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Antibacterial of Eight Macrofungi Species Against

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Abstract

Indonesia is a newly developing country, with many problems, one of which is health problems related to medicinal ingredients (antibiotics). Many microorganisms, especially bacteria, are resistant to various types of antibiotics. Various efforts have been made by the government to find new antibiotics, to reduce the use of semisynthetic or synthetic antibiotics which are very dangerous. One of them uses macrofungi.The antibacterial activity of eight species of macrofungi (*Ganoderma applanatum*, *G. boninense*, *Ganoderma* sp1, *Ganoderma* sp2, *Trametes* sp1, *Trametes* sp2, *Trametes* sp3, *Microporus xanthopus* and *Suillus* sp1) against *Escherichia coli* and *Staphylococcus aureus* has been carried out, using the diffusion method. The results showed that six species were able to inhibit the growth of Staphylococcus aureus. The species were *Ganoderma applanatum, Ganoderma* sp1, *Trametes* sp.1, *Trametes* sp.2, *Trametes* sp.3, and *Suillus* sp. The resulting limiting zone ranged from 6.5-11 mm. The zone of inhibition produced by *Trametes*, the smallest inhibition zone of *Ganoderma* sp1. The higher the concentration of the extract, the greater the ability to inhibit the growth of the test bacteria.

Keywords: antibacterial, macrofungal, drug

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INTRODUCTION

Medicine is one of the necessities used in an effort to support and maintain public health. The need for medicinal ingredients in Indonesia is always increasing, especially when it is associated with population growth which is always increasing, which directly affects the health level of the population. Currently, many microorganisms, especially bacteria, have become resistant to various types of medicinal substances, especially antibiotics. Various efforts have been made by the government to look for new antibiotics, one of which is semisynthetic or synthetic antibiotics which are mostly derived from chemicals, but this creates new problems. To overcome this problem, one solution that is currently very appropriate to be researched and developed is to look for new alternative materials, such as macrofungi as medicinal ingredients.

Many species of macrofungi are edible and are also used by the community, as medicinal ingredients. Some examples of macrofungi that can be efficacious are shiitake mushroom *(Lentinula edodes*) producing lentinan, Suehirotake or *split gill* (*Schizophyllum commune*) producing schizophyllan. Both types of mushrooms are efficacious as immunomodulators, which can stimulate the immune system to increase the human body against diseases, such as cancer (Hendritomo 2010).

Furthermore, Gunawan (2000) in several countries such as Japan and China have used the fungus *Ganoderma lucidum* and *G, aplanatum* to increase the body's immune system. *Coriolus hirsutus, C, versicolor, Flamulina velutipes, Pholiota nameko*, *Pleurotus ostreatus*, and *Tricholoma matsuke*, are efficacious in inhibiting sarcoma 180 tumors. *Grifola frondosa* (maetake) to prevent tumors and cancer, and is also used to inhibit the growth of HIV (Human Immudeficiency Virus), increase natural killer cell products in the body, treat high blood pressure, diabetes, and hepatitis. Handrianto and Science (2017), Lingzhi mushroom (*Ganoderma lucidum*) is a herbal remedy that has antibiotic or antibacterial activity. Lingzhi mushroom triterpenoids contain compounds that possess antibacterial activity by reacting with porin mechanisms (transmembrane protein) on the outer membrane of the bacterial cell wall, forming strong bond polymers that cause damage to porin.

Microorganisms, especially bacteria, are closely related to human life and other living things. The presence of bacteria in life in addition to providing positive benefits for humans, some small groups that can cause some dangerous infectious diseases. Infectious diseases are diseases that can cause death, one example is diarrheal disease, which is caused by foodborne infections and waterborne infections by the bacteria *Salmonella* spp., *Campylobacter jejuni, Staphylococcus aureus, Bacillus cereus, Clostridium perfringens*, and Enterohemorrhagic *Escherichia coli* (EHEC) (Zein. et al. . 2004).

