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Isolation and Screening of β-Cyclodextrin Glucanotransferase-Producing Bacteria from Papaya Rhizosphere Soil

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Abstract

Cyclodextrin glucanotransferase (CGTase) is an enzyme produced by several microorganisms and has an important role in the conversion of starch into cyclodextrins, including β-cyclodextrin. Soil is a diverse microbial habitat and represents a potential source for the exploration of microorganisms with useful enzymatic activities. This study aimed to isolate and screen β-CGTase-producing bacteria from papaya (Carica papaya L.) rhizosphere soil and to characterize selected isolates based on their phenotypic and biochemical properties and β-cyclodextrin production. Rhizosphere soil samples were collected from three papaya plantations at depths of approximately 5–20 cm, with five sampling points from each plantation. Bacterial isolation and screening were performed using Horikoshi medium under alkaline conditions. Eight bacterial isolates were obtained, of which three isolates, designated CG-P1, CG-P2, and CG-P3, showed a yellow hollow zone indicative of CGTase activity. The selected isolates were Gram-positive, rod-shaped, spore-forming, catalase-positive, and motile, with phenotypic characteristics consistent with members of the genus Bacillus. The selected isolates showed active growth during approximately 8–24 h of incubation, with 24 h identified as the optimum incubation time for CGTase production under the tested conditions. β-Cyclodextrin production reached 94.129%, 93.620%, and 94.320% for CG-P1, CG-P2, and CG-P3, respectively. CG-P3 showed the highest β-cyclodextrin production and produced the largest yellow hollow zone, with a diameter of 3.2 cm. Optimization of the reaction period showed that β-cyclodextrin formation was optimal at approximately 30 minutes. These results indicate that papaya rhizosphere soil is a promising source of β-CGTase-producing bacteria, with CG-P3 representing the most promising isolate for further molecular and enzymatic characterization.

Keywords: *Bacillus*; β-cyclodextrin; CGTase; papaya rhizosphere; screening.

Abbreviations: β-CD: β-cyclodextrin; CGTase: cyclodextrin glucanotransferase.

INTRODUCTION

Soil is one of the most diverse microbial habitats and contains microorganisms with a wide range of metabolic capabilities. This diversity makes soil an important source for bioprospecting, particularly for the discovery of microorganisms and enzymes with potential applications in biotechnology. Microbial bioprospecting has increasingly focused on exploring underutilized environments and microbial communities to identify biological resources with useful functional properties (Boddu et al. 2022; Selvakumar et al. 2024). Agricultural soils are of particular interest because their microbial communities are influenced by plants, soil characteristics, and cultivation practices, creating opportunities to obtain microorganisms with distinctive metabolic activities.

One enzyme of considerable biotechnological interest is cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19). CGTase catalyzes the conversion of starch into cyclic oligosaccharides known as cyclodextrins. The principal products are α-, β-, and γ-cyclodextrin, which contain six, seven, and eight glucose units, respectively. Among these products, β-cyclodextrin is particularly useful because its hydrophobic cavity enables the formation of inclusion complexes with various guest molecules. This property has contributed to the broad application of cyclodextrins in pharmaceutical, food, cosmetic, and other industrial processes (Pardhi et al. 2023).

CGTase-producing microorganisms have been reported from various environments, particularly alkaline soils. Members of the genus *Bacillus* are among the most frequently reported bacterial groups associated with CGTase production. However, the production characteristics of CGTase may vary substantially among isolates. Differences in growth conditions, incubation period, enzyme characteristics, and reaction conditions can influence the amount and type of cyclodextrin produced (Saini et al. 2022; Pardhi et al. 2025).

Therefore, exploration of new microbial sources remains relevant for obtaining bacterial isolates with favorable β-cyclodextrin-producing characteristics.

