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The Potential of *Streptomyces* spp. from Mangrove Land to Control Root-Knot Nematodes

Chowasil Ma'budah[1], Penta Suryaminarsih[1,✉], Wiwin Windriyanti[1]

[1] Department of Agrotechnology, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya, INDONESIA.

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Corresponding Author:
✉ penta_s@upnjatim.ac.id
(Penta Suryaminarsih)

ABSTRACT

Nematode attacks on tomato plants can be controlled using Streptomyces spp. The search for new Streptomyces isolates in mangrove lands is important because they can produce secondary metabolites potential to suppress nematodes. This study aims to determine the ability of Streptomyces spp. to control nematodes in vitro and inhibit root-knot formation. The in vitro test study was arranged in a factorial completely randomized design with three replications. The first factor was Streptomyces isolates from 2 different mangrove lands, namely Gunung Anyar (Surabaya) and Banyurip (Gresik.). The second factor was application concentration (10% and 20%). Data was analyzed using ANOVA continued with DMRT a confidence level of 95% (α = 5%). Results showed that Streptomyces have significant nematicidal activity against J2 of the nematode M. Incognita with Juvenile 2 (J2) mortality rates found to be higher at a concentration of 20% compared to 10%, and mortality rates increased for up to 48 h. The study showed that Streptomyces spp. from the mangrove lands has the potential as a nematicide for J2 root-knot nematode in cherry tomato plants. Treatment with Streptomyces spp. at a concentration of 20% resulted in the highest mortality of 60.21% for Gunung Anyar isolate and 58.33% for Banyurip isolate.

1. INTRODUCTION

Nematode attacks on tomato plants are one of the serious challenges in agricultural production. Nematodes in tomato plants are reported to cause crop losses of up to 29% because tomato plants have a softer root structure so nematode penetration into tomato roots is easier (Wulandari *et al*., 2019). Nematode infection in plants will cause a decrease in the function of the root system and disruption of the vascular bundle tissue. Currently, nematode control uses a lot of chemical pesticides because they kill nematodes quickly (Wuryani *et al*., 2014). Although chemical pesticides can provide a quick solution to nematode attacks, their use poses risks and negative impacts, such as loss of biodiversity, decreased populations of beneficial organisms and adverse impacts on the environment such as water and soil pollution, disrupting the ecosystem of soil biota and causing health problems in humans (Gill & Garg, 2014). Considering the negative impacts that may arise, it is important to find alternatives to more sustainable and environmentally friendly nematode control.

Streptomyces is a Gram-positive bacteria that can be found in mangrove soil. *Streptomyces* spp. has the ability to produce antibiotic compounds in the form of secondary metabolites that are antifungal, antibacterial and antiviral to survive from antagonistic organisms (Suryaminarsih & Mujoko, 2020). One of the characteristics of the genus *Streptomyces* is that its colonies are covered with free air mycelium and hyphae surrounded by a nuclear sheath (Raharini *et al*., 2012). The genus *Streptomyces* is able to produce extracellular hydrolytic enzymes such as chitinase to degrade cell walls (Suryaminarsih & Mujoko, 2020).

Streptomyces research results (Rashad *et al*., 2015) showed significant nematicidal activity against J2 of the

nematode M. *Incognita* with Juvenile 2 (J2) mortality rates found to be higher at a concentration of 20% compared to 10% and mortality rates continued to increase for up to 48 hours. Chitinase activity has been reported to be able to destroy the cuticle of adult nematodes and chitin in nematode eggs (Raharini *et al.*, 2012). *Streptomyces* spp has the ability to produce antibiotic compounds in the form of secondary metabolites that are antifungal, antibacterial and antiviral to survive from its antagonistic organisms (Suryaminarsih & Mujoko, 2020). With this important role, research and development of the search for new *Streptomyces* isolates on a large scale in mangroves began because they were able to produce secondary metabolites that had significant potential. With this important role, *Streptomyces* began to be researched and developed to search for new *Streptomyces* isolates on a large scale in mangroves because they were able to produce secondary metabolites that had significant potential. This study aims to determine the ability of *Streptomyces* spp from mangrove land to control nematodes in vitro.

