

Hydrolysis activity of sweet potato flour (*Ipomoea batatas* L.) by bacterial isolates from the rhizosphere of cat's whiskers (*Orthosiphon stamineus*)

Trivena Sinaga¹, Finna Piska^{2*}, Razoki²

Abstract

Background: Starch is a polysaccharide that can be hydrolyzed into simpler sugars by amylase-producing microorganisms.

Objective: This study evaluated the starch-hydrolyzing activity of six bacterial isolates from the rhizosphere of cat's whiskers (*Orthosiphon stamineus*) using purple sweet potato (*Ipomoea batatas* L.) starch as the substrate.

Methods: The isolates, designated RKK-1 through RKK-6, were screened using a modified disc diffusion method on starch agar. Amylolytic activity was assessed by iodine staining and measuring the clear-zone diameter after 24, 48, and 72 hours of incubation at 37°C.

Results: Five isolates, RKK-1 through RKK-5, produced detectable clear zones, whereas RKK-6 showed no hydrolysis zone. Clear-zone diameters generally increased with incubation time. After 72 hours, RKK-3 demonstrated the highest activity, with a clear-zone diameter of 20.34 mm, followed by RKK-2 with 18.32 mm.

Conclusion: These findings indicate that most of the tested rhizosphere bacterial isolates can hydrolyze purple sweet potato starch and may serve as potential sources of amylase. However, quantitative enzyme assays, appropriate controls, molecular characterization, and safety evaluations are required before pharmaceutical or industrial applications can be considered.

Keywords: amylase; rhizosphere bacteria; starch hydrolysis; purple sweet potato; *Orthosiphon stamineus*

Introduction

Starch is a carbohydrate composed primarily of amylose and amylopectin and serves as an important energy source for humans and animals. Amylose consists of linear glucose chains linked by α -1,4-glycosidic bonds, whereas amylopectin has a branched structure containing α -1,4-glycosidic bonds in its linear regions and α -1,6-glycosidic bonds at the branch points. Starch can be extracted from carbohydrate-rich plants, including purple sweet potatoes (*Ipomoea batatas* L.).¹

Purple sweet potatoes are important food crops widely cultivated, particularly in tropical regions.² In addition to serving as an energy source, they contain important nutrients, including starch, protein, vitamin C, and vitamin A in the form of β -carotene. They are also rich in anthocyanins, which function as natural antioxidants and give the tubers their characteristic purple color.³ The starch in purple sweet potatoes can serve as a carbohydrate substrate for microorganisms, including rhizosphere bacteria associated with cat's whiskers (*Orthosiphon stamineus*).

Bacteria can adapt to diverse environmental conditions and utilize available carbohydrates as energy sources for growth.⁴ This study used bacterial isolates obtained from the rhizosphere of cat's whiskers, designated RKK-1, RKK-2, RKK-3, RKK-4, RKK-5, and RKK-6. Rhizosphere bacteria can produce metabolites that vary according to their soil and root habitats.⁵ These isolates have also been evaluated for their ability to produce antibacterial

Affiliation

¹Undergraduate Program in Clinical Pharmacy, Universitas Prima Indonesia

²Department of Clinical Pharmacy, Universitas Prima Indonesia

*Correspondence

finnapiska@unprimdn.ac.id

bioactive compounds against *Escherichia coli* and *Staphylococcus aureus*.⁶ Their production of secondary metabolites may depend on habitat conditions and nutrient availability.⁷

Rhizosphere bacteria associated with cat's whiskers may also produce amylase, an enzyme capable of hydrolyzing starch. Amylase has potential as a starch-hydrolyzing agent for industrial and pharmaceutical applications.⁸ Amylase-based preparations may also contribute to intestinal microbiota balance, and digestive enzyme supplementation has been reported to improve nutrient utilization in patients with digestive disorders.⁹ Therefore, investigating the ability of rhizosphere bacteria to increase nutrient availability through the utilization of purple sweet potato starch is relevant to the development of sustainable and environmentally friendly food-processing approaches.