The rhizosphere is an especially interesting environment for microbial exploration because plant roots interact continuously with surrounding microorganisms. These interactions may influence the composition and functional characteristics of the microbial population in soil. Previous research has demonstrated the feasibility of isolating β-CGTase-producing bacteria from plantation soils. Kurniadi et al. (2024a) reported the isolation of a β-CGTase-producing bacterium from plantation soil, while Kurniadi et al. (2024b) characterized β-CGTase-producing bacteria obtained from sweet potato plantation soil. These findings suggest that agricultural rhizosphere soils may represent useful sources for the isolation of CGTase-producing bacteria.

Papaya (*Carica papaya* L.) is widely cultivated in tropical and subtropical regions, including Indonesia. The rhizosphere of papaya provides a distinct ecological environment in which microorganisms interact with plant roots and soil components. However, information concerning β-CGTase-producing bacteria specifically isolated from papaya rhizosphere soil is still limited. Exploring this habitat may therefore provide additional bacterial candidates with potential for β-cyclodextrin production.

The present study aimed to isolate β-CGTase-producing bacteria from papaya rhizosphere soil, screen the obtained isolates based on CGTase activity, characterize selected isolates using phenotypic and biochemical tests, and evaluate their ability to produce β-cyclodextrin. The study also aimed to identify the isolate with the most promising β-cyclodextrin-producing potential for further investigation.

MATERIALS AND METHODS

Study area

The study used rhizosphere soil collected from three papaya plantations. Samples were collected from the soil surrounding papaya roots at a depth of approximately 5–20 cm. Five sampling points were selected from each plantation. Isolation, screening, characterization, and β-cyclodextrin production analyses were conducted in the microbiology laboratory.

Rhizosphere soil sampling

Rhizosphere soil was collected from the area surrounding papaya roots at approximately 5–20 cm depth. Five sampling points were taken from each of three papaya plantations. Samples were collected using sterile sampling equipment and placed in sterile containers before being transported to the laboratory for bacterial isolation.

Bacterial isolation

Bacterial isolation was performed using Horikoshi medium under alkaline conditions. Soil samples were inoculated onto the selective medium and incubated under conditions suitable for the growth of alkalophilic bacteria. Morphologically different colonies were selected and purified through repeated subculturing until individual bacterial isolates were obtained.

The use of alkaline selective medium was based on the established approach for obtaining alkalophilic CGTase-producing bacteria from soil. Similar isolation procedures have successfully been applied to plantation soil and resulted in the recovery of β-CGTase-producing bacterial isolates (Kurniadi et al. 2024a; Kurniadi et al. 2024b).

Screening of CGTase-producing bacteria

The obtained bacterial isolates were screened for CGTase production using Horikoshi agar medium. A yellow hollow zone surrounding the bacterial colony was used as a qualitative indicator of CGTase activity. The formation of this zone is associated with cyclodextrin production and the interaction of cyclodextrin with phenolphthalein in the medium.

Isolates showing a visible yellow hollow zone were selected for further characterization. The diameter of the yellow hollow zone was measured as an indicator of relative CGTase activity under the screening conditions.

Phenotypic and biochemical characterization

Selected isolates were characterized based on colony morphology, including colony shape, margin, elevation, surface characteristics, and colony color. Microscopic characteristics were determined using Gram staining, cell morphology observation, and spore staining. Biochemical characterization included catalase, motility, and glucose utilization tests.

The obtained characteristics were compared with phenotypic characteristics reported for previously described CGTase-producing bacteria (Kurniadi et al. 2024b; Saini et al. 2022). Because phenotypic and biochemical characteristics alone are insufficient for reliable species-level identification, the selected isolates were not assigned to a specific species.

Growth profile and CGTase production

The growth pattern of the selected isolates was observed during the incubation period. Measurements were used to identify the period associated with active bacterial growth. The effect of incubation time on CGTase production was evaluated at different incubation periods. The optimum incubation time was determined based on the highest β-cyclodextrin formation obtained from the corresponding enzyme preparation under the tested conditions.

