International Seminar on Plant Protection
Faculty of Agriculture, University of Bengkulu
Department of Plant Protection, Faculty of Agriculture, University of Bengkulu, Indonesia

Volume 1, No. 1, June. 2025

Antagonistic Test of *Streptomyces* sp. Against the Growth of *Pestalotiopsis* sp. the Causal Agent of Rubber Leaf Fall Diseases in Vitro

Dwina Alif' Atia*[1], Ismed Setya Budi[2]
[1]*Student of Plant Protection, Faculty of Agriculture, Lambung Mangkurat University*
[2]*Plant Protection, Agriculture Faculty, Lambung Mangkurat University*
*Corresponding author: dwinaalifatia@gmail.com

Abstract

Rubber (*Hevea brasiliensis Muell*. Arg) plays a significant role in Indonesia's economy. However, its production has declined due to several factors, one of which is the leaf fall diseases caused by *Pestalotiopsis* sp. This disease infects all rubber clones, reducing latex yield up to 45%. Therefore, sustainable yet effective and efficient control methods are needed. One of the methods is biocontrol using antagonistic microorganisms. A potential biocontrol agent is the bacterium from the genus Streptomyces, known for producing antibiotic compounds. This study aims to investigate the initial potential of *Streptomyces* sp. to inhibit the growth of *Pestalotiopsis* sp. in vitro. A completely randomized design (CRD) was employed, including four treatments, each replicated five times, two petri dishes per experimental unit and the dual culture method. Results showed that different *Streptomyces* sp. isolates were capable of inhibiting the growth of *Pestalotiopsis* sp., with the highest inhibition observed in isolate PTJ at 50.66%, producing a clear zone of 19.5 mm, with the lowest in isolate PPT at 23.98%. An overgrowth mechanism was also observed, where *Pestalotiopsis* sp. hyphae grow over *Streptomyces* sp. Furthermore, *Streptomyces* sp. not only has the potential to suppress plant diseases but also to enhance plant growth, including height, weight, and leaf numbers. Future research is recommended to explore in vivo potential of *Streptomyces* sp. in inhibiting *Pestalotiopsis* sp., the causal agent of rubber leaf fall disease.

Keywords: Antagonist; *Streptomyces* sp. ; leaf falls; rubber; *Pestalotiopsis* sp.

Introduction

Rubber plants (*Hevea brasiliensis Muell*. Arg) are tropical trees that originally come from the Amazon forest and in Indonesia are included in the second largest rubber producing countries in the world after Thailand (Ministry of Agriculture, 2015). Based on rubber production data in South Kalimantan, out of 13 regencies, only Banjarmasin is not a rubber producing regency. In 2020, rubber production was at 205,646 tons. Then, the following year, namely 2021, it was recorded at 212,956 tons, an increase from the previous year. However, rubber production in South Kalimantan experienced a significant decline in 2022, namely to 198,193 tons (South Kalimantan Communication and Information Service, 2023).

More than 20 types of diseases that cause potential damage to rubber plants, namely in the roots, tapping areas, stems or branches and leaves. Currently, there is one type of leaf disease that is widespread, namely rubber leaf fall disease caused by the fungus *Pestalotiopsis* sp. (Kusdiana, 2020).

The presence of this rubber leaf fall disease attack can cause economic losses or very large ones. Therefore, effective and efficient control is needed in controlling this rubber leaf fall disease. Control of this rubber leaf fall disease is often carried out with chemicals, but chemicals can have negative effects on the environment. Therefore, environmentally friendly control is carried out but can still be said to be effective and efficient in controlling rubber leaf fall disease, one of which is biological control, namely with microorganisms that have antagonistic properties (Tylova *et al*., 2023).

Control by using *Streptomyces* sp. as a biological control agent is a good choice in suppressing the development of rubber leaf fall disease caused by the fungus *Pestalotiopsis* sp.. This *Streptomyces* sp. biological agent functions as a biofungicide that cannot pollute the environment and does not contain any chemicals so it does not cause environmental damage. Even *Streptomyces* sp. bacteria are able to suppress and inhibit the development of pathogenic fungi in the soil and do not cause adverse effects on plants.