2. MATERIALS AND METHODS

2.1. Research Implementation

The research was conducted from May to August 2024 at the Plant Health Laboratory I, Faculty of Agriculture, UPN "Veteran" Jawa Timur and the Wonorejo Seed Garden greenhouse. The materials included mangrove soil samples suspected of containing parasitic nematodes *Meloidogyne* spp. Chemicals for analysis consisted of alcohol 70%, GNA media (glucose 1 gr), KH_2PO_4 (1.75 gr), $NaNO_3$ (0.85 gr), KCl (0.75 gr), $MgSO_4.7H_2O$ (2.5 gr), agar (20 gr), sterile distilled water (1000 ml). The tools used in this study included petri dishes, autoclaves (all American Model), shakers (IKA Yelloe line RS 10), LAF (Envirco), micropipettes (Fisherbrand), beakers, Erlenmeyer flasks, glass stirrers, nematode hooks, droppers, bunsen, slides, cover glasses, knives, nematode filters, Baerman funnels, analytical scales (ACIS Model AD 6001), nematode specimen bottles, hot plates (Thermo Scientific).

2.2. Design of Experiment

In vitro test research was conducted according to factorial completely randomized design. The first factor was a four isolates *Streptomyces* spp. consisting of KS1 (*Streptomyces*best spp. 1 from Land 1), KS2 (*Streptomyces*best spp. 2 from Land 1), KG1 (*Streptomyces*best spp. 1 from Land 2), and KG2 (*Streptomyces*best spp. 2 from Land 2). The second factor was the difference in application concentrations consisted of: S1 (10%), and S2 (20%).

2.3. Exploration and Isolation of *Streptomyces* spp.

Exploration of *Streptomyces* spp. was carried out in two different places, namely the Gunung Anyar mangrove, Surabaya and the Banyurip mangrove, Gresik. Sampling was carried out using the purposive sampling method by taking soil between healthy plants and diseased plants with a depth of 10-15 cm to maximize the potential of the soil containing *Actinomycetes* (Hidayatullah, 2020). Then the soil was air-dried before being isolated.

Isolation of Streptomyces spp. was performed using the enrichment method described by Pratista (2019). Dilution was carried out by preparing 6 test tubes filled with 10 ml and 9 ml of sterile distilled water. Then 1 gram of soil was taken and put into a test tube containing 10 ml of sterile distilled water, then vortexed for 1 minute, the soil suspension is a 10^0 dilution. The dilution result was then taken 1 ml using a micropipette and inserted into a test tube containing 9 ml of sterile aquades and becomes 10^{-1}. This activity was carried out until the dilution is 10^{-5}. The dilution levels used are 10^{-4} and 10^{-5}. The dilution result was then taken as much as 0.2 ml using a micropipette and planted in the media GNA (Sodium Glucose Agar), with the spread plate method and leveling the suspension using a spreader then incubated for 24 h. The results of isolation from dilutions of 10^{-4}, and 10^{-5} *Streptomyces* spp. colonies generally have a smooth, floury surface and are white to gray in color (Dhanasekaran & Jiang, 2016).

2.4. Identification of *Streptomyces* spp.

Identification of *Streptomyces* spp. referred to the key book of Waksman & Lechevalier (1953) and was carried out by conducting macroscopic and microscopic observations. Macroscopic observation was done by observing the colony shape and color of *Streptomyces* spp. While microscopic observation was done to observe the shape of *Streptomyces* spp spores. According to Raharini *et al.* (2012), the microscopic form of *Streptomyces* spp has small and branched hyphae, producing spores that form chains and clusters.

2.5. Exploration of *Meloidogyne* spp.

Exploration of *Meloidogyne* spp was carried out by taking soil samples from tomato plants in Pacet District, Mojokerto. Sampling points were taken based on plants showing symptoms of being attacked by *Meloidogyne* spp in the form of swelling in the roots of the plant and damage or reduction or loss of root hairs. Five sampling points were determined a zigzag manner.

Isolation of *Meloidogyne* spp was carried out using the Baermann funnel method. This method is carried out by weighing 100 grams of soil and 10 grams of roots, then each sample is placed on a Baermann funnel set sieve and filled with water until it stagnates. The suspension that has been left for 24-48 hours is taken for later identification.

