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Tolerance of Rice Plants to Mercury Stress through the Application of Exopolysaccharide-Producing Bacteria

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A B S T R A C T

This study aimed to analyse the effect of exopolysaccharide-producing (EPS) bacteria on rice growth and yield under mercury stress induced by applying 40 mg $HgCl_2$ to each bucket containing 10 kg of soil. The research was conducted on a plastic-house scale in Sijunjung Regency, West Sumatra, Indonesia, from December 2025 to March 2026. The experimental design used was a two-factor factorial completely randomized design. The first factor was rice variety (IR 64 and Sertani). The second factor was no bacterial treatment versus bacterial consortium treatment (*Priestia megaterium, Priestia aryabhattai, Pseudarthrobacter defluvii, and Burkholderia vietnamiensis*). Each treatment was replicated three times. The results showed that there was no interaction between bacterial treatment and the tested rice varieties. In the IPB 9G and Sertani cultivars, the bacterial consortium increased tillers per hill, total productive tillers per hill, root dry weight, and shoot dry weight. It also increased 1,000-grain weight, filled-grain weight per panicle and per hill, and the number of filled grains per panicle. Compared with the control, the bacterial consortium treatment increased filled grain weight per hill by 25.76% in the IPB 9G variety and by 60.77% in the Sertani variety. Based on PCA, the variables contributing to PC1 were panicle length (PL) (0.925), PW (0.869), and PH (0.859). Correlation analysis showed that panicle weight (PW) and number of filled grains per panicle (NFGPP) (r = 0.98).

Contribution to Sustainable Development Goals (SDGs):
SDG 2: Zero Hunger
SDG 3: Good Health and Well-being
SDG 6: Clean Water and Sanitation
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action
SDG 15: Life on Land

1. INTRODUCTION

1.1. Research Background

Due to human discharge, especially from gold mining operations, mercury (Hg), a highly hazardous metal, often accumulates in flooded soils. Mercury is used in the amalgamation stage of gold extraction to separate gold from related mineral contaminants, increasing the risk of environmental pollution. Sijunjung Regency in West Sumatra is recognized as one of the gold-producing regions where mining activities are predominantly conducted illegally. Mining operations that were previously concentrated in river basin areas have now expanded into community rice fields. Gold mining systems in this region are carried out through two primary methods: (1) open-pit mining using excavators, which causes large-scale land clearing, and (2) closed mining by creating small excavation holes, from which mined materials are extracted, resulting in substantial environmental degradation in surrounding rice field areas, particularly through mercury contamination.

There are several types of mercury (Hg), including accumulative organic mercury molecules like methylmercury (MeHg: CH_3Hg^+) and bioavailable inorganic mercury (Hg^{2+}). Methylmercury can build up in rice plants and enter the food chain directly. It is commonly found in rice paddy soils contaminated with mercury. MeHg should not be consumed in excess of 0.1 g/kg body weight per day because it can cause serious neurotoxic problems in people. [1]. Plants exposed to mercury may show a variety of symptoms, including inhibited root growth, poor photosynthesis, reduced crop production, disrupted water transport and absorption systems, leaf chlorosis (yellowing of the leaves), necrosis (tissue death), and suppressed potassium uptake. Mercury exposure, particularly in rice plants, can reduce rice's nutritional quality and affect its safety and acceptability for human consumption. It may also reduce plant height, alter root morphology, cause leaf chlorosis, and reduce grain yield. Plants can absorb mercury from contaminated soils through their roots, which they subsequently store in their roots, stems, leaves, and grains, among other plant organs [2].

The movement of mercury (Hg) and its derivatives plays a vital role in transferring this toxic element into groundwater, vegetation, and ultimately the food chain, with profound consequences for human health. Consequently, remediation in polluted environments is indispensable to safeguard both ecosystems and human communities. Numerous studies have demonstrated that employing microorganisms and various materials offers a dependable strategy for stabilizing mercury [3]. EPS-producing bacterial species that can bind heavy metals include *Burkholderia vietnamiensis, Pseudarthrobacter defluvii, Priestia megaterium, and Priestia aryabhattai.* Hg, and are seen to be viable options for aiding remediation initiatives. These bacteria serve as nitrogen fixers and phosphate solubilizers in addition to binding heavy metals.

EPS consists of a diverse blend of high-molecular-weight biopolymers released by microorganisms, mainly including proteins, polysaccharides, uronic acids, humic substances, lipids, and nucleic acids [4]. The capacity of EPS to adsorb metal cations is attributed to the presence of negatively charged functional groups (carboxyl, phosphoryl, and hydroxyl) within its macromolecular structure. These functional groups can establish electrostatic interactions and bind positively charged metal ions [5].

