Selection of fungi that potentially produces IAA (Indole Acetic Acid) hormone origin of Takalar sugar factory waste

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Abstract. This research aimed to obtain fungi isolates from the laboratory collection Mycology Department of Plant Pests and Diseases Hasanuddin University which has the potential to produce hormones Indole Acetic Acid (IAA). The isolate used is the result of isolation from Takalar sugar factory waste. Measurement The fungi isolate was inoculated in the media of Potato Dextrose Broth (PDB) enriched L-Tryptophan and then added the Salkowski Reagent. The results showed a change in pink color showed the fungi can produce the IAA hormone. High and low content of IAA produced by fungi isolates was shown with pink color. The pinker the suspense color, the higher the IAA content. IAA concentration produced by the isolates was measured using a spectrophotometer with a wavelength of 520 nm. The IAA value is calculated by comparing it with the absorbance value of the standard curve. The results showed that the isolate D4 code produced the highest IAA hormone concentration of 10.17 ppm.

1. Introduction
Plants in the process of growth and development are supported and influenced by a hormone [1]. In agronomy, the existence of hormones is very important. The signaling molecules that act as chemical messengers to facilitate growth and promote plant development are phytohormones. Auxin is the most physiologically active hormone in nature and is the main requirement hormone for almost all plant species. One type of auxin hormone is the indole acetic acid (IAA) hormone [2].

The production of secondary metabolites produced by fungi such as gibberellins, auxins, abscisic acid, and organic acids, provides abiotic protection and environmental stress to plants [3]. Fungi are known to produce auxin such as Indole-acetic acid (IAA) and are beneficial for plant growth. The fungi lives in symbiosis with plants and support plant growth and the plants return to provide sugar and amino acids for their survival. The fungi are called plant growth-promoting fungi (PGPF) [4]. The IAA hormone plays a role in the development process, root elongation and the formation of xylem and phloem tissues and plays a role in the enlargement of plant cells [5], regulating plant cell development,
and contributing to xylem growth and phloem [6].

Compound Indole Acetic Acid (IAA) is one phytohormone with auxin activity that governs the process of plant cell development as well as contributes to xylem growth and phloem [6]. IAA is a product of the metabolism of L-tryptophan produced by several the microbes around the roots act as carrier signal communication molecule between plants and microbes as well as support plant growth. IAA helps produce roots being longer by increasing the number of hair roots and lateral roots involved in taking nutrients [6].

According to Wang [7], some Trichoderma species can produce or degrade IAA, consequently plants grow optimally. [8] concluded that endophytic fungi can increase plant growth under stress conditions and increase disease resistance because fungi have the potential to produce bioactive metabolites and increase host growth. So this research needs to be done to get fungi that has the potential to produce growth hormones Indole Acetic Acid (IAA) so it is caused effectively for biological fertilizer formulations and provides an alternative environmentally friendly strategy in modern agricultural systems.

2. Materials and methods

2.1. Tools and materials
This research was carried out in December 2019 at the Laboratory of Bioscience and Plant Reproduction Biotechnology, Faculty of Agriculture, Hasanuddin University. The isolates used in this study were isolates of Laboratory Fungi Mycology Department of Plant Pests and Diseases Hasanuddin University. The material used were PDA medium (Potato Dextrose Agar), medium PDB (Potato Dextrose Broth), Salkowski reagent (concentrated H₂SO₄, FeCl₃,6H₂O, Aquades), 70% alcohol, Spiritus, Tryptophan, Methanol, Aquades.

The tools used in conducting this research were analytical scales, reaction tubes, Erlenmeyer, drip pipettes, volume pipettes, micropipettes, autoclaves, spectrophotometers, centrifuges, shaker incubators, ovens, aluminum foil, hot plates, stirrers, tube shelves, bunsen, ose, stirring rods, digital cameras, and stationery.