Incubation time is an important factor in enzyme production because microbial growth and extracellular

enzyme production may not occur at the same rate. Optimization studies have demonstrated that CGTase production can vary according to the cultivation period and other environmental conditions (Pardhi et al. 2025).

β-Cyclodextrin production assay

β-Cyclodextrin production was determined using a phenolphthalein-based spectrophotometric method. The assay is based on the formation of an inclusion complex between phenolphthalein and cyclodextrin. The formation of cyclodextrin reduces the measurable absorbance of phenolphthalein and can therefore be used to estimate cyclodextrin production.

The β-cyclodextrin production percentage was calculated from the change in absorbance relative to the control. The reaction period was also evaluated to determine the time required to obtain optimum β-cyclodextrin formation. Phenolphthalein-based approaches have been widely used in the evaluation of cyclodextrin production, while reaction conditions can influence the catalytic performance and stability of CGTase (Cunha et al. 2024).

Data Analysis

The data were analyzed descriptively by comparing the phenotypic and biochemical characteristics, yellow hollow zone diameter, growth pattern, optimum incubation time, β-cyclodextrin production, and optimum reaction time among the selected isolates. The isolate showing the most favorable combination of qualitative CGTase activity and β-cyclodextrin production was considered the most promising candidate for further study.

RESULTS AND DISCUSSION

Isolation and screening of CGTase-producing bacteria

Isolation of bacteria from papaya rhizosphere soil resulted in eight isolates capable of growing on Horikoshi medium under alkaline conditions. The isolates showed variation in their colony characteristics, indicating the presence of different bacterial populations within the sampled rhizosphere soil.

Screening of the eight isolates based on the formation of a yellow hollow zone resulted in three positive isolates. These isolates were designated CG-P1, CG-P2, and CG-P3. The yellow hollow zone was considered an initial indication of CGTase activity and was used to select isolates for subsequent characterization and production studies.

Table 1. Isolation and screening of bacteria from papaya rhizosphere soil.

Parameter	Result
Soil source	Papaya rhizosphere
Number of plantations	3
Sampling depth	Approximately 5–20 cm
Sampling points	5 points per plantation
Initial isolates	8 isolates
Selected isolates	3 isolates
Isolate codes	CG-P1, CG-P2, CG-P3
Screening indicator	Yellow hollow zone

The recovery of CGTase-producing bacterial candidates from papaya rhizosphere soil supports the use of agricultural soil as a source for microbial enzyme exploration. Soil contains highly diverse microbial populations, and microbial bioprospecting has become an important strategy for finding microorganisms with useful metabolic capabilities (Boddu et al. 2022). Agricultural environments can be particularly valuable because plant-associated microbial communities may contain functional traits influenced by their local ecological conditions (Selvakumar et al. 2024).

The present result is also consistent with previous studies on CGTase-producing bacteria isolated from plantation soils. Kurniadi et al. (2024a) successfully isolated a β-CGTase-producing bacterium from plantation soil, while Kurniadi et al. (2024b) reported the isolation and characterization of β-CGTase-producing bacteria from sweet potato plantation soil. However, the present study used papaya rhizosphere soil as the microbial source, providing a different ecological setting for the exploration of β-CGTase-producing bacteria.

Phenotypic and biochemical characteristics of selected isolates

The three selected isolates showed broadly similar morphological and biochemical characteristics. All isolates were Gram-positive and rod-shaped, produced spores, were catalase-positive, and showed motility. The colonies were generally round and white to yellowish-white in color, although differences were observed in colony margin, elevation, and surface characteristics.

Table 2. Phenotypic and biochemical characteristics of selected CGTase-producing isolates.