Research Method

This study was conducted using a Completely Randomized Design (CRD) with a single factor. The treatments consisted of three different combinations along with one control, each treatment was repeated five times, and each experimental unit consisted of two Petri dishes. Therefore, a total of 40 Petri dishes were used in the experiment, including 10 dishes for the control treatment.

Table 1. Treatment table for each isolate

No.	Purification	Isolate Code
1.	Purification of Isolates frompoint	PPT
2.	Purification of Isolates from Gurang Hirang	PGH
3.	Purification of Isolates fromLanding Point	PTJ

This test was conducted on PDA media using the dual culture method between Actinomycetes bacteria and the pathogenic fungus *Pestalotiopsis* sp. With the cork borer method, namely by placing the pathogen in the middle of the petri dish. While the isolate from Actinomycetes was scratched lengthwise with a distance of 3 cm from the edge of the petri dish to be incubated at room temperature after 7 days the percentage of inhibition was then calculated using the PIRG (Percentage Inhibition of Radial Growth) calculation. The percentage of inhibition of each treatment of *Streptomyces* sp. bacteria will be measured using a caliper. The clear zone that will be produced by *Streptomyces* sp. bacteria is calculated using the percentage inhibition formula (%).

Data analysis

The research data is tested for homogeneity using the Barrlet test. If the Barrlet test results show that the data is homogeneous, then the data is then continued with the ANOVA test to determine whether the treatment has a significant effect. Data analysis is continued with the mean difference test (LSD).

Results and Discussion

Isolation of Pathogens Causing Rubber Leaf Fall Disease.

Isolates were obtained from rubber leaves with symptoms, (Figure 1) on land in the area of Jeruk Street Sungai Ulin, South Banjarbaru District, Banjarbaru City, South Kalimantan. The leaves were then identified using cube media with the characteristics of white hyphae accompanied by black spots which are pycnidia of the pathogen *Pestalotiopsis* sp. which causes rubber leaf fall.

Table 2. Sampling places and symptoms

No.	Isolate	Sampling Place	Symptoms of the disease
1.	*Pestalotiopsis* sp.	Jeruk street Sungai Ulin, Banjarbaru Selatan Subdistrict, Banjarbaru City, South Kalimantan.	The presence of uneven or slightly jagged round spots with the color of the spots in the middle being slightly darker than the edges of the spots and with the presence of very small black spots in the middle of the spots.

Figure 1. Sampling and isolation of leaves showing symptoms of rubber leaf fall.

In sampling symptomatic plants (Figure 1), there were 2 circular spots on the leaves with slightly faded brown leaf spots on the edges of the spots and a slightly darker brown color in the middle, then in the middle of the spots there were very small black spots and many were continued with the isolation of rubber leaves to obtain the fungus that causes leaf fall disease in rubber plants.

Confirmation of *Pestalotiopsis* sp. Isolate

Based on the morphology of the colony seen after 6 days (Figure 2) shows that the colony is white which will turn gray over time, and the growth in the media resembles the shape of a flower, another macroscopic characteristic that will be seen is the emergence of a fruiting body at the top of the hyphae of the isolate called pycnidia.

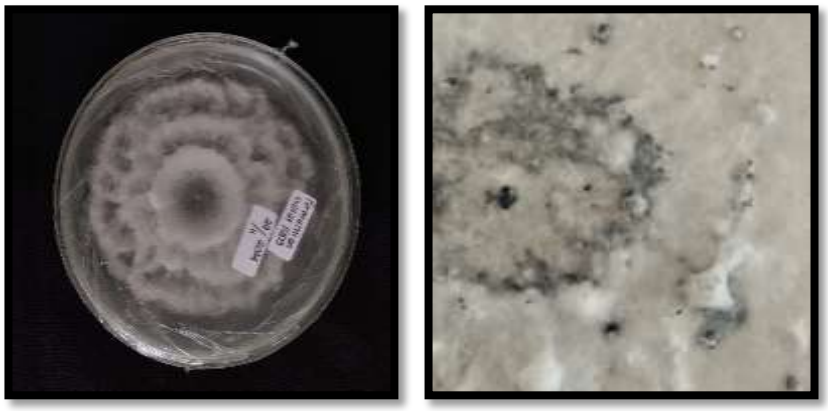


Figure 2. Results of rubber leaf isolation from rubber plants with leaf fall symptoms and macroscopic characteristics of rubber leaf isolates with leaf fall symptoms.