1.2. Research Objective

This study aimed to analyse the effects of EPS-producing bacteria on rice growth and yield under mercury stress, and to provide recommendations for applying EPS-producing bacteria as a bioremediation strategy and a means to enhance food security in regions affected by heavy metal contamination.

2. MATERIALS AND METHODS

This study was conducted on a plastic-house scale in Sijunjung Regency, West Sumatra, Indonesia, from December 2025 to March 2026. The experimental design employed was a two-factor factorial completely randomized design. The first factor ***was rice variety*** (V1 = IPB 9G and V2 = Sertani), while the second factor comprised B0 = no bacterial treatment and B1 = bacterial consortium treatment (Priestia megaterium, Priestia aryabhattai, Pseudarthrobacter defluvii, and Burkholderia vietnamiensis). Each treatment was replicated three times.

The planting medium used was soil free from Hg contamination. We collected soil samples from 0 to 20 cm below the surface. We placed the planting medium in transparent polybags measuring 30 × 35 × 15 cm. We then transferred a total of 10 kg of soil at field-capacity conditions into 18-litre buckets. We arranged the buckets according to the prepared experimental layout. Subsequently, we treated the planting medium in each bucket with $HgCl_2$ at a concentration of 40 mg bucket-1 (10 kg soil).

Seedling preparation involved preparing nursery media consisting of soil and manure at a 10:1 ratio. The nursery medium was thoroughly mixed until homogeneous and then placed in seedling trays. After preparing the nursery medium, rice seeds were soaked in water for 24 hours and then air-dried for another 24 hours. The seeds were then ready for sowing and were distributed in the trays. Transplanting was performed when the seedlings were 15 days after sowing, with two seedlings planted per planting hole into the prepared planting medium.

The bacterial consortium was applied at a rate of 5 L ha^{-1} as follows: first, 5 L of bacterial consortium formulation was diluted in 500 L of water. Second, based on the plant population per hectare, the required volume was calculated as 3.16 mL per plant. Third, the seedlings were soaked in the bacterial solution for 30 minutes. The remaining soaking solution was then poured into the polybags. The inorganic fertilizers applied were urea, SP36, and KCl. Fertilizer application rates were based on recommendations from Balitbangtan (2020). Urea was applied at 250 kg ha^{-1}, with 50% administered at planting and the remaining 50% at 45 days after transplanting (DAT). SP-36 was applied at a dose of 100 kg ha^{-1}, while KCl was also applied at 100 kg ha^{-1}. Harvesting was conducted when ≥95% of the rice panicles had turned yellow, including the sample plants.

Plant height, total tiller number, productive tiller number, panicle length, number of filled and unfilled grains per panicle, filled and unfilled grain weight per panicle, filled and unfilled grain weight per plant, and yield per hectare were among the variables that were observed.

Analysis of variance (ANOVA) at the 5% significance level was performed using Minitab 19 software. Principal Component Analysis (PCA) and correlation heatmap analysis were conducted using the R software package.

3. RESULT AND DISCUSSION

3.1 Rice Plant Morphology

The evaluation of two rice varieties treated with EPS-producing bacteria under $HgCl_2$ stress at a concentration of $bucket^{-1}$ was assessed based on plant height, number of tillers per hill, total productive tillers per hill, root dry weight, and shoot dry weight (Figure 1). The results of ANOVA analysis ($P > 0.05$) showed no significant interaction between rice varieties and bacterial treatment for all observed variables. The effects of EPS-producing bacteria were consistent across both varieties, indicating that improvements in growth variables were primarily

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due to the bacterial treatment itself rather than to specific variety–bacteria combinations. The number of tillers per hill in the IPB 9G variety treated with bacterial consortium (V1B1) was higher compared to the treatment without bacterial application (V1B0), and a similar trend was observed in the Sertani variety treated with bacterial consortium (V2B1). Plant height values were relatively similar under both consortium and non-bacterial treatments, whereas root dry weight in the IPB 9G variety was higher, exceeding 40 g, under consortium treatment. Likewise, the Sertani variety also exhibited higher root dry weight under bacterial consortium treatment (V2B1).

