The potency of culturable fungi from tidal and non-tidal swamplands in Indonesia

D N Susilowati¹, S Widawati², Suliasih², N Arrofaha³, N Radiastuti³ and E Yuniarti⁴

¹ Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development, Jl. Tentara Pelajar 3A Bogor 16111, West Java, Indonesia
² Research Center for Biology, Research Organization for Life Science, National Research and Innovation Agency (BRIN) Bogor 16911, West Java, Indonesia
³ Faculty of Science and Technology, Syarif Hidayatullah State Islamic University Jakarta, Ciputat 15412, Banten, Indonesia
⁴ Indonesian Soil Research Institute, Jl. Tentara Pelajar 12 Bogor 16111, West Java, Indonesia

*Corresponding author e-mail: d_nengsus@yahoo.com

Abstract. Tidal and non tidal swampland in Indonesia is a relatively large sub-optimal land resource and has not been used optimally for agricultural land due to various problems. Technological innovation for swamp land, especially for fertilization (biofertilizer) must pay attention to the indigenous microbial community, so that the application of biofertilizer can be effective. The aim of this research was to culturable fungal diversity and their potential of the tidal swamp soil in South Kalimantan and non tidal swamp soil in South Sumatra. The research was conducted in the Microbiology laboratory – ICABIOGRAD. Two samples were isolated from tidal and non tidal swamp soils in South Kalimantan and South Sumatra. Serial dilution methods and molecular identification were used to assess the diversity of fungal species. Production of IAA, siderophores, and ACC Deaminase was evaluated. Results showed there are 11 species consisting of Trichoderma asperellum KS.26.1, Penicillium sp. KS.26.2, T.asperellum KS.26.3, P.simplicissimum SS.27.1, P.janthinellum SS.27.2, Talaromyces calidicanius SS.27.3, P.citrioviride SS.27.4, P.citrioviride SS.27.5, P.citrioviride SS.27.1 PDA, P.citrioviride SS.27.2 PDA, P.citrioviride SS.27.3 PDA were identified from both tidal and non-tidal swamplands. Potential analysis showed that all fungal isolates were able to produce IAA, siderophores, and ACC Deaminase. The fungal diversity was higher in the non-tidal swampland than the tidal swampland as revealed by the differences in percentage occurrence of fungal species. There are potential indigenous fungal in the swampland to be used to improve productivity of the soils.

Keywords: swampland, siderophore, ACC deaminase, IAA.

1. Introduction
Swampland in Indonesia is a relatively large sub-optimal land resource and has not been used optimally. The typology of swampland consists of tidal and non tidal swampland which has the potential for development as agricultural land, especially based on food producers [1]. Tidal swamp land is land that is influenced by the ebb and flow of water or rivers. Moreover non tidal swamp land is land that is inundated with water for a certain period (at least one month) and the water is affected by rainfall, upstream and underground flooding both locally and in the surrounding area.
Swampland in Indonesia is recorded as ± 34.93 million ha or 18.28% of Indonesia’s land area, most of which are located on two islands, namely Sumatra ± 12.93 million ha and Kalimantan ± 10.02 million ha [2]. Swamp land in Kalimantan, especially in South Kalimantan, has an area of 4,969,824 ha, of which consists of tidal swamp land [1]. Most of the swamp land on the island of Sumatra is in South Sumatra which consists of non tidal swamp land covering an area of 285,941 ha [3]. The extent of swamp land in South Kalimantan and South Sumatra is the background for the need for efficient management of swamp land to increase agricultural production land.

Management of tidal and non tidal swamp land to be developed as agricultural land is faced with various problems. The biophysical problems of tidal swamp land for the cultivation of food crops include soils that have low fertility levels, high soil acidity, the presence of pyrite, high levels of Al, Fe, Mn, and organic acids, P deficiency, low base cations such as Ca, K, Mg, and suppression of microbial activity. The risk of drought in the dry season, flooding in the rainy season, low fertility and productivity are biophysical problems of lowland swamp land. These conditions lead to a narrower planting period and the types of plants cultivated [4].