Method

Study design

This study used a descriptive experimental design to screen the starch-hydrolyzing activity of six rhizosphere bacterial isolates, designated RKK-1, RKK-2, RKK-3, RKK-4, RKK-5, and RKK-6, obtained from cat's whiskers (*Orthosiphon stamineus*). A modified disc diffusion method was used as an initial screening method to evaluate amylase production using purple sweet potato starch (*Ipomoea batatas* L.) as the carbohydrate substrate. This method was selected to compare the relative hydrolytic activity of the isolates under standardized inoculum conditions. The study was conducted at the Integrated Research Laboratory, Universitas Prima Indonesia, Medan, Indonesia, from March to July 2025. This period included sample preparation and experimental procedures.

Samples

The samples consisted of six rhizosphere bacterial isolates, designated RKK-1 through RKK-6, obtained from cat's whiskers (*O. stamineus*). Fermentation was performed in liquid medium supplemented with purple sweet potato starch as the carbohydrate source.

Equipment and materials

The equipment included Petri dishes, a Labnet incubator, a Hirayama autoclave, beakers, inoculating loops, a refrigerator, a digital caliper, a Shimadzu analytical balance, a Shimadzu ultraviolet-visible spectrophotometer, a hot-plate stirrer, a vortex mixer, and micropipettes. The primary materials included purple sweet potato starch, nutrient agar (NA), distilled water, the six rhizosphere bacterial isolates, nutrient broth (NB), physiological saline, iodine solution, sodium nitrate, magnesium sulfate, zinc sulfate, manganese sulfate, potassium chloride, dipotassium hydrogen phosphate, and agar.

Collection and preparation of purple sweet potato starch

Purple sweet potatoes (*I. batatas* L.) were purchased from the MMTC central market in Medan and originated from Sidikalang, Dairi Regency. Intact tubers at harvest maturity were selected, peeled, and washed under running water. The tubers were then sliced into uniformly thin pieces using a slicer. The slices were blended until smooth and mixed with 100 mL of distilled water. The homogenate was filtered through a filter cloth to separate the pulp from the filtrate. The filtrate was allowed to stand at room temperature for 24 hours to permit complete starch sedimentation. The supernatant was carefully discarded, and the sedimented starch was dried in an oven at 60°C for 24 hours. The dried starch was ground to remove clumps and passed through a 60-mesh sieve to obtain a uniform particle size. The resulting starch powder was stored in a tightly closed container in a dry location until use as the substrate for starch-hydrolysis testing.

Preparation of bacterial cultures in nutrient broth

Nutrient broth powder weighing 2.4 g was dissolved in 1,000 mL of distilled water and heated on a hot-plate stirrer until boiling. The medium was sterilized in an autoclave at 121°C for 15 minutes. The sterile NB was dispensed into six sterile tubes. Each tube was inoculated with 0.1 mL of one bacterial isolate, RKK-1 through RKK-6, using a micropipette. The tubes were vortexed until homogeneous and incubated for 24 hours. Turbidity of the medium indicated bacterial growth.

Preparation of nutrient agar

NA powder weighing 3.36 g was dissolved in 1,000 mL of distilled water in a beaker and heated on a hot-plate stirrer until boiling. The medium was then sterilized in an autoclave at 121°C for 15 minutes and used for bacterial subculture.

Bacterial subculture

The six rhizosphere bacterial isolates were subcultured on sterile NA using the T-streak method. The plates were incubated at 37°C for 24 hours.

Preparation of the purple sweet potato starch-hydrolysis medium

Purple sweet potato starch weighing 7.5 g was mixed with 0.75 g of sodium nitrate, 0.25 g of magnesium sulfate, 0.25 g of zinc sulfate, 0.25 g of manganese sulfate, 0.125 g of potassium chloride, 0.125 g of dipotassium hydrogen phosphate, 0.005 g of NB, and 4.25 g of agar. The mixture was dissolved in 250 mL of distilled water in a 500-mL beaker and heated on a hot-plate stirrer until boiling. The medium was sterilized in an autoclave at 121°C for 15 minutes and then incubated for 24 hours to verify sterility.