The characteristics produced from (Figure 2) clearly show that there is a fruit body that is often called pycnidia. Pycnidia are the fruit bodies of fungi in the form of black, protruding round spots in very large numbers and have a soft texture.

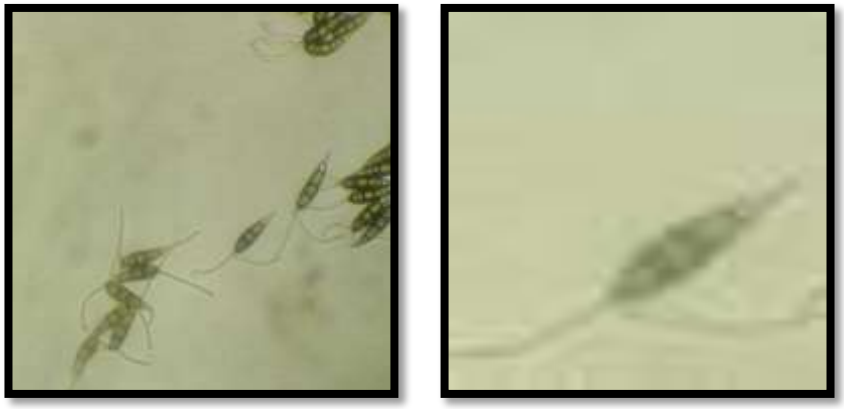


Figure 3. Microscopic characteristics of *Pestalotiopsis* sp. isolates causing rubber leaf fall disease.

The pycnidia described in (Figure 2) are quite numerous in black and have a soft texture when viewed in a microscope and will appear clear as in (Figure 3) where the pycnidia produced are conidia found in the isolate of the fungus *Pestalotiopsis* sp. which has the characteristics of a swollen oval shape with an apical size that is much longer than the basal size, has as many as 4 septa which are black and narrow in size, accompanied by the presence of a spatula. As stated in Febbyanti's 2021 research, the macro characteristics are in the form of fruiting bodies (subepidermal pycnidia accompanied by sori) which are black in color which usually develop around lesion spots in the middle of the leaf (generally towards the leaf veins) which are brown to dark brown in color while the micro characteristics of the *Pestalotiopsis* sp. fungus are in the form of conidia and hyphae. The conidia of the *Pestalotiopsis* sp. fungus are characterized by having 4 septa, the number of septa and the pigment color in the

median cell of the *Pestalotiopsis* sp. fungus with 2 members, even up to 4 cells that appear in the apical part which will be visible if observed microscopically.

Antagonist Test of *Streptomyces* sp. on the growth of *Pestalotiopsis* sp.

In the results of the antagonistic test of *Streptomyces* sp. against the growth of *Pestalotiopsis* sp., each isolate has a different mechanism in suppressing the growth of the fungus *Pestalotiospsis* sp. where the isolate with the isolate code is able to produce quite large inhibition with a very visible clear zone, then the mechanism produced by the PPT and PGH isolates has an overgrow mechanism with fungal hyphae growing on the upper part of the bacteria.

Figure 5. Results of the inhibition test of *Streptomyces* sp. isolate code PTJ against the growth of *Pestalotipsis* sp. which causes rubber leaf fall.

In the test of the isolate *Streptomyces* sp. (Figure 5) it was shown that the inhibitory power produced by the isolate *Streptomyces* sp. PTJ on the last day of observation was 50.33% with the mechanism produced by *Streptomyces* sp. the isolate code PTJ has quite a large potential in inhibiting the growth of *Pestalotiopsis* sp. and produces a clear zone which is an antibiotic produced by the isolate Streptomyces sp. of 19.5 mm. which is an antibiotic released from the isolate *Streptomyces* sp. It can be concluded that the isolate *Streptomyces* sp. It is stated to be able to suppress the growth of *Pestalotiopsis* sp. Causes of Rubber Leaf Fall Disease.