Several solutions to overcome swampland problems include soil and water management, development of agricultural machinery for swamps, improvement of cultivation technology such as the use of adaptive varieties of swampland and fertilization technology [5]. Technological innovation for swampland, especially for fertilization such as biological fertilization, must pay attention to the indigenous microbial community on land so that the application of biological fertilizer technology can be effective. The existence of different soil types from the two types of swampland will affect the structure of the microbial community where the microbial composition will have differences from one location to another [6]. Thus, it is necessary to identify indigenous microbes, in this case fungi, from both types of swampland, both cultivating and non-cultivating.

Fungi can improve the biological quality of the land. Some fungi can play a role in breaking down cellulose, fixing nitrogen, dissolving phosphorus, producing siderophores, producing ACC deaminase enzymes, controlling high salinity stress, increasing plant growth substances, and producing phytohormones indole acetic acid. The higher the number and types of microbes in land can be an indication that the land is on the outskirts with sufficient availability of organic matter in the soil, appropriate temperature, adequate air availability, and supportive soil ecological conditions [7].

The potential of fungi is quite large in improving agricultural ecosystems, so a diversity of fungi is needed that contributes to increasing soil and plant productivity. Various methods have been applied to analyze the genetic diversity of fungi in a field, namely growing fungi that can be cultured.

The aim of this study was to analyze the fertility of the tidal swampland of South Kalimantan and non tidal swampland of South Sumatera based on the diversity and composition of the fungal community and the potential of fungi to produce siderophores, IAA hormones and ACC deaminase enzymes.

2. Materials and Methods
2.1. Material
The materials used in this study were soil samples from tidal swamps in South Kalimantan and non-tidal swamps in South Sumatera with soil physical and chemical properties in Table 1, aquades, potato dextrose agar (PDA), potato dextrose broth (PDB), rose bengal, indole acetic acid (IAA), Salkowski reagent, Dworkin-Foster (DF) minimal medium, ammonium sulfate, 1-aminocyclopropane-1-carboxylate (ACC), Fe-Crome azurol sulfate (Fe-CAS/ C₃H₃C₁₂O₅SNa₃) medium KIT Nucleon-Phytopure (Illustra DNA Extraction KIT PhytopureTM), Tris-Acetic acid-EDTA (TAE) buffer, 1x, chloroform, isopropanol, ethanol 70%, IHC1 0,1 N, NaOH 0,1 N, GoTag® Green Master Mix (Promega), loading dye (Promega), nuclease free water (NFW), DNA ladder 100 bp, primer reverse ITS 4 (5’TCC TCC GCT TAT TGA TAT GC 3’) dan primer forward ITS 5 (5’GGA AGT AAA AGT CGT ACG AAG G3’), NaCl 0,85%, agarose, and 1,5 % gel red.
Table 1. Physical and chemical properties of tidal swampland in South Kalimantan and non swampland in South Sumatra.

| Soil Properties          | Tidal swampland from South Kalimantan (KS) | Non-tidal swampland from South Sumatra (SS) |
|--------------------------|--------------------------------------------|-------------------------------------------|
| Texture                  |                                            |                                           |
| -Sand (%)                | 0                                          | 0                                         |
| -Silt (%)                | 39                                         | 46                                        |
| -Clay (%)                | 61                                         | 54                                        |
| pH (H2O)                 | 3.15                                       | 4.05                                      |
| Organic matter           |                                            |                                           |
| C (%)                    | 15.85                                      | 7.22                                      |
| N (%)                    | 0.48                                       | 0.54                                      |
| C/N (me/100 g)           | 33.02                                      | 13.37                                     |
| P2O5 (mg/100 g)          | 132.32                                     | 24.53                                     |
| K2O (mg/100 g)           | 5.07                                       | 9.10                                      |
| Cation Exchange Rate     |                                            |                                           |
| -Ca (cmole/kg)           | 1.29                                       | 4.89                                      |
| -Mg (cmole/kg)           | 4.98                                       | 2.37                                      |
| -K (cmole/kg)            | 0.10                                       | 0.16                                      |
| -Na (cmole/kg)           | 2.81                                       | 0.22                                      |
| Cation Exchange Capacity - | 88.44                                      | 48.19                                     |
| CEC (me/100 g)           |                                            |                                           |
| Base Saturation - BS (%) | 10.38                                      | 15.85                                     |
| Al3+ (cmole/kg)          | 25.34                                      | 7.50                                      |
| H+ (cmole/kg)            | 2.28                                       | 0.36                                      |

