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Antibacterial Activity of Endophytic Fungi Isolated From *Portulaca Oleracea L*

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Abstract. *Portulaca oleracea L* is an annual grassy plant that is distributed in many parts of the world. Endophytes represent a complex community of microorganisms colonizing asymptomatically internal tissues of higher plants. Several reports have shown that endophytes enhance the fitness of their host plants by direct production of bioactive secondary metabolites. This study investigated the antibacterial activity of endophytes isolated from *Portulaca Oleracea L.* Isolation was carried out on *potato dextrose agar chloramphenicol*. Antibacterial activity using disc diffusion methods. Isolation results obtained as many as 8 endophytic fungi isolate with code IFK 1-IFK 8 isolates. The antibacterial activity test showed that the isolates with the IFK 6 had the best activity in inhibiting the growth of Staphylococcus aureus (14,03 mm) and Escherichia coli (15,93 mm). Then isolate IFK6 identified by PCR test. The results showed that the molecular characteristics of *Portulaca oleracea* isolates of IFK6 were genus *Aspergillus Sp* had similar species based on the results of sequencing, namely *Aspergillus* versicolor. Based on macroscopic and molecular characteristics, IFK6 isolates had similarities with the fungus Aspergillus versicolor and has antibacterial activity against Staphylococcus aureus and Escherichia coli.

INTRODUCTION

Endophytic fungi are present in plant tissue without causing infection[1]. It is estimated that every single living plant species is associated with about 1-4 types of endophytic fungi[2]. Endophytes had metabolic impacts on host plants, further suggests the possibility of regulating the biochemical status of host plants with fungal endophytes [3]. They also possess the potential to produce unique secondary metabolites, which can be exploited in pharmaceutical, agricultural and other industries. Thus, there is a growing interest of researchers in bioprospecting of endophytic microbial communities inhabiting the plants from various ecosystems [4].

Parts of plants that can be isolated to obtain endophytic fungi are the leaves, seeds, flowers, fruit stems[5]. Isolation of endophytic fungi from their host plants is one strategy that can be used to optimize the benefits of these endophytic fungi. One of them is the plant Portulaca oleracea L.

Portulaca has been widely distributed in other temperate and tropical areas of the world [6,7]. As a folk medicine, Portulaca oleracea has been used in many countries, such as a febrifuge, antiseptic, vermifuge, and so forth [8]. It shows various effects of pharmacology, including antimicrobe [9], and anti-inflammatory [10]. The chemical ingredients contained in Portulaca oleracea are fatty acids, terpenes, alkaloids, coumarins, flavonoids, and essential oils [7].

Flavonoids are one of the most abundant and important active constituents of Portulaca oleracea Kaempferol and apigenin have been mainly isolated from leaf and stem [11] Several alkaloids have been isolated from different parts of Portulaca oleracea such as Dopamine, noradrenaline [12]. Portulaca oleracea has a high potential to be utilized as a pharmacological agent in medicine. Moreover, knowledge about the endophytic fungal community in Portulaca oleracea is insufficient. The purpose of this study is to investigate the antibacterial activity of endophytes isolated from *Portulaca Oleracea L.*

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RESEARCH METHOD

This study has been workde in a Laboratory of Microbiology Faculty of Pharmacy, Universitas Muslim Indonesia. The sample used was Portulaca Oleracea leaves.

Isolation and culturing of endophytic Fungi

Fresh and healthy green leaves were selected. The plant samples were stored in the sealed plastic bags at 4°C until processed. Healthy mature leaves of Portulaca oleracea L were washed thoroughly under running tap water, then the samples were sterilized by dipping them in 75% ethanol for 30 s, followed by immersing in 3% sodium hypochlorite for several times, rinsed in sterile distilled water, and finally dried on sterile filter paper on a petri dish. A piece of each leaf was removed with a sterile scalpel then cut into small pieces about 1 cm, each piece was put on a Petri plate containing Potato Dextrose Agar (PDA) medium + Chloramphenicol and incubated at room temperature (27-28°C) to promote fungal growth and sporulation. After 3 days, individual hyphal tips of actively growing fungi were picked up for subculturing by inoculating it onto new PDA medium plate individually and incubated at room temperature (27ºC) for one week. The purified fungal isolates were labeled for further use.

Antibacterial Activity

The endophytic fungi isolates were cut into small ±1 cm pieces. Samples were placed on the surface of the Nutrient Agar (NA) medium containing the tested bacteria. Furthermore, it was incubated for 1 x 24 hours at 37ºC. Each isolate was observed for its ability to inhibit bacterial growth.

Identification of endophytic fungal isolates.

Identification was based on morphological characteristics such as color of the colony and medium, margin character, configuration, and elevation. Obtained data were then compared with the descriptions of endophytic fungi and identified based on Refai [13] .