Standardization of bacterial suspensions

The bacterial suspensions were standardized by measuring absorbance at 660 nm using a UV-Vis spectrophotometer. A small amount of each bacterial colony grown on NA was transferred with an inoculating loop into a test tube containing 7 mL of sterile physiological saline. Each suspension was vortexed until homogeneous and adjusted to an absorbance of 0.1 at 660 nm. Sterile paper discs were then immersed in the standardized bacterial suspensions and placed on the starch-hydrolysis medium. The plates were incubated at 37°C for 24, 48, and 72 hours.

Starch-hydrolysis assay

After 24, 48, and 72 hours of incubation, iodine solution was added to the plates containing the paper discs. The formation of a clear zone surrounding a disc indicated starch hydrolysis by extracellular amylase. Clear-zone diameters were measured using a digital caliper. The zones were observed and recorded using a colony counter when appropriate. The 72-hour incubation period was selected to monitor the progressive development of hydrolysis zones. However, prolonged incubation may result in the accumulation of secondary metabolites or changes in the medium caused by bacterial metabolism, potentially affecting clear-zone size. Further studies should therefore determine the optimal incubation period for amylase activity.

Data analysis

The clear-zone diameters were measured using a digital caliper. The data were tabulated and presented descriptively. The results were then interpreted narratively and used to formulate the study conclusions.

Results

The six rhizosphere bacterial isolates obtained from cat's whiskers (*Orthosiphon stamineus*) were evaluated for amylase production using purple sweet potato starch (*Ipomoea batatas* L.) as the carbohydrate substrate. Amylolytic activity was indicated by the formation of clear zones surrounding the paper discs after iodine staining. Each treatment was performed in triplicate, and clear-zone diameters were measured after 24, 48, and 72 hours of incubation using a digital caliper.

Table 1. Clear-zone diameters produced by rhizosphere bacterial isolates on purple sweet potato starch medium

Isolate code	24 hours (mm)	48 hours (mm)	72 hours (mm)	Zone shape
RKK-1	7.03	13.06	15.19	Circular
RKK-2	7.17	16.46	18.32	Circular
RKK-3	7.58	19.25	20.34	Circular
RKK-4	7.03	11.06	16.93	Circular
RKK-5	7.05	12.50	15.76	Circular
RKK-6	No clear zone	No clear zone	No clear zone	Not formed

Note: RKK denotes rhizosphere isolate from cat's whiskers.

Five isolates, RKK-1 through RKK-5, produced clear zones, whereas RKK-6 did not produce a detectable zone during any observation period. Clear-zone diameters generally increased with longer incubation. At 72 hours, RKK-3 produced the largest zone, measuring 20.34 mm, followed by RKK-2, which produced a zone measuring 18.32 mm. RKK-4, RKK-5, and RKK-1 produced zones measuring 16.93, 15.76, and 15.19 mm, respectively.