2.2. Equipment
The equipment used including analytical balance (Scaltec), autoclave, shaker, vortex, oven, incubator, microcentrifuge (Microfuge 22R Beckman CoulterTM), laminar airflow, hot plate (Cimarec), pH meter (Metrohm), spin down, refrigerator, freezer -20º C, Eppendorf tube, nanodrop (Thermo), thermal cycler (Swift Maxi ESCO), horizontal electrophoresis (Mupid-EXU Sub Marine Electrophoresis System), spectrophotometer UV-Vis (Hitachi U-2800) and UV transilluminator.

2.3. Procedure
2.3.1. Soil Sampling
The soils were sampled from two locations, namely (i) tidal swamp at Jerapat Baru Village, Tamban Subdistrict, Barito Kuala Regency, South Kalimantan Province (03° 14’ 47.2” LS - 114° 30’ 44.9” BT) and (ii) non-tidal swamp at Tapus Village, Pampangan Subdistrict, Ogan Komering Ilir Regency, South Sumatra Province (03° 13’ 00.0” LS - 104° 58’ 23.2” BT) by using simple random sampling. The samples were taken at the end of 2019 with a depth of ± 20 cm each from both locations.

2.3.2. Fungal Isolation and Purification
The soil as much as 10 grams was diluted with 0.85% NaCl solution 90 ml and then it was diluted until a dilution 10⁻⁵. Soil samples were homogenized and 100µl of the solution was transferred into a plate with PDA and Rose Bengal medium (0.5 g MgSO₄.7H₂O; 10 g glucose; 1 g KH₂PO₄; 5 g peptone; 25 g bacto agar; aquades 1000 mL). Incubation was done at 27º C for 5-7 days. After the incubation period was completed, the fungal colonies growing on rose bengal media were purified. Fungal colonies obtained from isolation with different characteristics based on morphological appearances such as color, colony shape and colony surface, were further purified by transferring the fungal isolates to rose bengal
media and incubated at 27º C for 5-7 days. The pure isolate was then transferred to PDA media for further testing.

2.3.3. Molecular Identification of Fungal Isolation of fungal DNA samples using KIT Nucleon-Phytopure (Illustra DNA Extraction KIT PhytopureTM) consisting of Reagent I, Reagent II, and Resin according to the procedure according to the kit protocol. DNA concentration and purity were measured using nanodrops. Amplification of fungal is carried out by thermal cycler (with stages such as initial denaturation at 94° C for 5 minutes, denaturation at 94° C for 1 minute, then annealing at 55° C for 1 minute, extension at 72° C for 2 minutes, final extension at 72° C for 10 minutes and the storage temperature at the final stage was 4° C. The PCR amplification process lasted for 35 cycles) with reaction mixture including the master mix (dNTP, DNA Polymerase enzyme, enzyme buffer), ITS 4 primer pair as a reverse primer (5´TCC TCC GCT TAT TGA TAT GC 3´) and ITS 5 (5´-GGA AGT AAA AGT CGT AAC AAG G-3’) as a forward primer and NFW was prepared by mixing homogeneously with the following compositions: 8.5 L ddH2O (NFW), 12.5 L Go Taq Green Master Mix 2x, 1 L ITS primer 4, 1 L primer ITS 5 and 2 L of DNA were isolated so that the total volume was 25 L. And then, the results of DNA amplification were migrated through electrophoresis apparatus, continued with DNA visualization and sequenced at PT Genetika Science.