DNA Extraction

DNA extraction is carried out to separate the DNA genome from other molecules in the cell. The kits used in this study were kit Quick DNATM fungal/bacterial miniprep (Zymo Research, D6005).

Amplification DNA

The target regions of ITS rDNA were amplified by PCR (95oC for 3min, followed by 35 cycles of 94ºC for 30 sec, 52oC for 30 sec, and 72ºC for 30 sec, and final elongation at 72ºC for 10min) using the following primers: ITS1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') [24]. Amplifications were performed using My Taq HS Red Mix (Bioline, BIO 25047). Positive and negative controls (no DNA template) were included in all experiments. The PCR products were tested in 1.2% (w/v) agarose gel. The sequencing process was carried out by first base through Genetics Science. The sequencing was carried out in a "Single Pass DNA Sequencing" which uses the same primers as amplification genes on the PCR process. The sequencing results are processed using Bioedit application. BLASTn search of the nucleotide reference database (http://blast.ncbi.nlm.nih.gov/).

RESULT AND DISCUSSION

The results of isolation endophytic fungi Portulaca oleracea showed 8 isolates of endophytic fungi with different morphologies. Endophytic fungi that have grown on PDAC isolation media are then gradually purified one by one. Each pure endophytic fungus isolate was transferred to PDA media in Petri dishes. This purification aims to separate endophytic colonies with different morphologies to become separate isolates. Endophytic fungi from Portucala oleracea can be seen in figure 1.

