

Identification of Cadmium-tolerant Bacteria from Cd-Contaminated Rice Fields Based on Biochemical dan Molecular Characters

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Abstract. A number of cadmium (Cd)-tolerant bacterial isolates were successfully obtained from Cd-contaminated irrigated rice fields in Banyumas Regency, Central Java Province, Indonesia. Bacterial isolates can reduce Cd levels up to 70 percent. Bacterial isolates have the potential to be used as bioremediation agents for Cd-contaminated land. Potential bacterial isolates need to be identified. The purpose of the study was to identify Cd-tolerant bacterial isolates based on biochemical and molecular characters. Biochemical characters of potential bacterial isolates were obtained through a number of tests such as oxidase, catalase, urease, glucose fermentation, motility, starch hydrolysis, and indole production. Molecular characters were obtained through 16S rDNA sequencing. The results showed that five bacterial isolates showed positive results for biochemical characters such as oxidase, motility, and starch hydrolysis. The five isolates showed negative results for biochemical characters such as catalase, urease, H₂S production, and indole. For glucose fermentation test, only isolate PLK1 showed negative results. 16S rDNA molecular characters show that bacterial isolates GML2, KBS1, and SRU2 are close to *Priestia megaterium*, while isolate JTI1 with *Neobacillus ginsengisoli*, and PLK1 with *Gottfriedia* sp. Biochemical and molecular characters can be used to identify potential bacterial bioremediators of Cd-contaminated land.

Introduction

A number of irrigated rice fields in Banyumas Regency Central Java Indonesia contain cadmium (Cd) heavy metal contamination above the threshold [1]. Cadmium can inhibit the formation of leaf chlorophyll and reduce the rate of photosynthesis [2]. Cadmium in paddy fields can inhibit the growth and yield of rice plants, which can disrupt food security. Efforts to remediate heavy metal contaminated land can be done with the use of indigenous microbes [3]. Previous research successfully obtained five indigenous bacterial isolates from Cd-contaminated irrigated rice fields (codes: GML2, SRU2, JTL1, PLK1, and KBS1). The bacterial isolates are tolerant to Cd up to 100 ppm. Bacterial isolates can also reduce Cd levels up to 70.02 percent in liquid media and reduce soil available Cd from 0.96 ppm to 0.02 ppm.

Bacterial isolates have the potential to be used as bioremediation agents for Cd-contaminated rice fields. Bacterial isolates need to be identified.

Identification of bacterial isolates can be done with various approaches. Identification based on biochemical characters can reveal the enzymatic abilities of bacterial isolates. Enzymatic reactions in bacterial cells can support the role of bacteria in reducing Cd levels or other abilities that can be utilized in agriculture. Bacterial identification can also use molecular approaches [4]. Molecular approach based on 16S rDNA sequence nucleotide reading is a fast and accurate method to identify bacteria up to genus or species level. The purpose of this study was to identify potential Cd-tolerant bacterial isolates for bioremediation of Cd-contaminated rice fields based on biochemical and molecular characters.

Result and Discussion

1.1 Biochemical Character

Biochemical characters of Cd-tolerant bacterial isolates are known through a number of tests (Table 1).

Table 1. Biochemical characters of Cd-tolerant bacterial isolates as potential bioremediators of cadmium-contaminated paddy fields.

Biochemical Characters	Bacterial Isolate Code				
	GML2	SRU2	JTL1	PLK1	KBS1
Oxidase activity	+	+	+	+	+
Catalase activity	-	-	-	-	-
Urease activity	-	-	-	-	-
Alpha-amylase activity (starch hydrolysis)	+	+	+	+	+
H ₂ S production	-	-	-	-	-
Indole production	-	-	-	-	-
Glucose fermentation	+(with gas production)	+(without gas production)	+(without gas production)	-	+(without gas production)
Motility	+	+	+	+	+
N ₂ fixing activity	+	+	+	+	+
Phosphate solubility activity	-	+	+	+	+
IAA (phytohormone) production activity	-	-	-	-	-