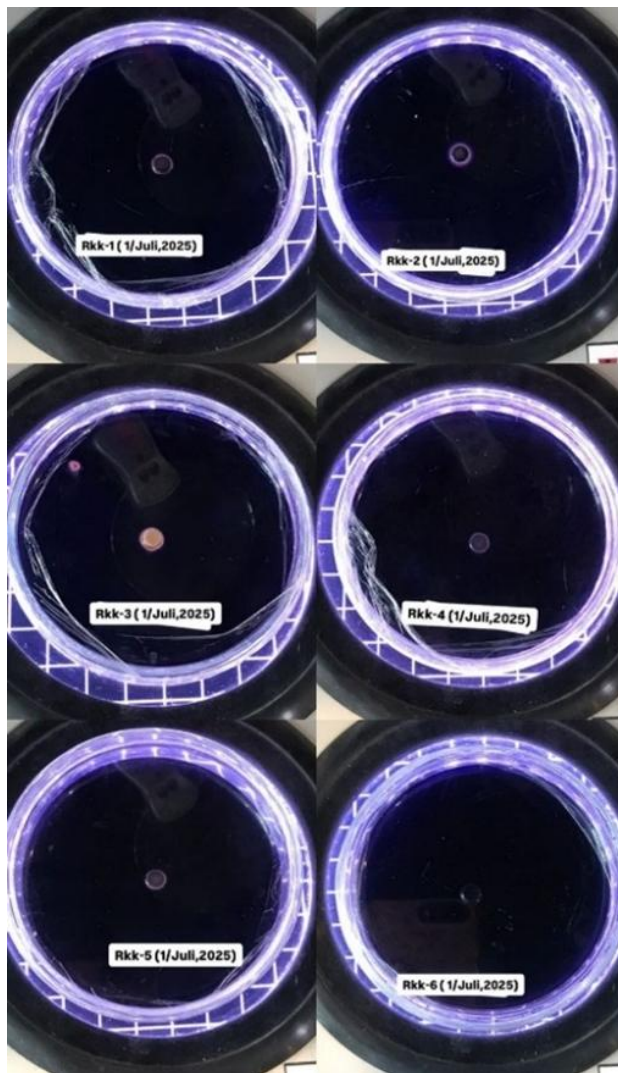


Figure 1. Clear-zone formation by isolates RKK-1 through RKK-6 after 24 hours of incubation

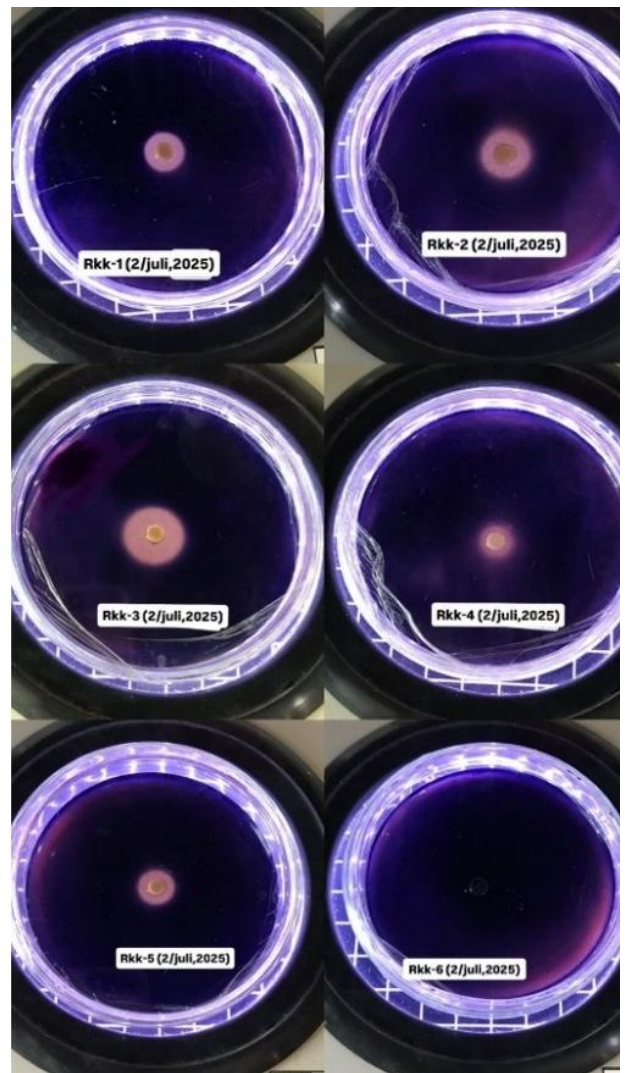


Figure 2. Clear-zone formation by isolates RKK-1 through RKK-6 after 48 hours of incubation

The hydrolysis results indicated that RKK-3 and RKK-2 exhibited the highest activity throughout the observation period. At 24 hours, the clear-zone diameters produced by RKK-3 and RKK-2 were 7.58 and 7.17 mm, respectively. These diameters increased to 19.25 and 16.46 mm at 48 hours and to 20.34 and 18.32 mm at 72 hours.