2.3.4. IAA-Producing Fungi Potential Test Fungal isolate was grown on 9 ml PDB media that enriched with L-tryptophan for 7 days at 27°C. Furthermore, 5 mL of fungal culture was centrifuged at 6,000 x g at 4°C for 15 minutes and 2 ml of supernatant was taken and transferred to a test tube to add 4 mL of Salkowski reagent (150 mL concentrated H2SO4; 250 mL distilled water; 7.5 mL 0.5 M FeCl3.6H2O) incubated for 60 minutes at 28°C. Fungal that produce IAA in the supernatant were pink. The intensity of the color in the supernatant was measured using a spectrophotometer at a wavelength of 530 nm, while the absorbance values were recorded and then compared with the IAA standard solution curve. The IAA-producing bacteria test was carried out twice to obtain more accurate results.

2.3.5. Siderophores-Producing Fungi Potential Test The Fe-CAS agar media includes several solutions which are sterilized separately. First, the blue dye consists of three solutions (solution I, solution II, solution III). Solution I was consisted of 0.06 g of Chrome Azurol Sulfate dissolved in 50 mL of aquabidest. Solution II was consisted of 0.0027 g of FeCl3.6H2O dissolved in 10 mL of mM HCl. Solution III was consisted of 0.073 g of hexadecyltrimethylammonium bromide (HDTMA) dissolved in 40 mL of aquabidest. A total of 10 mL of solution II was placed into solution I and homogenized then, solution III was slowly added, stirred until the solution turned blue, and then poured into a container with 6 M HCl. The homogeneous blue dye solution was sterilized by autoclaving for 15 minutes at a temperature of 121°C with a pressure of 1 atm. Second, the preparation of a mixed solution consisting of a salt solution, a glucose solution and a Casamino acid solution was carried out. The salt solution was prepared by weighing 15 g KH2PO4, 25 g NaCl, and 50 g NH4Cl then dissolved in 500 mL aquabides. Glucose solution was made by weighing as much as 20 g of glucose and dissolved in 100 mL of distilled water. The glucose solution was filtered using a 0.22 m Millipore membrane filter and placed in a sterile bottle. Cassamino acid solution was prepared by dissolving 5 g of casamino acid in 45 mL of distilled water and then extracted with 3% 8-hydroxyquinoline in chloroform (v/v). Then the aqueous extract was filtered using a 0.22 m millipore membrane filter and placed in a sterile bottle.

Third, the preparation of Fe-CAS agar was made by dissolving 100 mL of minimum salt solution stock in 750 mL of distilled water. 32.24 g of pepperazine-N,N’-bis[2-ethanesulfonic acid] (PIVES) was added slowly while homogenizing with a magnetic stirrer. The Fe-CAS media was checked with a pH meter. If the media is too acidic, then NaOH is added until the pH of the media reaches 6.8 so that PIVES can dissolve completely. Then, as much as 25 g of agar powder was added to the media and sterilized.
by autoclaving for 15 minutes. After the sterilization process is complete, the temperature of the media is allowed to reach ± 50°C – 60°C and then added with 30 mL of casamino acid solution and 10 mL of glucose solution. A total of 100 mL of blue dye was added through the edge of the container while homogenizing. After thoroughly mixed, the media was poured into a sterile petri dish. Fe-CAS agar was inoculated with pure isolates of culturable fungi. Furthermore, Fe-CAS media containing fungal cultures was incubated at 27°C for 5-7 days. After the incubation period was completed, the fungal colonies were observed. Positive results of testing the potential for siderophore-producing fungi are indicated by yellow (orange) colonies.