The chitinase test was carried out to determine the ability of *Streptomyces* spp. isolates to degrade chitin. The implementation began by cutting the *Streptomyces* spp. isolate using a 0.5 mm diameter cork borer and move it to the center of a petri dish containing Colloidal Chitin Agar (CCA) media. The isolate was then incubated for 3 days, and then dripped with iodine solution to see the clear zone formed. The formula for calculating the chitinolytic index follows the formula from (Suryadi *et al*., 2020) as follows:

$$I = d_1/d_2 \quad (1)$$

where I is the chitinolytic index, d_1 is the diameter of clear zone, and d_2 is the colony diameter.

2.6. Calculation of Colony Density of *Streptomyces* spp.

The density of the *Streptomyces* spp. colony used was 10^7 (Rahmiyati *et al*., 2021). *Streptomyces* spp. that had been grown into GNA media in a 100 test tube was then poured with 10 mL of sterile auqadest and vortexed for 1 min, after that 1 ml of suspension was taken and reaction 10^1 was added. This was done repeatedly until dilution 10^7. After dilutions of 10^5, 10^6, and 10^7, 0.2 ml of suspension was taken then planted on GNA media and leveled using a spreader, then incubated for 10 days (Suryaminarsih & Mujoko, 2020) and the colonies that grew were counted.

2.7. Preparation of *Streptomyces* spp. Suspension

The preparation of *Streptomyces* spp. suspension was carried out using EKG media by taking the isolate of *Streptomyces* spp. then inserting it into the EKG media in an Erlenmeyer flask. The isolates used were the 2 best isolates from chitinase. This process was carried out in LAF to minimize the risk of contamination. The bacteria were incubated for 14 days using a shaker at a speed of 120 rpm (Agustin *et al*., 2023). *Streptomyces* spp from the EKG media culture were taken as much as 10 ml and 20 ml then dissolved in 100 ml of sterile distilled water.

2.8. In Vitro Nematicidal Ability Test of *Streptomyces* spp. Against Juvenile Root Knot Nematodes

The test of the nematicidal ability of *Streptomyces* spp. at different concentrations and lands was conducted in vitro in the Plant Health laboratory, Department of Agrotechnology, UPN "Veteran" East Java referring to the method of Chandel *et al*. (2014). A total of 30 juveniles were put into sterile eppendorf tubes, added with *Streptomyces* spp. suspension from 2 different lands with concentrations of 10% and 20%. Incubation was carried out at room temperature. Observations were made using a compound microscope (Olympus CX33). The level of nematicidal ability of *Streptomyces* spp. was carried out through observations of root knot nematode mortality.

2.9. Data Analysis

Data obtained from the nematicidal ability test of *Streptomyces* spp. against juvenile root-knot nematodes were then analyzed using Analysis of Variance (ANOVA). If it is known that there is a significant difference in the effect of the treatment, further testing will be carried out using Duncan with a confidence level of 95% ($\alpha = 5\%$). Data analysis was carried out using IBM SPSS Statistic 24 software.

3. RESULTS AND DISCUSSION

3.1. Isolation of *Streptomyces* spp. Exploration Results

Identification of *Streptomyces* spp. was done by macroscopic and microscopic observation. Macroscopic observation was done by directly observing the aroma, color, and shape of the colony. The results of isolation, macroscopic

observation and chitinase can be seen in table 1. From the results of the chitinase test, the *Streptomyces* spp. isolates tested had 4 isolates that had the highest chitinolytic index, namely in G1 of 7.4 G2 of 10, S1 of 8.8, and S2 of 8.4. The chitinolytic index is classified as high or strong if the value is > 2, and low or weak if < 2 (Sembiring *et al*., 2021; Hardoko *et al*., 2020; Setia & Suharjono, 2015).

Table 1. Macroscopic identification results of *Streptomyces* Spp.