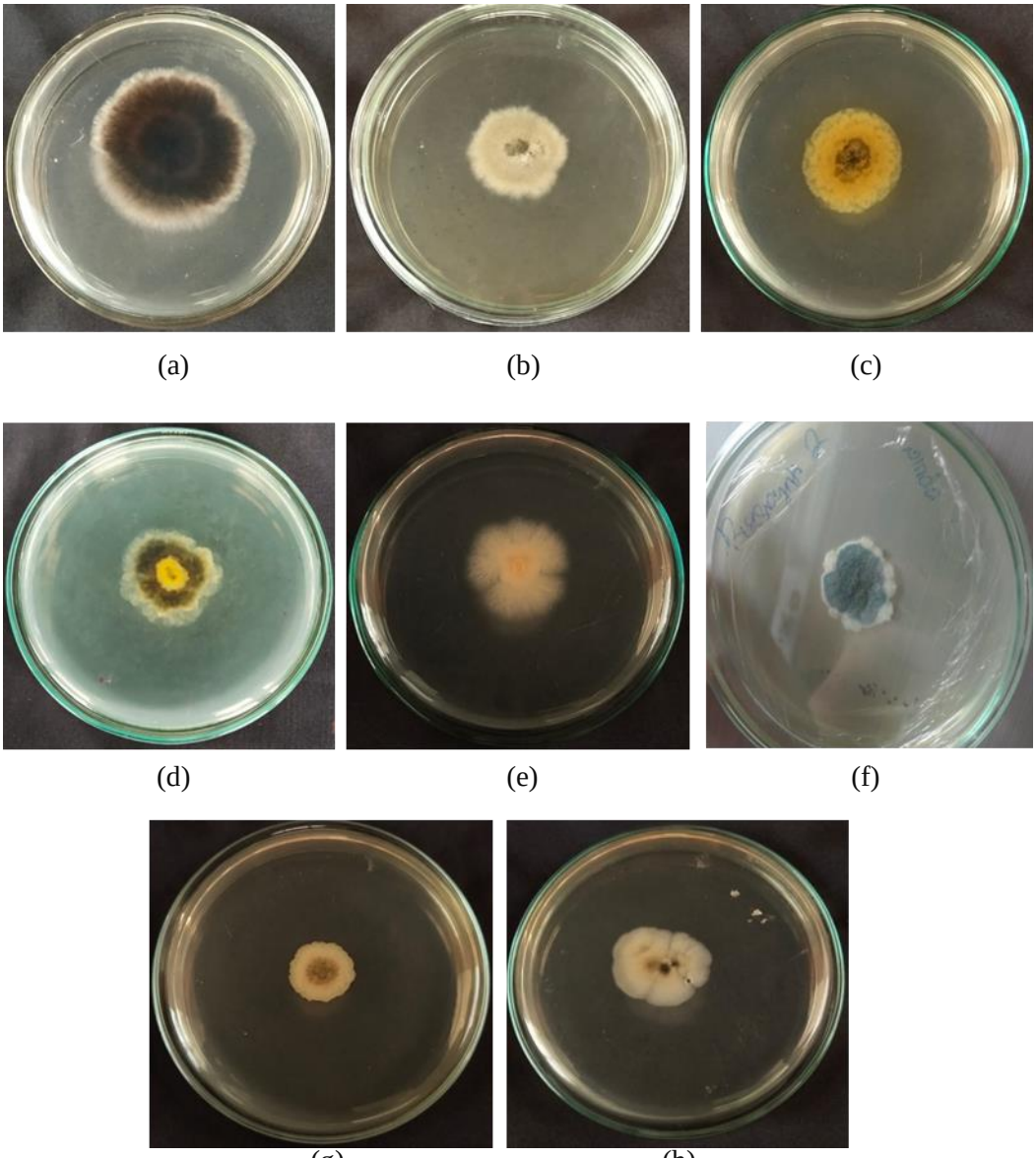


FIGURE 1. Endophytic fungi isolate Portulaca oleracea L. (a) IFK 1, (b) IFK 2, (c) IFK 3, (d) IFK 4, (e) IFK 5, (f) IFK 6, (g) IFK 7, (h) IFK 8

Results of the antibacterial activity test showed that the isolates with the IFK code 6 had the best activity in inhibiting the growth of Staphylococcus aureus and Escherichia coli bacteria which was characterized by the formation of the largest diameter of the inhibition zone. The results can be seen in figures 2.

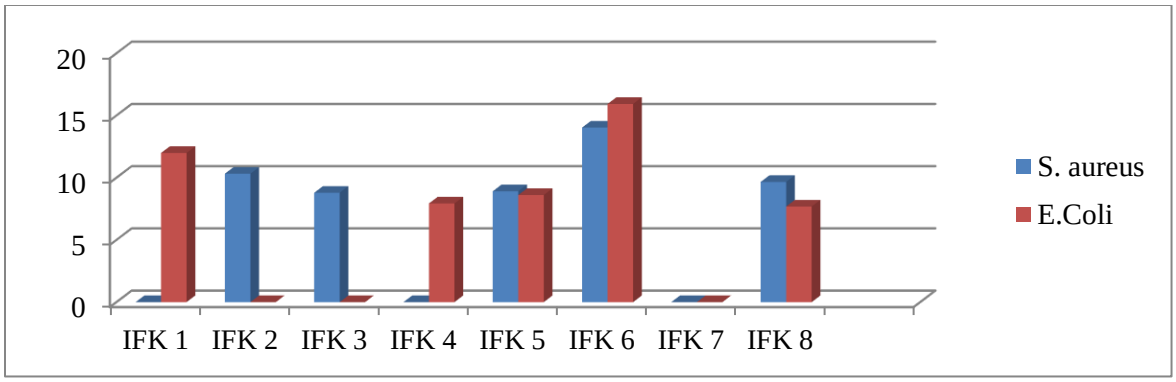
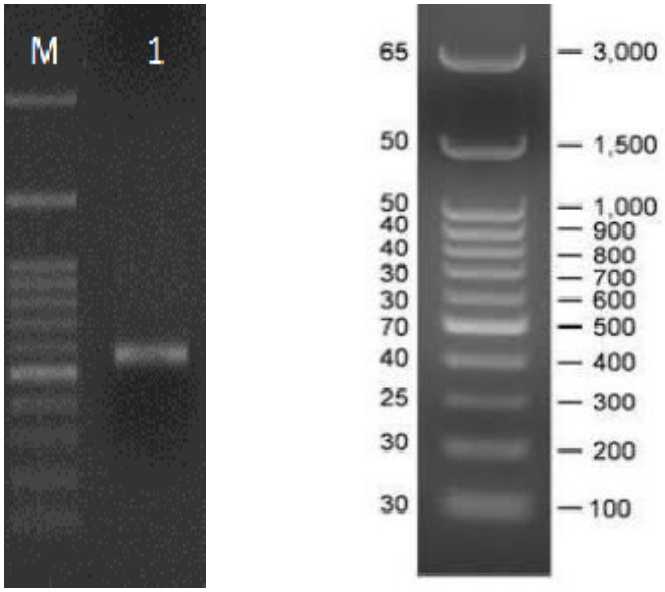


FIGURE 2. Antibacterial activity of endophytic fungi isolated from Portulaca oleracea L

Based on macroscopic characteristics, it was found that the colony color that grew on PDA medium was grayish-green. The colony color obtained was in accordance with the research conducted by Refai, 2014 that the Aspergillus versicolor colony was grayish-green and beige with white colony border color. The color of the lower surface of this mold colony is brownish yellow. This fungus has a smooth velvety texture, while the upper surface looks like a colony having a radial groove in the middle like the spokes of a bicycle tire. Colonies grow rapidly on PDA media at room temperature. From these characteristics, the colony color obtained was in accordance with the colony morphology of the Aspergillus Versicolor species.