2.2. Preparation medium of fungi
Weighed 19.5 grams of Potato Dextrose Agar (PDA), 10.5 grams of agar, 5 grams of glucose, 500 ml of distilled water, antibiotics, then placed in the Erlenmeyer. The solution was heated using a hotplate and stirred using a magnetic stirrer until homogeneous. Sterilized the media for ± two hours at a temperature of 121 °C using an autoclave. Laminar Airflow was sterilized then the media solution was poured it into a sterile petri dish and allow it to harden. One loop inoculation of fungal colonies were placed on PDA media was then stored in the incubator room for ± one week.

2.3. Preparation of Potato Dextrose Broth (PDB) liquid media
Potato Dextrose Broth (PDB) Liquid Media Material was weighed as much as 24 grams, L-tryptophan 0.1 gram, Glucose 20 grams, then added with Aquadest as much as 1000 ml then homogenized using a stirrer and sterilized in an autoclave for 15 minutes at 121°C.

2.4. Analysis of IAA production by fungi
The rejuvenated fungi were inoculated on 45 ml Potato Dextrose Broth (PDB) media with the addition of 0.1 g / L L-tryptophan and incubated using a shaker at room temperature for six days. Subsequently, measurements were taken to see the results of IAA hormone production by taking 10 ml of culture then centrifuged for 30 minutes at a speed of 8000 rpm. The supernatant was filtered with filter paper, then 1 ml the supernatant was obtained and added with 4 ml of Salkowski reagent (75 ml concentrated H₂SO₄, 3.75 ml FeCl₃,6H₂O 0.5 M, and 125 ml sterile Aquades) into each test tube. Incubated in a dark room for 30 minutes. The test tube was removed from the incubator after 30 minutes, then the sample changed color. Measurement of the concentration of IAA produced by the isolates was measured using a
spectrophotometer at a wavelength of 520 nm. The IAA concentration was calculated after being compared with the absorbance curve of the standard IAA.

2.5 Fungi identification
Identification of Fungi was carried out after pure fungi grew in PDA media for ± one week. Identification of Fungi refers to the fungus identification book. Identification of Fungi was carried out descriptively, determining the genus level by looking at the macroscopic characteristics including color, diameter, and texture, microscopic features including hyphae and spores. Microscopic identification of fungi was carried out by taking the fungi growing on PDA media using preparatory needles and placed on glass objects that had been dripped with distilled water and then closed using a glass deck, then observing the fungi used a microscope with 40x magnification. The images obtained were adjusted to the literature image.

3. Results
Fungi isolate which has been incubated for 6 days on Potato Dextrose Broth (PDB) Media added with 0.1 g / L L-Tryptophan to test its ability to produce IAA. Fungi isolate supernatant was added with Salkowski reagent. The results show a pink discoloration. The change of this color indicates that the fungi can produce the IAA hormone. High and low content of IAA produced by fungi isolates was shown with pink color. The pinker the suspense color, the higher the IAA content (figure 1).

![Figure 1](image1.png)

**Figure 1.** Changes in the color of the supernatant before (A) and after (B) addition of Salkowski’s reagent.

The results of measurements of IAA concentrations produced by isolates were measured using a spectrophotometer at a wavelength of 520 nm can be seen in the figure 2 below.

![Figure 2](image2.png)

**Figure 2.** Test results of the ability of isolates to produce IAA enzymes.
Based on the selection results, the best isolate that produced the Indole Acetic Acid (IAA) enzyme was D4 isolates which had the highest IAA concentration of 10.17 ppm while the isolate that had the lowest IAA concentration was isolated D1 with a concentration of 7.89 ppm (figure 2). The results of microscopic identification results are the best fungi come from the genus *Aspergillus* (figure 3).