Isolat	Macroscopic Characteristics				Chitinase
	Colony Color	Colony Form	Edge	Aroma	
Gresik 1	Old Grey	Irregular	Uneven	Soil	7.4
Gresik 2	Yellow	Irregular	Uneven	Soil	10
Gresik 3	Light Grey	Round	Flat	Soil	6.5
Gresik 4	Yellow Grey	Round	Uneven	Soil	5.5
Gresik 5	Dark Grey	Round	Flat	Soil	2.2
Surabaya 1	Brownish-Yellow	Round	Uneven	Soil	8.8
Surabaya 2	Pink	Round	Uneven	Soil	8.4
Surabaya 3	Yellowish Grey	Regular	Flat	Soil	4.2
Surabaya 4	Grayish Pink	Round	Flat	Soil	4.4
Surabaya 5	Grayish Yellow	Irregular	Uneven	Soil	3.8

The results of macroscopic observations show that the *Streptomyces* spp. isolates obtained have different characteristics between isolates. Figure 1 shows *Streptomyces* isolate (G1) has the characteristics of a thick, dark gray colony with a floury colony texture, an uneven surface. *Streptomyces* isolate (G2) has the characteristics of a yellow colony with a brownish center on the surface, the surface is flat. *Streptomyces* isolate (G3) has the characteristics of a light gray colony with a floury texture, an uneven and spreading surface, (G4) has an uneven colony shape with radial lines, yellow-gray in color. The surface of the colony is floury and smooth, *Streptomyces* isolate (G5) has a the circular shape of a floury colony with a gray color, and a flat colony surface, *Streptomyces* isolate (S1) has characteristics of a floury colony with a pink color, flat colony growth, *Streptomyces* isolate (S2) has characteristics of a floury colony with a flat colony surface and reddish yellow. *Streptomyces* isolate (S3) has a flat colony and is yellowish gray, *Streptomyces* (S4) has characteristics of a thick floury colony with a flat surface and is pinkish gray *Streptomyces* (S5) has characteristics of a floury colony with a flat surface and is yellow grayish.

Macroscopically, all *Streptomyces* isolates found have a round shape. However, they have different edges, some have flat edges, or not. The color of the *Streptomyces* colony is, gray, yellow to pink. The *Streptomyces* sp colonies found have a general shape of a round colony, a floury colony surface and uneven colony edges. The Actinomycetes group has different colony colors because the pigments that make up their cells are different. The presence of pigment in cells and spore chains indicates that the actinomycetes isolate found is a colony of *Streptomyces* spp. (Sari *et al*., 2019).

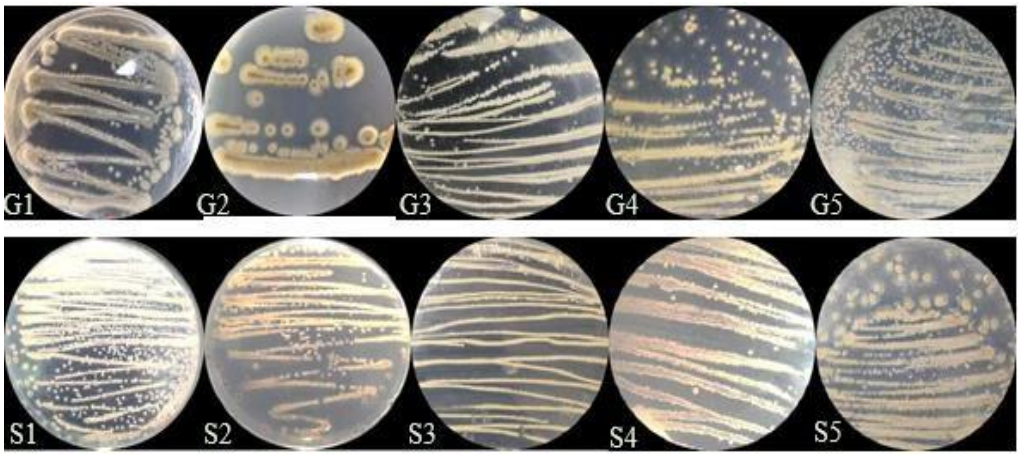

Figure 1. *Streptomyces*spp. Exploration Results

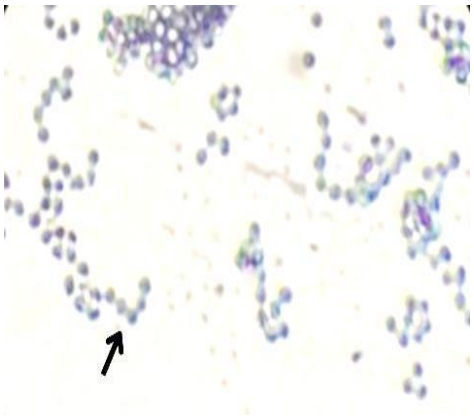

Figure 2. Chain of spores of *Streptomyces* spp. (Magnification 10x100)

(2019). The floury surface is a collection of hyphae consisting of many spores. The mycelium is initially white to brown, but over time it changes to a certain color.