2.3.6. ACC Deaminase Producing Fungi Potential Test
Screening the potential of ACC deaminase-producing fungi was carried out by inoculating fungal isolates on Dworkin-Foster (DF) minimal salt selective media (4 g KH₂PO₄, 6 g Na₂HPO₄, 0.2 g MgSO₄.7H₂O, 1 mg FeSO₄.7H₂O, 2 g glucose, 2 g gluconic acid, 2 g citric acid, 25 g agar powder and then dissolved in 1000 mL of distilled water) enriched with ammonium sulfate according to the Glick (2012) procedure. Preparation of media DF + ammonium sulfate and DF + ACC was made by adding ammonium sulfate or ACC as much as 2 g of ammonium sulfate and 0.3033 g of ACC, respectively. The ACC material was filtered using a 0.22 m millipore membrane filter.

The fungal isolates were inoculated into PDB media and agitated for 5-7 days at 27°C. Then, 5 μL of isolates were taken and grown on DF and DF + ammonium sulfate media. Isolates growing on DF media indicated that fungi could obtain a source of N by binding N₂ in the air. Isolates that were able to grow on selective media DF + ammonium sulfate were putative isolates that had the opportunity to produce the ACC deaminase enzyme. The putative isolates were grown on DF + ACC media. Positive results were indicated by the growth of fungal colonies which indicated the activity of ACC deaminase-producing enzymes.

2.3.7 Data Analysis
This study uses a descriptive analysis method with a culture approach. The data on the test results of siderophore-producing fungi, ACC deaminase and IAA are presented in the form of tables, graphs or pictures. Sequencing data in the form of forward and reverse sequences were combined to obtain a complete sequence of rDNA genes with the help of the Bioedit program to find overlapping areas of the two sequences so as to produce a complete set of promoter sequences.

The gene sequences from the sequenced (queried) were aligned with the DNA database on the GenBank using the BLASTn program on the http://www.ncbi.nlm.nih.gov/blast site. The results of the BLASTn analysis showed that the subjects with the largest percentage of similarity were determined as the sequences that had the closest relationship to the query sequence. The identity of each culturable fungi isolates was described by phylogenetic construction using MEGA X software. The phylogenetic tree construction method used the Neighbor Joining Tree which was tested statistically using the bootstrap method with 1,000 times replication.

3. Results and Discussion
3.1. Isolation and identification of culturable fungi.
The results of the isolation of KS and SS soil samples followed by purification obtained as many as 11 fungal isolates consisting of 3 isolates from KS and 8 isolates from SS with different morphological characteristics. Molecular identification was carried out after morphological observations to confirm the initial suspicion of the genus of pure isolates. DNA sequence analysis of the ITS region with a phylogenetic tree (Figure 1) showed that the culturable fungi SS. 27.5, SS. 27.2 PDA and SS. 27.3 PDA isolates were grouped into one clade with Penicillium citroviride strain PcHYSF1.2 and had a bootstrap value of 84%. The SS. 27.4 is in the same clade as the SS.27.5 SS. 27.2 PDA and SS. 27.3 PDA and Penicillium citroviride strain PcHYSF1.2 with a bootstrap value of 80%, so it can be seen that SS.27.4, SS. 27.5, SS. 27.2 PDA and SS. 27.3 PDA as Penicillium citroviride.
Figure 1. Phylogenetic tree of culturable fungi isolates in the tidal swamp land KS and non-tidal swampland SS.

The SS 27.1 PDA was in the same clade with *Penicillium citrioviride* OTU82 and *Penicillium citrioviride* strain PcH801 with bootstrap values of 90% (Figure 1), so SS 27.1 PDA was identified as *Penicillium citrioviride*. Meanwhile, SS. 27.1 was grouped into one clade with *Penicillium simplicissimum* psb 1 with a bootstrap value of 100% (Figure 1) and SS 27.1 isolate identified identically as *Penicillium simplicissimum*. Figure 1 shows that the KS. 26.2 isolate was grouped into a sister group with *Penicillium janthinellum*, *Penicillium simplicissimum* and *Penicillium citrioviride* which had a bootstrap value of 75% so that it could be seen that the KS. 26.2 belongs to *Penicillium*.
KS 26.2 and SS 27.2 isolates were grouped into one clade with *Penicillium janthinellum* strain ATCC 4845 with a bootstrap value of 89% (Figure 1), so SS 27.2 identified as *Penicillium janthinellum*. SS 27.3 was in the same clade with *Talaromyces calidicanius* T0046B with a bootstrap value of 96% (Figure 1). This clade forms a sister group with sequences *Talaromyces calidicanius* CBS 112002 type material and *Talaromyces calidicanius* strain CBS 112002 which has a bootstrap value of 92%. SS 27.3 isolate identified as *Talaromyces calidicanius*.