As stated in his research by Syahriyanor *et al*., 2024, the inhibition mechanism that occurs is thought to be due to the presence of antibiotic compounds produced by *Streptomyces* sp. bacteria. as evidenced by the presence of a clear zone in the test carried out with pathogenic fungi. This is supported by Prepagdee's opinion in Syahrianor *et al*., 2024 that *Streptomyces* sp. can produce hydrolytic compounds such as chitinase, which can degrade the cell walls of fungi so that they cannot grow normally. In addition to these mechanisms, antibiotic activity can also inhibit the formation of cell walls, changes in the permeability of target cells, and inhibit the work of enzymes that play a role in pathogen growth, disrupting protein and nucleic acid synthesis.

Figure 6. The results of the inhibition test of *Streptomyces* sp. isolate code PGH on the growth of *Pestalotipsis* sp. which causes rubber leaf fall.

In the test of the isolate *Streptomyces* sp. the PGH code was produced (Figure 6) that the inhibitory power produced by the isolate *Streptomyces* sp. on the last day of observation was 30.63%, without a clear zone then the pathogen grew towards the position of the bacteria and passed through the bacteria, The potential produced by *Streptomyces* sp. as explained in the study of Syahrianor *et al*., 2024 another mechanism is overgrowth where the hyphae of *Pestalotiopsis* sp. grow above the bacteria Streptomyces sp. and experience damage such as, the hyphae will become curly and the hyphae will swell. This is thought to be caused by the presence of compounds produced by *Streptomyces* sp. bacteria which causes a nutritional deficit so that it affects the development of hyphae to become abnormal (Pacios-Michelena et al., 2021). This PGH isolate was only able to inhibit well on the first to fourth day, and on the following day the pathogen began to grow on the upper part of the bacteria, therefore it can be said that *Streptomyces* sp. this PGH isolate is less effective in inhibiting the growth of *Pestalotiopsis* sp. However, this isolate is able to slow down the growth of *Pestalotiopsis* sp., the cause of Rubber Leaf Fall Disease.

Figure 7. Results of the inhibition test of *Streptomyces* sp. isolate code PPT on the growth of *Pestalotipsis* sp. which causes rubber leaf fall.

In the test of *Streptomyces* sp. isolate code PPT (Figure 7) it was shown that the inhibitory power produced by the *Streptomyces* sp. isolate on the last day of observation was 23.63%, in this test this isolate had the smallest inhibitory power compared to PTJ and PGH isolates. This isolate did not produce a clear zone the same as the PGH isolate, but there was another mechanism produced, namely overgrowth with the growth of hyphae on the upper part of the bacteria.

Table 3. Percentage results of the inhibitory power of *Streptomyces* sp. bacteria on the growth of *Pestalotiopsis* sp.

No.	Treatment	Inhibitory Power Percentage (%)
1.	Control	0.00
2.	*Streptomyces* sp. PTJ	50.66[c]
3.	*Streptomyces* sp. PGH	30.88[b]
4.	*Streptomyces* sp. PPT	23.98a

Description: The average value followed by different letters indicates that it is significantly different based on the BNT test at a level of 5%.

The results shown (Table 2) have quite a big difference between the test given each treatment with the control. The control treatment has a percentage of inhibition of 0% and does not have any mechanism, but the treatment of Streptomyces sp. PTJ isolate has a percentage of inhibition of 50.66%, the treatment of Streptomyces sp. PGH isolate has a percentage of inhibition of 30.88%, and the treatment of Streptomyces sp. PPT isolate has a percentage of inhibition of 23.98%.

Table 4. Antibiosis Test Results

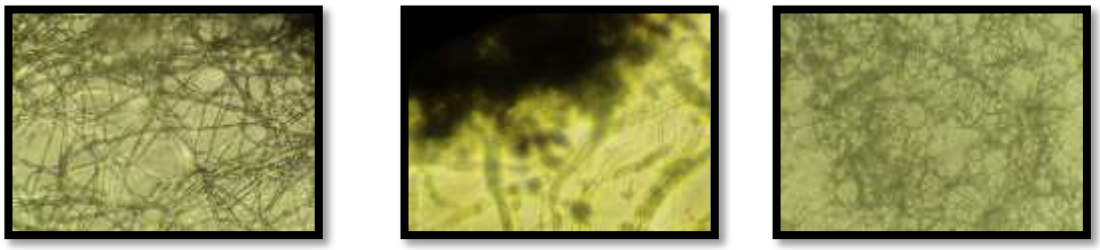
No.	Isolate Code	Clear Zone Width
1.	*Streptomyces* sp. PTJ	19.5 mm

In the research results (Table 4), the results of the antibiotic test produced in the test showed a clear zoba width of 19.5 mm for the isolate code *Streptomyces* sp PTJ.