Microscopic observation, *Streptomyces* spp. obtained have round-shaped spores, and form flexuous spore chains that cluster (Figure 2). This is in accordance with the statement Rahmiyati *et al*. (2021), that *Streptomyces* sp. spores are round and their conidia are in the form of chains that cluster at the ends of aerial hyphae. The growth of *Streptomyces* spp. is initially round with a smooth surface, then forms an aerial mycelium that changes into a flour-like shape. Differentiation of aerial hyphae into spores through cell division and maturation (Jones *et al*., 2013).

3.2. Effect of *Streptomyces* spp. on the Mortality of Root-Knot Nematodes

Table 2 shows effect of *Streptomyces* spp. treatment with different isolates and concentrations on root-knot nematode mortality. The results of the analysis of variance in the test showed a significant effect in increasing nematode mortality. The mortality ability of the treatment against juvenile nematodes *Meloidogyne* spp. ranged from 18.33% - 60.21%. Seen 24 hours after treatment and began to increase 48 hours after treatment in the initial observation after 24 hours, the treatment of *Streptomyces* spp. Surabaya land with a concentration of 20% (KS1S2) showed a fairly high mortality rate of 31.67%, compared to the control which was only 8.83%. After 48 hours of treatment it became increasingly clear, that treatment of *Streptomyces* spp. Surabaya land with a concentration of 20% (KS1S2) and *Streptomyces* spp. Gresik land with a concentration of 20% (KG2S2) reached the highest mortality rates, respectively 60.21% and 58.33%gave a significantly greater suppression effect than the control treatment which was only 38.33%.

The ability of *Streptomyces* to suppress the mortality of juvenile root nematodes cannot be separated from the role of the toxin compound content that is nemasticidal for juvenile nematodes. This is in line with Hallmann *et al*. (1997]) compounds produced by *Streptomyces*, one of which is a hydrolyzing enzyme such as chitinase, protease, cellulose, lipase and pectinase. In addition, the use of higher concentrations of *Streptomyces* ensures that there are enough bioactive agents in contact with nematodes, thereby increasing the possibility of cell wall degradation and ultimately

Table 2. Effect of *Streptomyces* spp. treatment with different isolates and concentrations on root-knot nematode mortality.

No	Treatment	24 hours	48 hours
1	Control	8.83 a	38.33 a
2	Gresik 1 10% (KG1S1)	23,33 bcd	43.33 ab
3	Gresik 1 20% (KG1S2)	26.67 cd	51.67 ab
4	Gresik 2 10% (KG2S1)	11.67 ab	41.67 ab
5	Gresik 2 20% (KG2S2)	33.33 cd	58.33 ab
6	Surabaya 1 10% (KS1S1)	18.33 abc	46.33 ab
7	Surabaya 1 20% (KS1S2)	36.67 d	60.21 b
8	Surabaya 2 10% (KS2S1)	18.33 abc	48.33 ab
9	Surabaya 2 20% (KS2S2)	23.33 bcd	53.33 ab

Note: Numbers accompanied by the same letters indicate no significant difference in the DMRT test at the 95 % confidence level.

killing nematodes. This is in accordance with Rashad *et al*. (2015) which states that a concentration of *Streptomyces* of 20% is sufficient to produce a significant nematicidal effect on root-knot nematodes. This concentration is effective in degrading nematode cell walls in a shorter time, usually within 24 to 48 h.