Parameter	CG-P1	CG-P2	CG-P3
Colony shape	Round	Round	Round
Margin	Wavy	Entire	Entire
Elevation	Convex	Flat	Convex
Surface	Slightly rough	Smooth	Wrinkled
Colony color	Yellowish-white	White	Yellowish-white
Gram reaction	Positive	Positive	Positive
Cell shape	Rod	Rod	Rod
Spore	Positive	Positive	Positive
Catalase	Positive	Positive	Positive
Motility	Positive	Positive	Positive
Glucose	Positive	Positive	Positive

The characteristics observed in the three isolates are compatible with those commonly reported for CGTase-producing *Bacillus* isolates. Gram-positive, rod-shaped, spore-forming, catalase-positive, and motile characteristics have been described among bacterial isolates producing CGTase (Kurniadi et al. 2024b). *Bacillus* species are also recognized as important microbial sources of CGTase because of their ability to produce extracellular enzymes under alkaline conditions (Pardhi et al. 2023).

Although the phenotypic characteristics suggest that the isolates may belong to the genus *Bacillus*, species-level identification cannot be established from these characteristics alone. Differences in colony morphology and biochemical reactions may also occur among closely related species. Therefore, molecular analysis, particularly 16S rRNA gene sequencing, would be necessary to determine the taxonomic identity of CG-P1, CG-P2, and CG-P3.

Growth profile and optimum incubation time for CGTase production

The selected isolates showed active growth during approximately 8-24 h of incubation. Based on the production analysis, 24 h was identified as the optimum incubation time for CGTase production under the tested conditions.

The optimum incubation period is influenced by the physiological condition of the microorganism and the relationship between biomass formation and extracellular enzyme production. CGTase production does not necessarily reach its maximum at the same time as maximum bacterial biomass. Changes in incubation time may therefore result in differences in enzyme production (Pardhi et al. 2025).

The optimum time observed in the present study also differs from some previously reported CGTase-producing isolates. Such differences are expected because enzyme production depends on the characteristics of the producing microorganism as well as the composition and physicochemical conditions of the culture medium. Kurniadi et al. (2024a) demonstrated that CGTase production by their plantation-soil isolate was affected by incubation time and alkaline cultivation conditions. These findings reinforce the importance of optimizing production conditions for each newly obtained bacterial isolate.

β-Cyclodextrin production by selected isolates

All three selected isolates were capable of producing β-cyclodextrin. However, the production levels differed slightly among isolates. CG-P3 produced the highest β-cyclodextrin level at 94.320%, followed by CG-P1 at 94.129% and CG-P2 at 93.620%.

Table 3. β-Cyclodextrin production by selected isolates.

Isolate	β-Cyclodextrin production (%)	Optimum Incubatio time
CG-P1	94.129	24 h
CG-P2	93.620	24 h
CG-P3	94.320	24 h

The relatively small difference in β-cyclodextrin production among the three isolates indicates that all selected bacteria possess promising β-CGTase-producing potential under the tested conditions. Nevertheless, CG-P3 showed the highest production value. This result was supported by the qualitative screening data, in which CG-P3 also produced the largest yellow hollow zone.

Yellow hollow zone development during incubation

The diameter of the yellow hollow zone increased progressively during incubation and reached its maximum at 24 h for all three isolates (Table 4). At 24 h, CG-P1, CG-P2, and CG-P3 produced yellow hollow zones measuring 2.4, 2.1, and 3.2 cm, respectively. No further increase in yellow hollow zone diameter was observed from 24 to 72 h, indicating that the screening response had reached a plateau under the tested conditions. CG-P3 consistently produced the largest yellow hollow zone, suggesting the strongest CGTase-associated activity among the selected isolates under the tested conditions.

Table 4. Yellow hollow zone diameter of selected isolates during incubation.