Based on the background and problems above, this study was conducted to know the ability of eight species of macrofungi extracts, namely *Ganoderma applanatum, G. boninense*, *Ganoderma* sp1, *Ganoderma* sp2, *Trametes* sp1, *Trametes* sp2, *Trametes* sp3, *Microporus xanthopus*, and *Suillus* sp1) in inhibiting the growth of pathogenic bacteria. The test bacteria (pathogenic) used in this study are bacteria that are commonly found in everyday life. It has a high infection rate and is opportunistic towards other organisms. These bacteria are Gram-positive *Staphylococcus aureus* which produces thermostable toxins and Gram-negative bacteria *Escherichia coli* which can infect the digestive tract. The hypotheses to be tested in this study are:

1. Extracts of *Ganoderma applanatum*, *G. boninense*, *Ganoderma* sp1, *Ganoderma* sp2, *Trametes* sp1, *Trametes* sp2, *Trametes* sp3, *Microporus xanthopus*, and *Suillus* sp1 have inhibitory properties against *Escherichia coli* and *Staphylococcus aureus*.
2. Differences in the concentration of macrofungi extracts will affect the inhibition zone formed.
3. The test bacteria had different responses to macrofungi extracts.

Urgency (Priority) of Research

1. It is known that the antibacterial activity of the macrofungi extracts of *Ganoderma applanatum, G. boninense, Ganoderma* sp1, *Ganoderma* sp2, *Trametes* sp1, *Trametes* sp2, *Trametes* sp3, *Microporus xanthopus*, and *Suillus* sp1 has antibacterial activity against test bacteria (Escherichia coli and Staphylococcus aureus).
2. It is known the type and the best concentration of macrofungi extract used to inhibit the test bacteria.
3. Can be pursued as an alternative in treatment, especially due to bacterial infections

METHOD

A. Research Place

The research was carried out at the Laboratory of Microbiology and Genetics, Faculty of Biology, Universitas Nasional, Bambu Kuning, Pasar Minggu, South Jakarta**.**

B. Research Materials

The materials used in this study included: extracts of the macrofungi *Ganoderma applanatum*, *Ganoderma baniense*, *Ganoderma* sp1, *Ganoderma* sp2, *Trametes* sp1, *Trametes* sp2, *Trametes* sp3, *Microporus xanthopus*, and *Suillus* sp1, isolates of Staphylococcus aureus and Escherichia coli, Mueller-Hinton media Agar (Oxoid), Bacto Agar (Difco), Nutrien Agar (NA) (Oxoid), Nutrien Broth (NB) media (Oxoid), 80% Methanol, 1% H2SO4 Solution, 1% BaCL2 Solution, Antibiotic Ampicillin 10 g (AMP 10) (Oxoid), and aquades.

The equipment used in this study included: blender, oven, autoclave, centrifuge, vaporizer (Evaporator Butchi 461), shaker, vortex, digital scale, Petri dish, Laminar air flow, incubator, Erlenmeyer flask, Beaker glass, electric stove, sterile swab, tweezers, measuring flask, volumetric pipette, filter paper, bunsen burner, test tube, stirring rod, loop needle, aluminum foil, measuring cup, cotton swab, lighter, label paper, marker pen, paper disc, universal pH, bulb, and caliper.

C. **Procedure**

1. Preparation of Growth Medium and Sterilization of Equipment

a. NA (Nutrient Agar)

Nutrien Agar (NA) media was prepared by: weighing 23 grams of Nutrien Agar powder, adding 5 grams of Bacto Agar, then dissolving in 1 L of distilled water, stirring until the mixture was smooth while heated until boiling. After that, it was put into a prepared test tube of 5 mL for oblique culture and the rest was put into an Erlenmeyer. Then covered with cotton, sterilized in an autoclave at 121 °C, a pressure of 1-2 atm for 15 minutes.

b. NB (Nutrient Broth)

Nutrient Broth (NB) media was prepared by: weighing 23 grams of sodium broth powder, then dissolving in 1 L of distilled water, stirring until the mixture was smooth while heated until boiling. After that, it was put into a prepared test tube of 5 mL for oblique culture and the rest was put into an Erlenmeyer. Then covered with cotton, sterilized in an autoclave at 121 °C with a pressure of 1-2 atm for 15 minutes.

c. Mueller-Hinton Agar

This medium was made by weighing 38 grams of Mueller-Hinton Agar (MHA), adding 6 grams of Bacto Agar, then dissolving in 1 L of distilled water and heated until boiling. The media was then sterilized by autoclaving for 15 minutes at 121 °C (1-2 atm pressure) for 15 minutes (Capuccino and Sherman, 1986). After sterilization, the media from the autoclave was allowed to stand until the temperature reached 45 °C and poured into sterile Petri dishes. This medium is used to test bacteria.

d. Sterilization

Petri dishes, test tubes, Erlenmeyer, and other glassware to be used are sterilized in an oven at 180^{o}C for 2 hours after previously being washed, dried, and wrapped in paper (Capuccino and Sherman, 1986, Pelczar and Chan, 1986).