KS 26.1 and KS 26.3 isolates were grouped in one clade with *Trichoderma asperellum* clone 597 and *Trichoderma asperellum* strain ATCC 52438 supported by a bootstrap value of 100%. The second clade of isolates formed a sister group with sequences *Trichoderma asperellum* strain CBS 43397 type material and *Trichoderma asperellum* strain CBS 125572 which had a bootstrap value of 99% (Figure 1). KS. 26.1 and KS 26.3 identified identically as *Trichoderma asperellum*.

### 3.2. Siderophores-Producing Fungi

The results showed that all tested culturable fungi isolates were able to produce siderophores qualitatively (Table 2) and produce an orange zone which is a hydroxamate type siderophore [8,9]. Siderophores are low molecular weight iron chelating ligands produced by almost all microorganisms [9]. The test isolates of culturable fungi that are able to produce siderophores have an important component in plant growth promoting fungi, because siderophores are able to bind iron (Fe$^{3+}$) into siderophores-iron bonds that become available to plants.

| Isolate Code | Fungal species               | Result | Zone  | Type of siderophores |
|--------------|------------------------------|--------|-------|----------------------|
| KS.26.1      | *Trichoderma asperellum*     | ++     | Orange| Hydroxamate           |
| KS.26.2      | *Penicillium janthinellum*   | ++     | Orange| Hydroxamate           |
| KS.26.3      | *Trichoderma asperellum*     | ++     | Orange| Hydroxamate           |
| SS.27.1      | *Penicillium simplicissimum* | ++     | Orange| Hydroxamate           |
| SS.27.2      | *Penicillium janthinellum*   | ++     | Orange| Hydroxamate           |
| SS.27.3      | *Talaromyces calidicanius*   | +++    | Orange| Hydroxamate           |
| SS.27.4      | *Penicillium citrioviride*   | +++    | Orange| Hydroxamate           |
| SS.27.5      | *Penicillium citrioviride*   | ++     | Orange| Hydroxamate           |
| SS.27.1 PDA  | *Penicillium citrioviride*   | +++    | Orange| Hydroxamate           |
| SS.27.2 PDA  | *Penicillium citrioviride*   | ++     | Orange| Hydroxamate           |
| SS.27.3 PDA  | *Penicillium citrioviride*   | ++     | Orange| Hydroxamate           |

### Table 2. Siderophores produced by each strain of culturable fungi isolates.

- + = produces weak siderophores;
- ++ = produces moderate siderophores;
- +++ = produce strong siderophores

The results showed differences in the ability of each test isolate to produce siderophores (Table 2) based on the diameter of the orange zone. The strongest siderophore production (+++) was shown by isolates from SS soil, namely isolates of *Talaromyces calidicanius* SS. 27.3, *Penicillium citrioviride* SS.27.4 and *Penicillium citrioviride* SS.27.1 PDA, while the other five isolates from soil SS were in the moderate category (++). The 3 isolates from KS soil were *Trichoderma asperellum* KS.26.1, *Penicillium janthinellum* KS.26.2, *Trichoderma asperellum* KS.26.3 is known to produce siderophores in the moderate category (++). Differences in the ability of fungal isolates to produce siderophores depended on the ability of each isolate to extract Fe [10]. Based on the siderophore zone in table 2, there was a change in the color of the Fe-CAS media to orange due to the ability of the test isolates to produce siderophores.
siderophores. CAS and HDTMA compounds form a strong complex with ferric causing the formation of a blue color on Fe-CAS agar media. If there is a strong ferrous metal chelating (Fe) such as siderophores, the chelating will take Fe from the blue dye complex so that the Fe-CAS medium will change color from blue to orange [10].