Discussion

Purification of the rhizosphere bacterial isolates was performed to ensure that the cultures used in the assay were free from contamination by other microorganisms. This step was intended to obtain bacterial colonies with consistent characteristics so that their enzymatic activity could be evaluated accurately. The T-streak method was performed on agar medium containing purple sweet potato starch to separate colonies according to their morphological characteristics.^{10,11} The resulting single colonies were subcultured and incubated again to confirm the purity and stability of the isolates before the enzyme-activity assay.

Starch-hydrolysis activity was evaluated qualitatively to determine the ability of the bacterial isolates to produce amylase.¹² The assay was based on the reaction between starch and iodine. Unhydrolyzed starch reacts with iodine and produces a dark blue to black color. In contrast, areas where starch has been hydrolyzed by amylase do not react with iodine, resulting in a clear zone around the bacterial growth.¹³ The presence and diameter of the clear zone provide an indication of the isolate's ability to produce and secrete amylase into the medium.

Amylase produced by rhizosphere bacteria catalyzes the hydrolysis of α -(1,4)-glycosidic bonds in starch.¹⁴ The extracellular enzyme hydrolyzes purple sweet potato starch into simpler sugars, such as maltose and glucose. These hydrolysis products can then be used by the bacteria as sources of carbon and energy to support growth and metabolic activity.¹⁵

In this study, isolates RKK-3 and RKK-2 demonstrated the greatest starch-hydrolysis activity. This finding may be related to genetic differences, metabolic capacity, and greater amylase-production capacity.¹⁶ In contrast, RKK-6 did not produce a detectable clear zone. However, this result does not necessarily indicate that the isolate is unable to utilize starch. The absence of a visible hydrolysis zone may reflect low extracellular amylase production, differences in amylase gene regulation, the bacterial growth phase, or suboptimal culture conditions. The amount of enzyme produced may also have been insufficient to generate a visually detectable zone. These possibilities should be evaluated using quantitative enzyme-activity assays.¹¹

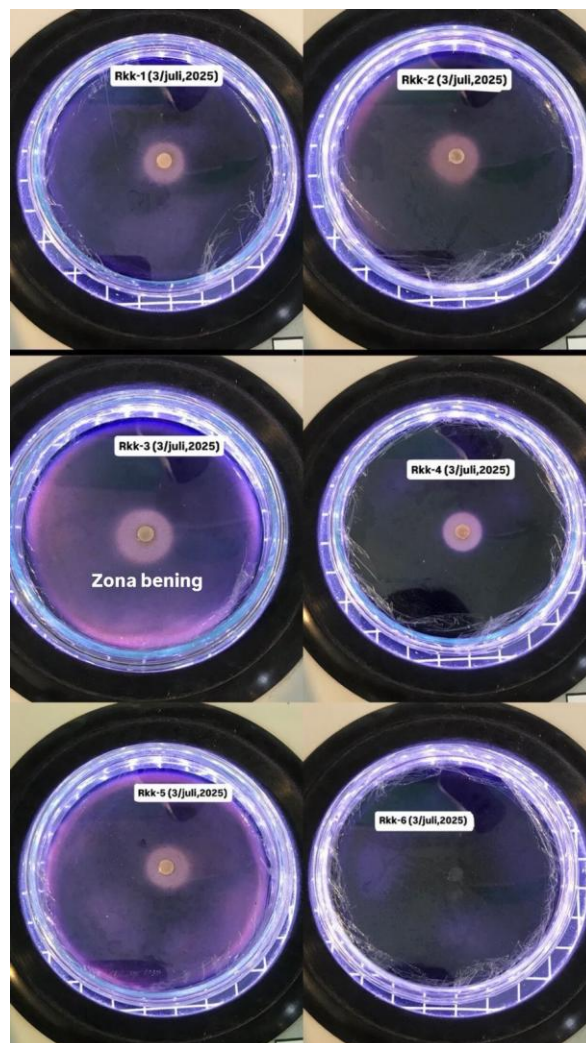


Figure 3. Clear-zone formation by isolates RKK-1 through RKK-6 after 72 hours of incubation