2. Sample Preparation

The fruit bodies of the macrofungi were first dried in the oven, then crushed using a blender until smooth, then sieved using the smallest size sieve with a diameter of 150 mesh

3. Macrofungi Fruit Body Extraction

The powdered sample was extracted by: weighing 100 grams of macrofungi powder and then adding 500 ml of 80% methanol as a solvent, then the sample was shaken for 12 hours and macerated for 24 hours at room temperature. The macerated macrofungi extract was filtered using filter paper so that two parts were formed in the form of filtrate and dregs. Furthermore, the macrofungi pulp was added with 250 ml of methanol and shaken, then macerated again as in the previous work, this was done three times.

The extracts were mixed and centrifuged at 3500 rpm for 15 minutes and then filtered again. The resulting supernatant was evaporated at a temperature of 40 °C with a vacuum rotary evaporator until it was dry which was marked by the presence of sediment. Furthermore, different concentrations of treatment were made, namely: 25%, and 50%.

4. Preparation of Test Bacteria

A total of one ose colony of test bacteria (*Staphylococcus aureus* and *Escherichia coli*,) was dissolved in 0.85% physiological NaCl solution and the number of test bacteria used was homogenized using the McFarlan standard 0.5 (bacterial density 1.5 x 108) on a black background and bright light. McFarlan turbidity standard 0.5 was prepared by mixing 0.5 ml of 1% BaCL2 solution with 9.5 ml of 1% H2SO4 (Barry, 1980 in Lorian, 1980).

The sterile swab was dipped into a mixture of test bacteria with 0.85% physiological NaCl, then drained by pressing the end of the swab and rotating it against the inner wall of the tube to remove excess fluid. Then the swab is applied to the surface twice, namely horizontally and vertically so that bacterial growth can be evenly distributed.

5. Antibacterial Test

This antibacterial test was carried out to determine the ability of macrofungal extracts to inhibit the growth of the test bacteria. Antibacterial test of the macrofungal extract against test bacteria (pathogens) using the Kirby-Bauer (disc) diffusion method. Each blank paper disc was heated in an oven at 70 °C for 15 minutes. Then each disc was given a solution of macrofungi extract of each concentration that had been previously determined using a 0.25 L micropipette. The discs were allowed to stand for 15 minutes before being placed on the test medium.

Antibiotics ampicillin 10µg (positive control) and 80% methanol (negative control) for each test bacteria. The Petri dishes were then incubated at 37 °C for 24 hours. The diameter of the inhibition zone (marked by the formation of a clear zone) formed around the disc was measured using a caliper. In measuring the inhibition zone, the diameter of the disc was also measured, concentrations having a diameter of 6 mm (equal to the diameter of the disc) were said to have no inhibition zone, while concentrations with a diameter of more than 6 mm were said to have an inhibition zone.