The results of the study in show that there are differences in the ability of each test isolate to produce siderophores. This depends on the ability of each isolate to take Fe. Several siderophores can be produced by microorganisms to obtain Fe from different sources [10]. Fungi produce various hydroxamic siderophores in response to iron-limited conditions [9]. In the upper soil layers under aerobic conditions, the solubility of Fe is low and is mostly found in the form of Fe3+ which is not available to plants [11]. One of the specific mechanisms developed by fungi is the synthesis of Fe3+ complexing compounds followed by Fe transport and accumulation. Siderophores are synthesized and excreted by soil fungi in response to iron deficiency in the environment and as a result fungal inoculants increase plant growth and resistance through absorption of Fe from the soil [8, 12].

Siderophores have high affinity for Fe3+ and can facilitate cellular iron transport. Siderophores will significantly reduce the presence of ferric ions which cause inhibition of the growth of pathogenic fungi. Siderophores are useful in agriculture for plant disease management and also for enhancing plant growth [13]. Siderophores hydroxamate found in the rhizosphere effectively increase the availability of Fe and P elements in acid soils. The chelating ability of Fe in acid soils with high Fe and phosphate content has implications for the supply of P to plants as well as increasing resistance to pathogens.

3.3. ACC Deaminase - Producing Fungi.

The results of the examination of ACC deaminase activity are presented in Table 3 which shows the growth of 11 fungal isolates on DF+ ACC media. All isolates were able to use ACC which demonstrated its capacity to inhibit ethylene stressors that inhibit plant development [14].

| Isolate Code | Fungal Species               | DF  | DF+AS | DF+ACC |
|--------------|------------------------------|-----|-------|--------|
| KS.26.1      | *Trichoderma asperellum*    | +   | +     | +      |
| KS.26.2      | *Penicillium janthinellum*  | +   | +     | +      |
| KS.26.3      | *Trichoderma asperellum*    | +   | +     | +      |
| SS.27.1      | *Penicillium simplicissimum*| +   | +     | +      |
| SS.27.2      | *Penicillium janthinellum*  | +   | +     | +      |
| SS.27.3      | *Talaromyces calidicanius*  | +   | +     | +      |
| SS.27.4      | *Penicillium citrioviride*  | +   | +     | +      |
| SS.27.5      | *Penicillium citrioviride*  | +   | +     | +      |
| SS.27.1 PDA  | *Penicillium citrioviride*  | -   | +     | +      |
| SS.27.2 PDA  | *Penicillium citrioviride*  | +   | +     | +      |
| SS.27.3 PDA  | *Penicillium citrioviride*  | -   | +     | +      |

ACC deaminase is a cytoplasmic enzyme produced by some soil microorganisms to degrade ethylene producers. The ACC deaminase enzyme will break down ACC as an ethylene precursor into ammonia and-cetobutirate which is a nitrogen and carbon source for microorganisms [15] and which results in a decrease in ethylene levels in plants so as to stimulate root/shoot growth [16] (Glick, 2014). Several studies have shown the activity of the ACC deaminase enzyme from several fungal species. The biosynthetic activity of ethylene and ACC of *T. asperellum* T203 has already evaluated [17]. The study showed that the isolate was able to produce ACC deaminase which stimulated root elongation.
**Trichoderma** species were able to promote plant development up to 300% through the activity of ACC deaminase-producing fungi and IAA production [18].

Microorganisms isolated from highly contaminated soil or stressed environments were mostly ACC deaminase-producing agents and were more resistant under abiotic stress conditions [19]. ACC deaminase activity is not only a growth promoter in plants but can also play a multi-level role in various plant processes. Plants produce higher levels of phytohormones ethylene which means higher levels of ACC under stress conditions [20].