Table 2. Hydrolytic activity of the most active isolates, RKK-3 and RKK-2

Incubation period	RKK-3 (mm)	RKK-2 (mm)	Zone shape
24 hours	7.58	7.17	Circular
48 hours	19.25	16.46	Circular
72 hours	20.34	18.32	Circular

Amylase has several potential pharmaceutical applications, particularly as an ingredient in digestive enzyme preparations or supplements.¹⁷ By facilitating the breakdown of complex carbohydrates into simpler sugars, amylase may support carbohydrate digestion and nutrient absorption. Enzyme supplementation may be beneficial for individuals with impaired digestive enzyme production, although its clinical effectiveness and safety require appropriate evaluation.¹⁸ Therefore, amylase produced by rhizosphere bacterial isolates may have potential for development as an active ingredient in enzyme-based pharmaceutical products. Further studies should include quantitative activity assays, enzyme purification, stability testing, molecular identification of the isolates, and assessment of safety and functional performance.

Conclusion

Five of the six rhizosphere bacterial isolates from cat's whiskers, namely RKK-1, RKK-2, RKK-3, RKK-4, and RKK-5, hydrolyzed purple sweet potato starch, as indicated by the formation of clear zones after iodine staining. Isolate RKK-3 exhibited the highest hydrolytic activity, followed by RKK-2. These findings indicate that the tested rhizosphere isolates may serve as potential sources of amylase. However, quantitative enzyme assays, appropriate positive and negative controls, molecular characterization, and further functional and safety evaluations are required before their pharmaceutical or industrial applications can be considered.

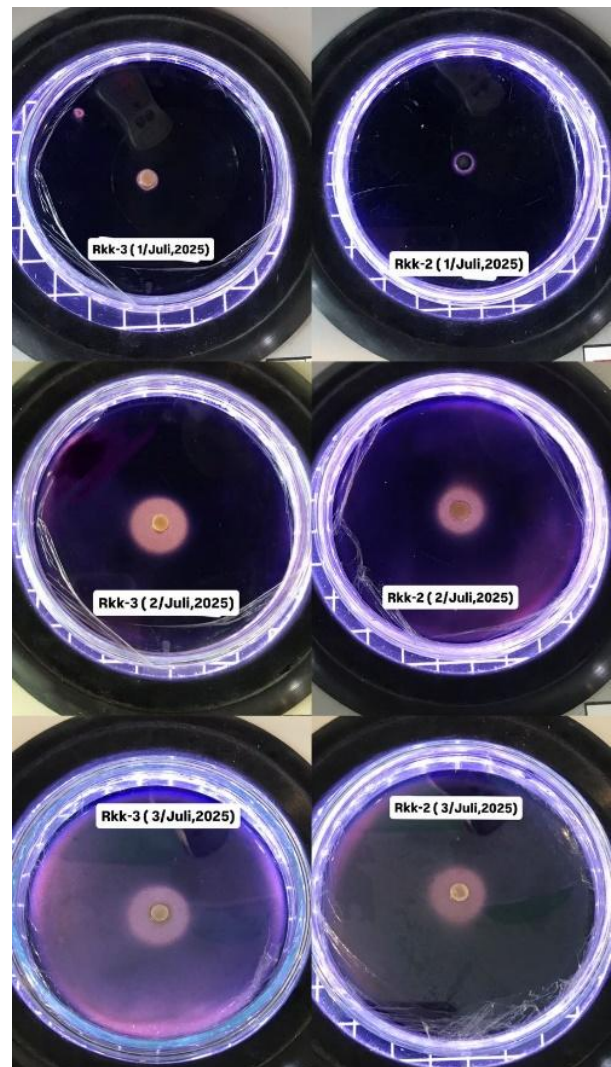


Figure 4. Clear-zone diameters produced by the most active isolates, RKK-3 and RKK-2

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