Incubation time (h)	CG-P1 (cm)	CG-P2 (cm)	CG-P3 (cm)
0	0.0	0.0	0.0
4	0.6	0.7	0.7
8	1.1	1.1	1.1
12	1.5	1.5	1.5
18	2.2	1.9	2.3
24	2.4	2.1	3.2
30	2.4	2.1	3.2
36	2.4	2.1	3.2
42	2.4	2.1	3.2
48	2.4	2.1	3.2
72	2.4	2.1	3.2

The agreement between the yellow hollow zone screening and β-cyclodextrin formation supports the selection of CG-P3 as the most promising isolate. However, yellow hollow zone diameter and β-cyclodextrin formation represent different measures and should not be considered equivalent to direct enzyme activity. The yellow hollow zone provides a qualitative or semi-quantitative indication of CGTase-associated activity, whereas β-cyclodextrin formation reflects product formation in the reaction system. Direct determination of CGTase activity in enzyme units would provide a more robust comparison of the isolates.

CGTase catalyzes the conversion of starch through an intramolecular transglycosylation reaction, producing cyclodextrins with characteristic hydrophobic cavities. These structural properties are responsible for the ability of cyclodextrins to form host–guest complexes with various compounds (Pardhi et al. 2023). The production of β-cyclodextrin by the isolates obtained in this study therefore provides a basis for further exploration of their potential in applications requiring cyclodextrin-mediated complex formation.

The results are consistent with the known variability among CGTase-producing microorganisms. Saini et al. (2022) demonstrated that CGTase-producing bacteria can differ in enzyme production, characterization, and product specificity. Similarly, Pardhi et al. (2025) showed that optimization of culture conditions is important for obtaining favorable CGTase production from alkaliphilic bacteria. Thus, the relatively high β-cyclodextrin formation observed for CG-P3 represents an important preliminary finding, although further enzymatic characterization is required to determine its specific catalytic properties.

Optimization of reaction time for β-cyclodextrin formation

Optimization of the reaction period showed that β-cyclodextrin formation was optimum at approximately 30 min under the tested conditions. The relatively short optimum reaction period indicates that the CGTase preparation was able to catalyze the conversion of starch to β-cyclodextrin effectively during the early stage of the reaction.

Reaction time is an important parameter in cyclodextrin production because the catalytic reaction is influenced by enzyme concentration, substrate availability, reaction conditions, and enzyme stability. Modification of reaction conditions can affect both enzyme activity and the amount of cyclodextrin formed (Cunha et al. 2024). Differences in optimum reaction time between isolates are therefore expected.

The optimum reaction time observed in this study may differ from previously reported CGTase-producing bacteria because each enzyme preparation has distinct biochemical properties. Further studies should evaluate the effect of substrate concentration, pH, temperature, enzyme concentration, and reaction time simultaneously to obtain a more comprehensive production profile.

Overall, CG-P3 was identified as the most promising isolate among the three selected bacteria. The isolate produced the largest yellow hollow zone and the highest β-cyclodextrin production. The consistency between qualitative screening and product formation supports the selection of CG-P3 for further investigation. However, molecular identification and quantitative enzyme activity assays are still required to establish its taxonomic identity and biochemical characteristics.

CONCLUSIONS

Three potential β-CGTase-producing bacterial isolates, CG-P1, CG-P2, and CG-P3, were obtained from papaya rhizosphere soil. All selected isolates were Gram-positive, rod-shaped, spore-forming, catalase-positive, and motile, with phenotypic characteristics suggesting affiliation with the genus *Bacillus*. The optimum incubation time for β-cyclodextrin formation under the tested conditions was 24 h. β-Cyclodextrin production reached 94.129%, 93.620%, and 94.320% for CG-P1, CG-P2, and CG-P3, respectively. CG-P3 showed the highest β-cyclodextrin formation and produced the largest yellow hollow zone, measuring 3.2 cm. The optimum reaction time for β-cyclodextrin formation was approximately 30 minutes. These findings demonstrate that papaya rhizosphere soil is a promising source of β-CGTase-producing bacteria, with CG-P3 representing the most promising isolate for further molecular and enzymatic characterization.

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interpretation of the results. Helvi Ramadhani: ethical clearance coordination. All authors: manuscript revision and approval of the final version.

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