D. Research Design

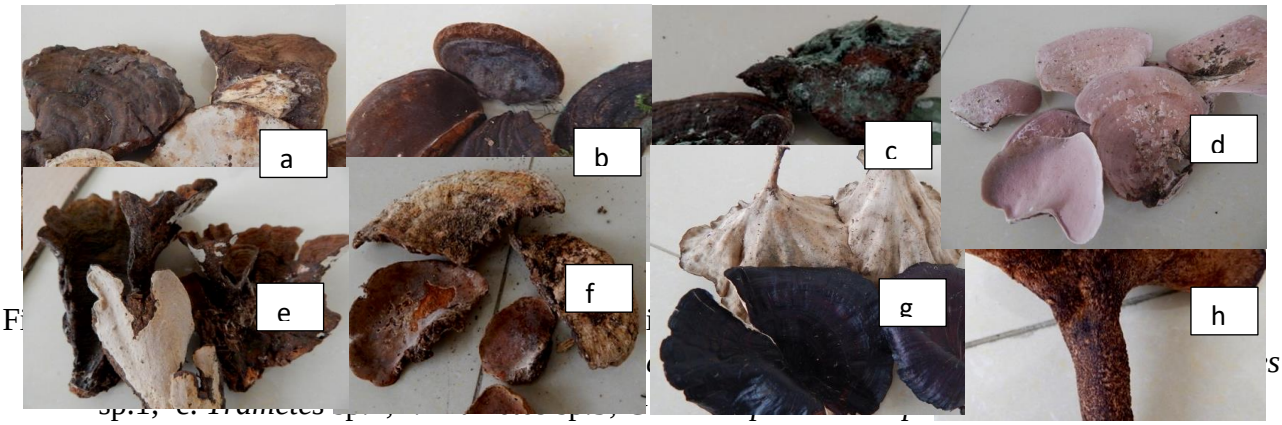
The design used in this study was a Factorial Completely Randomized Design (CRD). As factor A were 6 (six) macrofungal extracts, Factor B concentration of macrofungi extract (25% and 50%, ampicillin as positive control and methanol as negative control), and 2 types of test bacteria (*E. coli* and *S. aureus*). The antibacterial test was carried out with 2 replications. The number of

treatments was 48. The inhibition zone formed was observed as a parameter and analyzed descriptively.

RESULT

A. Macrofungi Species Tested for Antibacterial Activity

Eight types of macrofungi tested for their antibacterial activity belong to the Phylum Basidiomycota, with the species *Ganoderma applanatum, Ganoderma boninense, Ganoderma* sp1, *Trametes* sp.1, *Trametes* sp.2, and *Trametes* sp.3, *Microporus xantophus*, and *Suillus* sp, (Figure 1).



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B. Antibacterial Activity of Extracts of 8 Macrofungi Species Against Bacteria *Staphylococcus aureus* and *Eschericia coli*.

The antibacterial activity of extracts of 8 species of macrofungi against *Eschericia coli* and *Staphylococcus aureus* is shown in Table 1 and Figures 2-3.

Table 1. Average Diameter of Inhibitory Zone Extracts of 5 Macrofungi species Against Growth of *Eschericia coli* and *Staphylococcus aureus*

No	Species macrofungi	Concentration	*Eschericia coli*	*Staphylococcus aureus*
1	*Ganoderma applanatum,*	50%	0	10
		25%	0	7.5
2	*Ganoderma boninense*	50%	0	0
		25%	0	0
3	*Ganoderma* sp1	50%	0	6.5
		25%	0	0
4	*Trametes* sp. *1*	50%	0	11
		25%	0	10,5
5	*Trametes* sp.2	50%	0	10
		25%	0	7,5
6	*Trametes* sp.3	50%	0	7,5
		25%	0	7

7	*Microporus xantophus*	50%	0	0
		25%	0	0
8	*Suillus* sp	50%	0	8,25
		25%	0	7

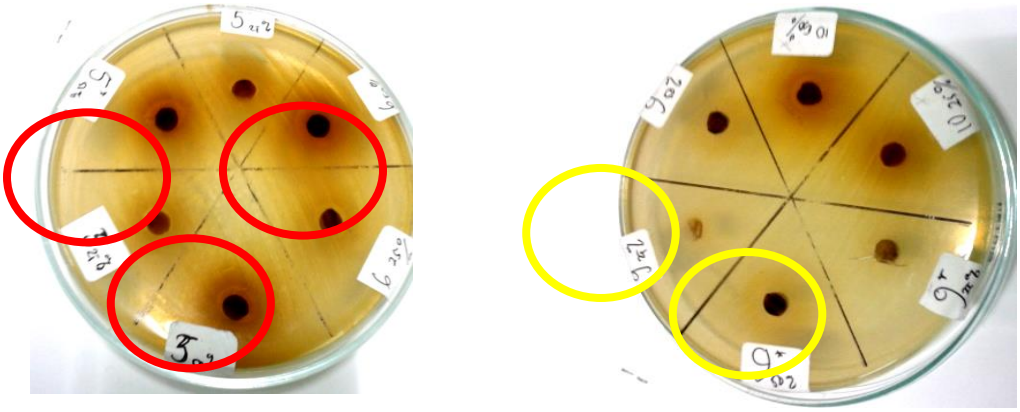

Figure 2. Antibacterial activity of macrofungal extract against bacterial growth *Staphylococcus aureus.*

Description : * The red circle indicates the inhibitory zone of *Ganoderma applanatum* extract against *Staphylococcus aureus growt*.
**Green circle indicates the inhibition zone of *Ganoderma* sp 1 extract against *Staphylococcus aureus* growth.

