* had been reported to produces a lipopeptide antibiotic compound [38] and E2 antifungal protein compounds [39], both of these compounds have strong antifungal activity against *Fusarium oxysporum*. Results of research conducted by Souza et al. [40] also showed that *Bacillus subtilis* subsp. *Subtilis* isolated from banana plants also have antifungal activity against *Fusariumoxysporum* [40]. It is also reinforced by isolates of *Bacillus subtilis* 55C1-1 endophytic bacteria isolated from leaves of *Citrus aurantifolia* Swingle have antifungal activity against *Fusariumoxysporum*. Based on this information, it can be stated that *Bacillus subtilis* is one species of endophytic bacteria that can be

developed as a biocontrol agent to controlling *Fusarium oxysporum.* Therefore, more research in laboratory scale and field scale need to be donein order to obtain data that strongly support the application of these microbes towards plants.

Table 1: The observation for characteristic of endophytic bacteria isolates

Isolates Code	Number of Cells (CFU/gr)	Colony Morphology	Microscopic Observation	Biochemical Test															
				Glucose Fe1rmentation	Lactose Fermentation	Manitol Fermentation	H_2S Production	Nitrat Reduction	Indol Production	Methyl Red	VogesPraskruer	Citrate Utilization	Urease	Catalase	Gelatin Hydrolysis	Lipid Hydrolysis	Starch Hydrolysis	Casein Hydrolysis	Motility	Litmus Milk
MC 1	2.0×10^2	Irregular; Raised; Undulate; White	Gram +; Bacil	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	+	LR
MC 2	1.0×10^2	Circular; Umbonate; Erose; Yellow	Gram +; Bacil	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	+	ACGLR
MC 3	2.0×10^2	Punctiform; Raised; Undulate; White	Gram +; Bacil	+	+	+	-	-	-	-	-	+	-	+	+	-	+	-	+	LR

*Abbreviation: LR = Litmus Reduction; ACGRL: Acid, curd, gasand litmus reduction + : Positive result; - : Negative result

Table 2: Result of 16S rRNA sequence analysis from endophytic bacteria isolates

No	Isolates Code	16S rRNA Sequence	Result of BLAST NCBIand Homology
1	MC 1	CGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTA AGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAA AGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAA GGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC GGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAA GGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGT ACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCG GAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGG AGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGC GTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGC GTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGT TTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTA CCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCA TGGTTGTCGTCAGCTCGTGTCCTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGC CAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATC ATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGG TTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCG CTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACG AGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTAGGAGCCAGCCGCCGAAGGTGGGACAGATGAT	*Bacillussubtilis* subsp. *inaquosorum* MER_77 (KT719652.1) 99%
2	MC 2	GCAGTCGAGCGGACCTCTTCGGAGGTCAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGA CTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATACTATGTCAAACCGCATGGTTTGACATTCAAAGA CGGTTTCGGCTGTCACTTACAGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGC GACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG AGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTT TTCGGATCGTAAAACTCTGTTGTCAGGGAAGAACAAGTGCCGGAGTAACTGCCGGCGCCTTGACGGTACCTG ACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATT ATTGGGCGTAAAGCGCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGT	*Bacillus* sp. SG3-2 (KP992136.1)

		CATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGA GATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGG GAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCG CCCTTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAG GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAG GTCTTGACATCCTTTGCCACTTCTAGAGATAGAAGGTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATG GTTGTCGTCAGCTCGTGTCCTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCC AGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCA TCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCGAGACCGCGAGGTT TAGCCAATCCCATAAAACCATTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCTGGAATCGCT AGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAG AGTTTGCAACACCCGAAGTCGGTGGGGTAACCGCAAGGAGCCAGCCGCCTAAGGT	99%
3	MC3	GGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAA GACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAA GGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAG GCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAG GTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTA CCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG AATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGA GGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCG TAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGGAGCGAAAGCG TGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTT TCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCA AAGGAATTGACGGGGGCCCGCACAACCGGTGGAACATGTGGTTTAATTCAAAGCAACGCGAAGAACCTTAC CAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAGGTGGTGCAT GGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGC CAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATC ATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACAGAACAAAGGGCAGCGAAACCGCGAGG TTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCG CTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACG AGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTAGGAGCCAGCCGCCGAAGGTGGGACAGATG	*Bacillus* sp. CC-YY22 (KU534259.1) 99%

Table 3: Observation result of antifungal activity and inhibitory effect against *Fusarium oxysporum*

No.	Isolate	Antifungal Activity	Inhibitory Effect (%)
1	*Bacillus subtilis* sub sp. *inaquosorum* MER_77	+	76.7
2	*Bacillus* sp. SG3-2	-	AnD
3	*Bacillus* sp. CC-YY22	+	33.3

*Abbreviation: + : Showed antifungal activity, - : No antifungal activity, AnD: Are not done

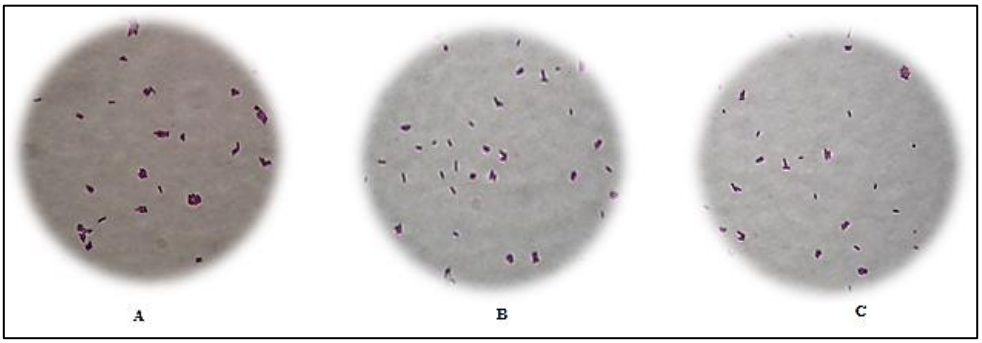

Figure 1: Microscopic characteristic of endophytic bacteria isolates with 1000x magnification (A=MC1; B=MC2; and C=MC3)