Table 5. Control mechanism of *Streptomyces* sp. bacteria.

No.	Isolate Code	Control Mechanism		
		Antibiosis	Hyperperasite	*Overgrowth*
1.	PTJ	+	-	-
2.	PGH	-	-	+
3.	PPT	-	-	+

Description: the positive symbol indicates that a mechanism occurs during testing in each treatment.



(a) (b) b(c)

Figure 8. Overgrowth control mechanism (note: a. *Pestalotiopsis* sp. hyphae grow normally. b. *Pestalotiopsis* sp. hyphae grow above Streptomyces sp. bacteria. hyphae become swollen. c. *Pestalotiopsis* sp. hyphae grow above *Streptomyces* sp. bacteria. hyphae become curly).

The results found in (Figure 8) show the difference between normal *Pestalotiopsis* sp. hyphae (Figure 8, a) without the antagonistic bacteria *Streptomyces* sp. with hyphae that experience the impact of the Overgrowth mechanism where the hyphae grow on the top of the antagonistic bacteria and experience swelling in the hyphae (Figure 8, b) and also the hyphae experience curling (Figure 8, c).

Conclusion

The results of testing the *Streptomyces* sp antagonist were found to be able to inhibit the growth of *Pestalotiopsis* sp. with an inhibition percentage above 50%. The highest inhibition percentage is PTJ isolates of 50.66%, while PGH isolates have an inhibition percentage of 30.88% and PPT isolates only have an inhibition percentage of 23.98%. There are 1 isolates that produce clear zones that are suspected of having antibiotic compounds and 2 of them have an Overgrowth mechanism.

References

Dinas Komunikasi dan Informatika Provinsi Kalimantan Selatan. 2023. Data Statistik Sektoral. Dinas Komunikasi dan Informatika Provinsi Kalimantan Selatan.

Kementerian Pertanian. 2013. Pedoman Pengenalan Klon Karet. Jakarta: Kementerian Pertanian.

Kusdiana, A, P, J. 2020. Diagnosis penyakit gugur daun karet (Hevea brasiliensis Muell. Arg.). Jurnal Penelitian Karet, 165-178.

Sahriyanor, A., Mariana, M., & Budi, I. S. (2024). Uji Streptomyces sp. Isolat Lahan Rawa Untuk Menekan Pertumbuhan Colletotrichum sp. Asal Cabai Rawit Varietas Hiyung Secara In Vitro. *JURNAL PROTEKSI TANAMAN TROPIKA*, *7*(2), 922-933.

Tylova, V. N., Bahri, S., Juanda, B. R., & Kusdiana, A. P. J. 2023. Eksplorasi Bakteri Endofit Terhadap Cendawan Pestalotiopsis microspora Penyebab Penyakit Gugur Daun Pada Tanaman Karet (*Hevea brasiliensis* Muell. Arg.). *Jurnal Ilmu-Ilmu Pertanian Indonesia*, 25(1), 51-58.

Pacios-Michelena, S., Aguilar González, C. N., Alvarez-Perez, O. B., Rodriguez-Herrera, R., Chávez-González, M., Arredondo Valdés, R., Ascacio Valdés, J. A., Govea Salas, M., & Ilyina, A. (2021). Application of Streptomyces Antimicrobial Compounds for the Control of Phytopathogens. Frontiers in Sustainable Food Systems, 1-13. https://doi.org/10.3389/fsufs.2021.696518.

Prapagdee, B., Kuekulvong, C., & Mongkolsuk, S. (2008). Antifungal Potential of Extracellular Metabolites Produced by Streptomyces Hygroscopicus Against Phytopathogenic Fungi. International Journal of Biological Sciences, 4(5), 330-337. https://doi.org/10.7150/ijbs.4.330.