**Figure 3.** Macroscopic observation on the best fungi (A); microscopic identification (B)

4. **Discussion**

Based on the test results the ability to produce Indole Acetic Acid (IAA) shows that fungal isolates can produce IAA hormones with different concentrations. The ability of fungal isolates to produce IAA hormones can be seen when fungal isolates are inoculated on Potato Dextrose Broth (PDB) enriched L-Tryptophan and Salkowski reagents show a pinkish color change. According to Sukmadewi [10] Bacteria that can produce IAA will turn red/pink after adding the Salkowski Reagent, due to interactions between IAA and Fe forming complex compounds [Fe2 (OH) 2(IA)4]. The combination of Fe and sulfuric acids (H2SO4) as a single reagent can increase sensitivity in the formation of IAA [11].

The results of research conducted by Isolate D4 produced the highest IAA concentration of 10.17 ppm. The results obtained are lower than the results of the Larekeng [12] study that the concentration of isolates produced by isolates from the Mahoni root area in Takalar, obtained 3 isolates which had high IAA concentrations including 18.08 ppm, 19.52 ppm and 19.69 ppm with (*Penicillium* Genus Isolate), while 3 isolates had Low IAA concentrations include 10.27 ppm, 12.13 ppm (MT 1.1, MT 8.5, *Aspergillus* Genus) and 12.47 ppm (MT 10.4, Genus *Gliocladium*). This is due to the influence of environmental physicochemical variables on the biosynthesis of indole acetic acid metabolites (IAA).

According to Napitupulu [13], IAA production by *Trichoderma harzianum* InaCC F86 strain is influenced by the presence of L-tryptophan and its concentration, acidity, temperature, and salinity. This is in line with the results of endophytic bacteria selection in producing IAA [14] states that OS03 isolates produce maximum IAA in the medium, by giving an intense pink color with the highest OD value of 0.187 at 530 nm with 17.715 + 0.32 ug/ml IAA. Optimal IAA production in culture media on the 8th day, with a temperature of 37 °C and a pH of 7. Research Results from [15] IAA test results of isolates CAR4 (*Colletotrichum gloeosporioides*) treatment temperature 30°C, with growing media Potato Dextrose Broth (PDB) and pH 6 show the highest results.

In this study, the results obtained were higher when compared with the results obtained [12] which was 9,656 ppm with an incubation period of 3 days, but during the seven-day incubation period, IAA production decreased to 4,049 ppm. IAA production is also influenced by the incubation time, where the longer incubated isolates can cause a reduction in the number of nutrients present in the growth so that the IAA produced is consumed again for microbial growth [16]. IAA is the result of secondary metabolites produced by bacteria/fungi when entering the stationary phase or the end of the logarithmic phase and secondary metabolites produced by fungi are mostly formed in the stationary phase. Some secondary metabolites produced by fungi have biological activity, so they are often exploited commercially, which is use one of them in producing phytohormone in the form of IAA [17].

IAA concentrations produced by these isolates are very small but these isolates can stimulate plant
growth because these fungi produce plant phytohormone growth [12]. At low concentrations, IAA functions in the elongation of root cells, but at high concentrations, it can inhibit the lengthening of root cells [18].

The results of Microscopic identification results are the best fungi come from the genus *Aspergillus*. According to [18] *Aspergillus* sp. NPF7 obtained from the local wheat rhizosphere of Surat, Gujarat produced multiple plant growth-promoting traits like IAA, GA, and siderophore, solubilized phosphate, and promoted seed germination, root length, and shoot length in wheat and chickpea plants. Such multipotent fungi obtained from the local rhizosphere and used in the same conditions are expected to perform better due to their proper adaptation to the local soil environmental conditions and hence can be used as an eco-friendly and natural alternative to hazardous chemical fertilizers leading to sustainable agriculture.

5. Conclusion
This study concludes that from 9 isolates tested for the ability to produce Indole Acetic Acid (IAA) Hormone showed that isolate code D4 produced the highest IAA Hormone Concentration, 10.17 ppm come from the genus *Aspergillus*. Therefore *Aspergillus* sp has the potential to be used as biological fertilizer

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