Microorganisms that bind plant tissue usually utilize plant exudates as a source of nutrients. Under stress conditions, not only does the amount of ACC produced by plants increase, most of the rhizosphere microorganisms produce the phytohormone IAA which acts to loosen plant cell walls thereby facilitating root exudation. The production of IAA bacteria has also been shown to increase the expression of ACC synthase in plants [21]. Thus, microorganisms that can produce IAA and utilize ACC can have a competitive advantage over other soil microorganisms [22].

### 3.4. IAA Producing Fungi.

The results of the reaction analysis from the culture were taken the fungal isolate supernatant and reacted with Salkowsky's reagent. Based on Figure 2, it can be seen that all isolates of culturable fungi in the tidal swamp land of South Kalimantan and non tidal swamp land of South Sumatera have the potential to produce IAA. Each isolate has a different ability to produce IAA. The isolates of culturable fungi that produced the highest IAA were *Penicillium simplicissimum* SS 27.1 at 1.75 ppm and the lowest was *Penicillium citrioviride* SS 27.5 at 0.53 ppm. The results of this study are in line with previous research that the *Penicillium* isolated from the Galam rhizosphere (*Melaleuca cajuputi*) had a higher IAA content compared to other isolates with a concentration of 8.46 + 0.26 ppm 8 ppm on media supplemented with L-tryptophan [23]. This is influenced by the physiological properties of each mold in converting tryptophan to IAA [24].

![Figure 2](image)

**Figure 2.** The concentration of IAA hormone produced by fungal species of tidal swampland KS and non-tidal swampland SS.

The mechanism of fungi in producing IAA is carried out through an enzymatic process. Specific enzymes that are useful in producing IAA are expressed by the presence of certain genes that change the basic ingredient of tryptophan [25]. There are two pathways that can be applied by fungi to produce IAA, namely through Indole-3-pyruvate (IPA) and Indole-3-acetamide (IAM). Based on the research that has been done, the IPA pathway is the most widely used pathway by microorganisms in producing IAA [26,27].
The highest concentration of IAA produced by *Penicillium simplicissimum*. It may be associated with the pathogenicity of a fungi, since some pathogenic fungi are indicated to produce high amounts of auxin than others. Environmental conditions in culture media are too acidic, it will interfere with the performance of enzymes that catalyse L-tryptophan precursors into IAA. If the environmental conditions have a pH that tends to be acidic, it will affect the performance of the enzyme [28]. The pH can cause a denaturation process which results in decreased enzyme activity, including aminotransferase enzymes, IAAId oxidase activity will decrease. These enzymes require optimal conditions for the catalysis process to run optimally. Level pH will be related to the presence of hydrogen ions. The concentration of hydrogen ions greatly affects the activity of the enzyme, because the enzyme can be active if the amino acid is the active site of the enzyme in the right ionization state. pH is too acidic or alkaline will cause the enzyme to be denatured so that the enzyme is not active.

The concentration of IAA produced ranges in increasing plant growth. IAA is classified as a natural auxin that has an indole ring component and plays a role in cell division and cell elongation [29]. Exogenous IAA secreted by fungi can promote root growth directly by initiating plant cell elongation. IAA compounds that affect the activity of the fungus ACC deaminase. Stimulation of adventitious and lateral roots affected by ethylene caused by the hormone IAA will have an impact on increasing the number of roots and correlate with ethylene production. When ethylene production is high, it will affect growth. It is proven that when exogenous IAA levels are high, it will inhibit the growth of elongation in roots [30].

4. Conclusion
The results of the culturing technique obtained 11 isolates of fungi from both tidal and non-tidal swampland of South Kalimantan (KS) and South Sumatera (SS,) consisting of the genera *Trichoderma, Penicillium* and *Talaromyces*. All isolates of culturable fungi were able to produce siderophores, ACC deaminase enzymes and IAA phytohormones.

Acknowledgments
Authors wishing to acknowledge assistance or encouragement from colleagues, special work by technical staff Mrs. Aminah and Mr. Jajang.

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