Figure 3 shows the results of electrophoresis of IFK6 isolates. Based on Figure 3, amplification uses primary pairs of ITS1 (5'-TCC GTA GGT GAACCT GCG G-3 ') as forward, and primer ITS4 (5'-TCCTCC GCT TAT TGA TAT GC-3') as reverse. The visible results of electrophoresis are the formation of bands that are amplified DNA fragments and showpieces of the base. The results displayed using UV light show a clearly visible band at 543 bp.



(a) (b)

FIGURE 3. Electrophoresis of IFK6 isolates obtained a band of 543 bp

Figure 4 Shows the results of DNA sequencing. of Portulaca oleracea endophytic fungal isolates with the IFK6 code. DNA sequencing is a method used to determine the sequence of nucleotides or bases in a DNA fragment.

```
1       ACCTGCGGAA GGATCATTAC TGAGTGCGGG CTGCCTCCGG GCGCCCAACC TCCCACCCGT
61      GAATACCTAA CACTGTTGCT TCGGCGGGGA ACCCCCTCGG GGGCGAGCCG CCGGGGACTA
121     CTGAACTTCA TGCCTGAGAG TGATGCAGTC TGAGTCTGAA TATAAAATCA GTCAAAACTT
181     TCAACAATGG ATCTCTTGGT TCCGGCATCG ATGAAGAACG CAGCGAACTG CGATAAGTAA
241     TGTGAATTGC AGAATTCAGT GAATCATCGA GTCTTTGAAC GCACATTGCG CCCCCTGGCA
301     TTCCGGGGGG CATGCCTGTC CGAGCGTCAT TGCTGCCCAT CAAGCCCGGC TTGTGTGTTG
361     GGTCGTCGTC CCCCCCGGGG GACGGGCCCG AAAGGCAGCG GCGGCACCGT GTCCGGTCCT
421     CGAGCGTATG GGGCTTTGTC ACCCGCTCGA CTAGGGCCGG CCGGGCGCCA GCCGACGTCT
481     CCAACCATTT TTCTTCAGGT TGACCTCGGA TCAGGTAGGG ATACCCGCTG AACTTAAGCA
541     TAT
```

FIGURE 4. Sequence isolates of IFK6 Portulaca oleracea L.

Endophytic fungi isolated from medicinal plants may produce metabolites that are similar to their hosts and have been shown to be a rich source of precious bioactive compounds. Endophytes are ubiquitous organisms that are valued for their ability to synthesize various functional natural products [4,14,15] One of the plants that have antimicrobial benefits is portulaca oleracea. Research on the benefits of Portulaca oleracea has been developed, one of which is the antimicrobial and antifungal effects of Portulaca oleracea [9]. Biologically active compounds portulaca oleracea such as flavonoids (Apigenin, kaempferol, quercetin, luteolin, myricetin, genistein, and genistin), Alkaloids, Coumarins, anthraquinone glycoside, cardiac glycoside, and high content of ω-3 fatty acids.

In our course of finding antibacterial endophytes of Portulaca oleracea L, Eight fungal strains were isolated. Then the isolates were tested for antibacterial activity against Staphylococcus aureus and Escherichia coli bacteria. The results of the antibacterial activity test showed that IFK 6 was the isolate that had the greatest inhibition zone against Staphylococcus aueus and Escherichia coli test bacteria.

The amplification stage was carried out using the MyTaq HS Red Mix kit (Bioline, BIO-25047). The amplification stage using PCR aims to multiply isolated DNA fragments. In this amplification stage, the primer pairs ITS1 (5'-TCC GTA GGT GAACCT GCG G-3 ') are used as forward, and ITS4 primers (5'-TCCTCC GCT TAT TGA TAT GC-3') as reverse. This primer will amplify fungal DNA located at the ITS locus which is a conservative area for the fungal kingdom [16].

Based on the results of macroscopic and molecular tests, it can be identified that the IFK6 isolate has similarities with the Aspergillus Versicolor species. Aspergillus Versicolor produces new quinolone derivative compounds, namely aniduquinolones which have antibacterial activity against the Staphylococcus aureus bacteria [17].

CONCLUSION

The isolation results from Portulaca oleracea L obtained 8 isolates and IFK6 isolates were the isolates that had the largest inhibition activity. Macroscopic and molecular characteristics (PCR) obtained from the IFK6 endophytic fungal isolate were Aspergillus vesicolor. Based on macroscopic characteristics, it was found that the colony color that grew on PDA medium was grayish-green. The color of the lower surface of this mold colony is brownish yellow. From these characteristics, the colony color obtained was in accordance with the colony morphology of the Aspergillus Versicolor species. The visible results of electrophoresis are the formation of bands that are amplified DNA fragments and showpieces of the base.The results displayed using UV light show a clearly visible band at 543 bp.

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