Figure 3. Antibacterial activity of macrofungal extract on bacterial growth *Escherichia coli*.

Description: All discs containing macrofungal extract do not form zones of inhibition (zone of inhibition 0)

The diameter of the inhibition zone produced by the macrofungi extract against *Staphylococcus aureus* bacteria ranged from 6.5mm to 11mm. The largest inhibition zone was produced by the macrofungal extract of *Trametes* sp.1, the smallest inhibition zone was produced by the macrofungi extract of *Ganoderma* sp.1 (Figure 4).

Figure 4. The diameter of the largest inhibition zone produced by the extract *Trametes* sp.1 (A) and the smallest inhibition zone diameter were produced by *Ganoderma* sp.1 extract (B)

C. The Effect of Differences in Concentration of Macrofungi Extracts on Growth of *Staphylococcus aureus* and *Eschericia coli bacteria*.

The concentration of macrofungi extract used was 25% and 50%. The antibacterial activity of these macrofungi extracts increased with increasing concentrations given (Figure 5).

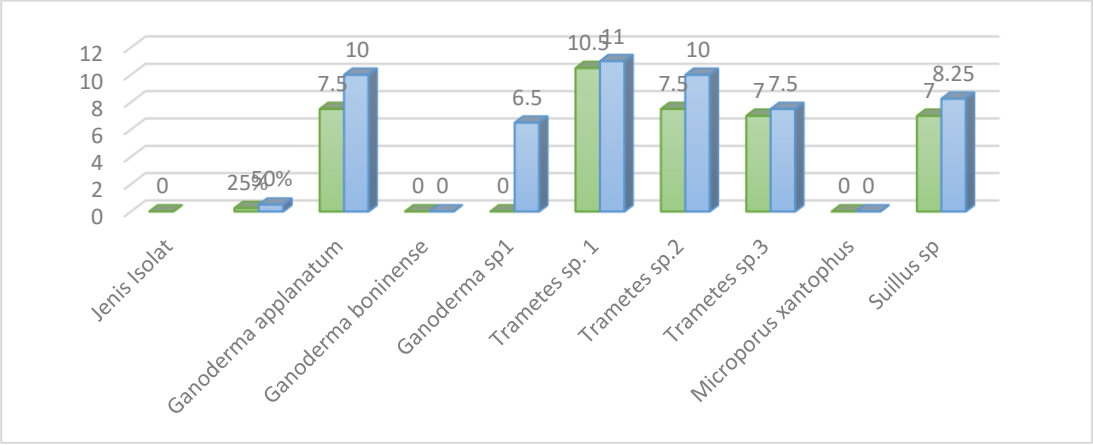

Figure 5. Bar Diagram of the Average Inhibitory Zone Diameter of Macrofungi Extract Against the Growth of *Staphylococcus aureus* Bacteria With Different Concentration

Description: Green color indicates 25% concentration, Blue color indicates 50% concentration

DISCUSSION

The ability of macrofungi extracts to inhibit the growth of test bacteria is influenced by many factors, including the type and nature of the test bacteria, the type, concentration, and the active bacterial compounds produced by the macrofungi. In this study, the bacteria used were representatives of Gram-positive bacteria (*S. aureus*) and representatives of Gram-negative bacteria (*E. coli*). These two species of bacteria have different abilities against the antibacterial materials used. *S. aureus* (Gram-positive) bacteria are resistant to physical treatment and not resistant to antibacterial agents, while *E. coli* (Gram-negative) bacteria are not resistant to physical treatment and resistant to chemical treatments (Radji, 2010). This is reflected in this study, where the growth of *S. aureus* bacteria was inhibited by the six species of macrofungal extracts used, while the macrofungal extracts were unable to inhibit the growth of *E. coli* bacteria.