Based on Table 1, the five bacterial isolates show similarities in a number of biochemical characters. All bacterial isolates have oxidase and alpha amylase enzyme activities. Oxidase activity in bacteria is related to the ability of the enzyme cytochrome c oxidase to catalyze the oxidation reaction of cytochrome c and the reduction of molecular oxygen to water [5]. This stage is an important part of the electron transfer chain that allows bacteria to use oxygen as the final electron acceptor in aerobic respiration. Amylase enzyme activity in soil bacteria

has an important role to determine the potential of bacteria in hydrolyzing starch into simple molecules [6]. Bacteria that have alpha amylase activity can use starch as a source of carbon and energy. Alpha amylase enzyme has an important role in agricultural activities, especially to help the decomposition process of starch-based organic matter (amylose and amylopectin) which is found in the biomass of dead plant residues into simple sugars such as dextrins that can be utilized by other microorganisms and plants as a source of nutrients for their growth.

Table 1 also shows that all bacterial isolates exhibit the same biochemical characteristics in converting atmospheric nitrogen (N₂) into ammonia (NH₃). This bacterial ability is due to the activity of nitrogenase enzymes in their cells [7]. Furthermore, the ammonia molecules produced can be converted into ammonium (NH₄⁺) by other soil bacteria. Ammonium is a form of available N that can be absorbed by plants for their growth. Phosphate solubilizing activity is also possessed by potential bacterial isolates, except GML2. Bacteria that show positive test results may have enzyme activity that can produce organic acids that can release PO₄³⁻ from the bonds of metal ions such as Ca²⁺, Fe³⁺, Al³⁺ [8].

In agriculture, the enzymatic character of bacteria converting atmospheric N₂ into NH₃ and releasing bound P into PO₄³⁻ is very important to provide nutrients for plant growth. Table 1 also shows that a number of bacterial isolates can perform glucose fermentation activities into organic acids, except PLK1. Organic acids produced from this group of bacteria can reduce the pH of the media and release nutrients that are absorbed by metals so that they are available to plants. These results are in line with other studies that show bacteria isolated from soil have the ability to ferment simple carbohydrates such as glucose to produce organic acids⁹. Bacterial isolates obtained from Cd-contaminated rice fields are classified as free-living bacteria that move freely and are not symbiotic with plant roots. This is evidenced by the results of the motility test which shows all bacterial isolates are motile.

1.2 Molecular Character

Molecular characters of Cd-tolerant bacterial isolates are known through 16S rDNA sequence nucleotides (Table 2).

Tabel 2. Molecular characters of Cd-tolerant bacterial isolates as potential bioremediators of cadmium-contaminated paddy fields.

Isolate Code	Length of amplified 16S rDNA Sequence (bp)	Blast-N Result				Identity of Genus/Species
		Max. score	Query cover	E value	Percentage of identity (%)	
GML2	1422	2621	100	0.0	99.93	<i>Priestia megaterium</i>
SRU2	1421	2619	100	0.0	99.93	<i>Priestia megaterium</i>
JTL1	1422	2627	100	0.0	100.0	<i>Neobacillus ginsengisoli</i>
PLK1	1421	2625	100	0.0	100.0	<i>Gottfriedia sp</i>
KBS1	1424	2630	100	0.0	100.0	<i>Priestia megaterium</i>

Based on Table 2, the length of the 16S rDNA sequence of all bacterial isolates is around 1400bp. These results are in accordance with other studies that show soil bacteria isolated from agricultural land have a 16S rDNA sequence length of around 1400 bp [9]. The 16S

rDNA gene sequence, which is commonly used for molecular identification of bacteria, has a length close to 1500 bp [10]. Based on Table 2, isolates GML2, SRU2, and KBS1 have a genetic closeness of 99.93% to 100% with the 16S rDNA sequence of *Priestia megaterium* species. While JTL1 isolate has 100% genetic closeness with *Neobacillus ginsengisoli* species and PLK1 with *Gottfriedia* sp. *P. Megaterium* is known as one of the bacteria that has good resistance and ability to biosorb Cd^{2+} so it has a good prospect for media remediation from Cd contamination [11]. The results of this study reveal another role of *N. ginsengisoli* and *Gottfriedia* sp as Cd-tolerant bacteria and can reduce Cd contamination. Previous studies have shown that this group of bacteria has a role in providing nutrients for plants [12].

1.3 Conclusions

Biochemical and molecular characters can be used to identify potential bacterial bioremediators of Cd-contaminated land.

References

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