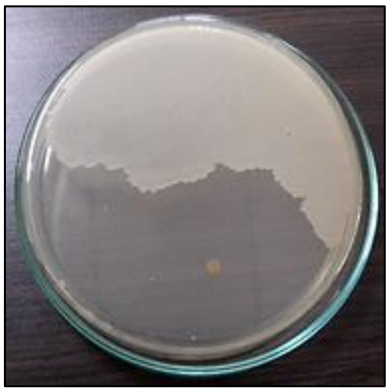

Figure 2: Isolate of endophytic bacteria

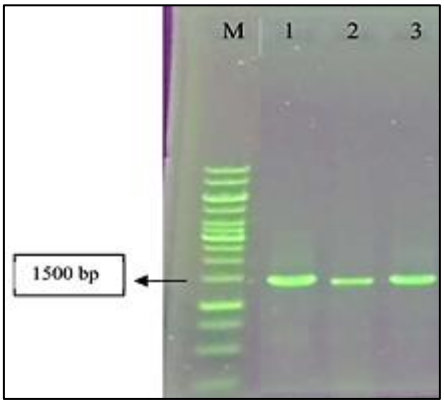

Figure 3: PCR purification result from 16S rRNA of endophytic bacteria (M=marker; 1=MC1; 2=MC2; 3=MC3)

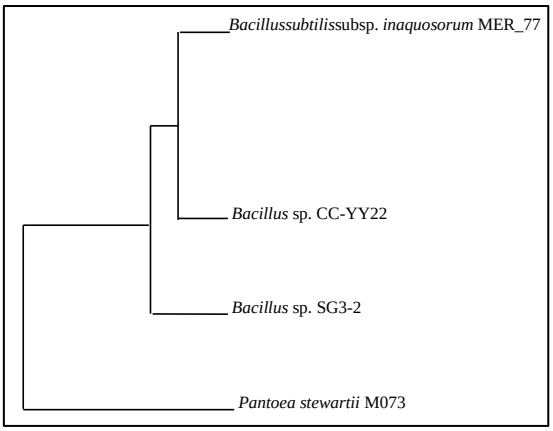

Figure 4: Phylogenetic tree of endophytic bacteria from 16S rRNA sequence

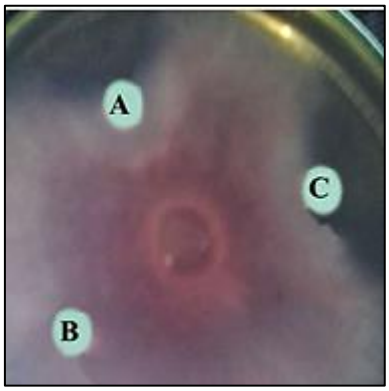

Figure 5: Antifungal activity against *Fusarium oxysporum* (A=*Bacillussubtilis* sub sp. Inaquosorum MER_77; B=*Bacillus* sp. SG3-2; and C=*Bacillus* sp. CC-YY22)

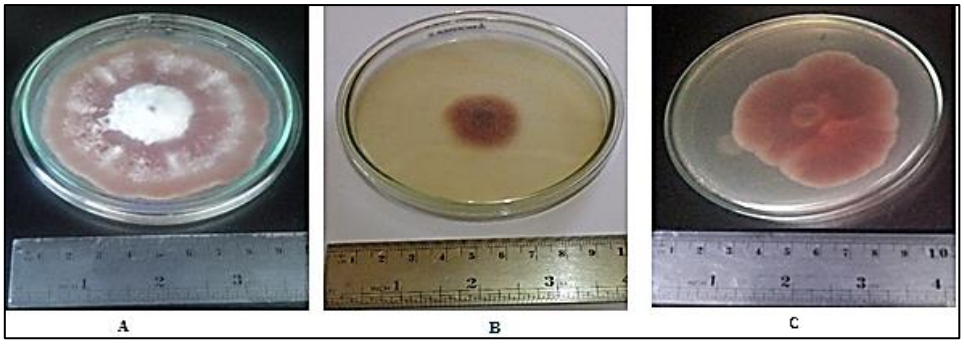

Figure 6: Inhibitory effect against *Fusarium oxysporum* (A=control; B=*Bacillus subtilis* sub sp. *inaquosorum* MER_77; and C=*Bacillus* sp. CC-YY22)

CONCLUSION

Three isolates was obtained, those are MC1 (2.0×10^2 CFU/g), MC2 (1.0×10^2 CFU/g), and MC3 (2.0×10^2 CFU/g). Results of identification by using analysis of 16S rRNA showed three consecutive isolate is *Bacillus subtilis* subsp. *Inaquosorum*MER_77, *Bacillus* sp. SG3-2, and *Bacillus* sp. CC-YY22. *Bacillus subtilis* subsp. *Inaquosorum* MER_77 and *Bacillus* sp. CC-YY22 showed antifungal activity against *Fusarium oxysporum* with inhibitory effect respectively 76.7% and 33.3%, while *Bacillus* sp. SG3-2 did not showed antifungal activity against *Fusarium oxysporum*.

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