The ability of *Streptomyces* to suppress the mortality of juvenile root nematodes cannot be separated from the role of the toxin compound content that is nemasticidal for juvenile nematodes. This is in line with Hallmann *et al*. (1997]) compounds produced by *Streptomyces*, one of which is a hydrolyzing enzyme such as chitinase, protease, cellulose, lipase and pectinase. In addition, the use of higher concentrations of *Streptomyces* ensures that there are enough bioactive agents in contact with nematodes, thereby increasing the possibility of cell wall degradation and ultimately killing nematodes. This is in accordance with Rashad *et al*. (2015) which states that a concentration of *Streptomyces* of 20% is sufficient to produce a significant nematicidal effect on root-knot nematodes. This concentration is effective in degrading nematode cell walls in a shorter time, usually within 24 to 48 h.

Observation results of *Meloidogyne* sp. mortality due to *Streptomyces* spp. parasitism showed that juveniles experienced mortality conditions with varying damage (Figure 3). The nematode body experienced degradation both in the body wall and in the internal organs of the nematode. Nematodes that did not experience damage still had serrations on the outer layer of the body clearly visible. Nematodes that died in the in vitro test showed damage caused by chitinolytic activity by the chitinase enzyme produced.

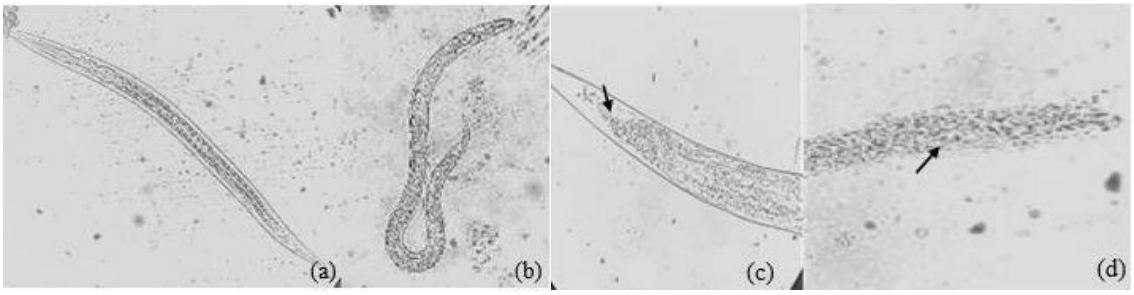

Figure 3. Effect of chitinase enzyme on nematode body. (a) Dead *Meloidogyne* spp. body without damage (Magnification 40 x 10), (b) Degraded and damaged inner body (Magnification 100 x10), (c) Living *Meloidogyne* spp. (Magnification 4- x10), (d) Lysed *Meloidogyne* spp. (Magnification 100 x10).

The mechanism of *Streptomyces* in suppressing the juvenile nematode population is that it is able to produce various secondary metabolites such as antibiotics, actinomycin, and other toxic compounds that are nematicidal. These metabolites can inhibit nematode mobility and even cause direct death in J2. In addition, according to Narayana & Vijayalakshmi (2009) *Streptomyces* also produces chitinase enzymes that can degrade cell walls. These enzymes help in directly damaging the structure of nematodes, especially at the J2 stage, thereby reducing resistance and reducing the nematode population in the soil and roots.

The chitinase enzyme produced by *Streptomyces* is used to penetrate *Meloidogyne* sp. and hydrolyze the eggs of the nematode *Meloidogyne* sp. which are mostly composed of chitin. Cuticle penetration is carried out by initiating spore growth on the cuticle. Bacterial spores attach to the nematode's body then germinate and penetrate the nematode's cuticle until they affect the nematode's nervous system and cause acetylcholine to accumulate (Istiqomah & Aisyah, 2023).

4. CONCLUSION

Based on the research on the potential of *Streptomyces* spp. mangrove land as a control of root-knot nematodes of cherry tomato plants, it can be concluded that. *Streptomyces* spp from the results of mangrove land exploration has the potential as a nematicide J2 for root-knot nematodes. Treatment of *Streptomyces* spp. using a concentration of 20% from both mangrove lands was the highest result which affected mortality by 60.21% and 58.33%.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
CM	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
PS	✓	✓		✓		✓	✓	✓		✓		✓	✓	
WW				✓			✓			✓		✓		

C: Conceptualization
M: Methodology
So: Software
Va: Validation
Fo: Formal Analysis
I: Investigation
D: Data Curation
R: Resources
O: Writing - Original Draft
E: Writing - Review & Editing
Vi: Visualization
Su: Supervision
Fu: Funding Acquisition
P: Project Administration

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