The ability of these six macrofungal extract species to inhibit the growth of *S. aureus* bacteria is related to the chemical compounds they produce which are antibacterial. The chemical compounds produced by macrofungi vary depending on the species. According to Suriawiria (2000), Ganoderma spp. produces Ganodermin (Ganodermic acid) which can help neutralize or reduce compounds that cause various diseases. In addition, Ganoderma also produces bioactive polysaccharides that have antitumor and antimicrobial properties, such as HIV infection in humans (Chang and Miles, 2004). Besides Ganoderma, another species, namely the culture of Trametes Versicolor mycelium, also produces protein-chain-polysaccharides consisting of 62% polysaccharides and 26% protein which are also efficacious as medicinal ingredients.

In this study, the antibiotic activities of 75 mushrooms collected in the area surrounding Oxford, Ohio (USA), were assayed for antibiotic activity against 6 bacterial strains (*Pseudomonas aeruginosa* reference strains PAO1 and PA14, *P. fluorescens, Bacillus subtilis, Staphylococcus epidermidis*, and *Micrococcus luteus*). Mushroom samples were identified by using DNA ribotyping. We used methanol and water extracts of mushrooms in agar diffusion assays to screen for antibiotic activity in each bacterial strain. A total of 25 mushroom species had antibacterial activity against at least 1 bacterium. Water extracts of *Polyporus squamosus, Ganoderma applanatum, Lentinellus subaustralis, Laetiporus sulphureus, G. lucidum*, and *Trametes versicolor* exhibited strong antibiotic activity against all bacterial strains tested. Water and methanol extracts from 25 mushroom species had significant activity against most of the bacteria tested. A minimum inhibitory concentration (MIC) against *S. epidermidis* was determined for all samples that exhibited antibiotic activity in the disk assay. The *G. lucidum* and *L. sulphureus* extracts displayed the strongest inhibition, with a MIC of 0.1 mg/mL (Orzali, et al. 2020).

Table 1 above shows that six of the eight species of macrofungal extracts tested were only able to inhibit the growth of *Saphylococcus aureus* bacteria, namely the macrofungal extracts of *Ganoderma applanatum, Ganpderma* sp1, *Trametes* sp1, *Trametes* sp2, *Trametes* sp.3, and *Suillus* sp. The antibacterial activity of the six macrofungi extracts against the growth of *Staphylococcus aureus* bacteria was indicated by the formation of an inhibition zone (clear area) around the disc used (Figure 2). Meanwhile, for macrofungi extracts that were not able to inhibit the growth of the test bacteria used, there would be no inhibition zone formed around the discs used (zero inhibition zone), this can be seen in Figure 3, where there is no inhibition zone for the macrofungal extract against the growth of *Escherichia Coli* bacteria.

Figure 5 above shows that the diameter of the inhibition zones of six species of macrofungi extracts increased with increasing concentrations of the given macrofungal extract on the growth of *S. aureus* bacteria. An increase in the zone was due to the higher the concentration of the extract given, the more antibacterial substances contained in it, so the higher the ability of the macrofungi extract to inhibit the growth of the test bacteria. According to Akinnibosun, et al. (2008), the antibacterial activity will increase in line with the increase in the concentration of the extract used.

CONCLUSION

Based on the results obtained from this research, it can be concluded:

1. Six of the eight macrofungal extracts tested were only able to inhibit the growth of *Staphylococcus aureus* bacteria
2. Macrofungi extracts capable of inhibiting the growth of *Staphylococcus aureus* bacteria are *Ganoderma applanatum*, *Ganoderma* sp1, *Trametes* sp.1, *Trametes* sp.2 and *Trametes* sp.3, and *Suillus sp*,
3. The average diameter of the inhibition zones of the six macrofungi extracts against *Staphylococcus aureus* bacteria ranged from 6.5-11 mm, the largest inhibition zone was produced by the *Trametes* sp.1 macrofungi extract with a concentration of 50% at 11 mm, the lowest range was produced by the macrofungi extract of *Ganoderma* sp. 1 concentration 50% by 6.5mm.
4. The higher the concentration of the extract given, the higher the ability of the macrofungal extract to inhibit the growth of the test bacteria.

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