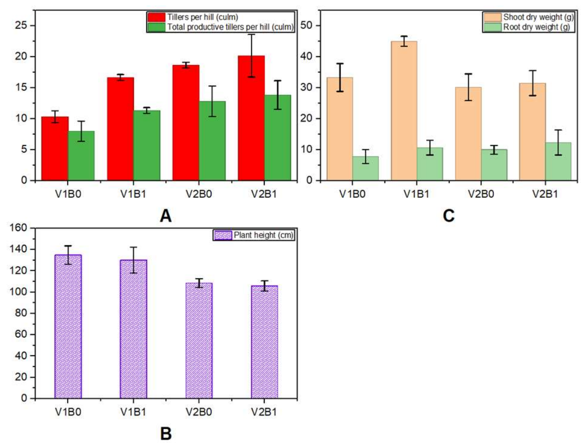

Fig 1. (A) Observed variables of tiller number and total productive tillers, (B) plant height, and (C) root dry weight and shoot dry weight. V1B0 = IPB 9G without bacteria, V1B1 = IPB 9G with bacterial consortium, V2B0 = Sertani without bacteria, and V2B1 = Sertani with bacterial consortium. Standard deviation was calculated using (n = 3).

In general, the EPS-producing bacterial consortium influenced the observed variables, including the number of tillers per hill, total productive tillers per hill, root dry weight, and shoot dry weight. This effect was attributed to the bacteria's ability to enhance phosphate and nitrogen availability through enzymatic activities. EPS reduced the toxic effects of Hg, thereby preventing disruption of nutrient transporters in the roots. As a result, plants were able to continue respiratory and photosynthetic processes even under heavy metal stress. The primary effects of EPS-producing bacteria were more evident in the root system and in physiological processes supporting tiller development than in stem elongation, as shown by the plant height remaining mostly unchanged. At this stage, the genetic factors of rice varieties also played an important role in determining morphological responses to mercury stress [2], [6], [7].

3.2 Rice Yield Components

The results of ANOVA analysis ($P > 0.05$) showed that there was no significant interaction between rice varieties and bacterial consortium treatment on panicle length, panicle weight, number of filled and unfilled grains per panicle, filled and unfilled grain weight per panicle, 1,000-grain weight, and yield per hectare (Tables 1 and 2). Panicle length and panicle weight were similar under both bacterial consortium treatment and no bacterial treatment, with the differences mainly attributed to varietal characteristics. This indicates that genetic factors strongly influenced panicle length and panicle weight variables. Under bacterial consortium treatment, both rice varieties had the highest number of filled grains per panicle: IPB 9G (247.60 grains) and Sertani (152.50 grains). The lowest number of unfilled grains in the IPB 9G variety was observed under no bacterial treatment (58.60 grains), whereas in the Sertani variety, the lowest value was found under bacterial consortium treatment (17.40 grains). Filled grain weight and unfilled grain weight per panicle were consistent with the number of filled and unfilled grains per panicle. The highest filled grain weight per panicle for the IPB 9G variety was also recorded under bacterial consortium treatment (6.77 g), and similarly, the Sertani variety showed the highest value under bacterial consortium treatment (5.02 g).

Table 1. Panicle length, panicle weight, number of filled and unfilled grains per panicle are components of rice yield.

Treatment	Panicle length (cm)	Panicle weight (g)	Number of filled grains per panicle (grains)	Number of unfilled grains per panicle (grains)
V1B0	29.24	7.06	214.60	58.60
V1B1	29.94	7.85	247.60	69.07
V2B0	23.45	4.46	146.28	35.54
V2B1	22.93	4.70	152.50	17.40

NOTE: V1B0 = IPB 9G without bacteria, V1B1 = IPB 9G with bacterial consortium, V2B0 = Sertani without bacteria, and V2B1 = Sertani with bacterial consortium

The highest filled grain weight per hill was observed under bacterial consortium treatment in both rice varieties, namely IPB 9G (48.67 g) and Sertani (53.36 g). A similar trend was observed for 1,000-grain weight, with IPB 9G reaching 24.15 g and Sertani 26.48 g under bacterial consortium treatment. In contrast, the IPB 9 G variety recorded the highest unfilled grain weight per hill under bacterial consortium treatment (4.95 g), whereas the Sertani variety had the highest value under no bacterial treatment (2.64 g).

Table 2. Components of rice yield based on 1,000-grain weight and filled and unfilled grain weight per panicle and filled and unfilled grain weight per hill.

Treatment	Filled grain weight per panicle (g)	Unfilled grain weight per panicle (g)	Filled grain weight per hill (g)	Unfilled grain weight per hill (g)	1000-grain weight (g)
V1B0	5.69	0.41	38.70	3.14	23.85
V1B1	6.77	0.55	48.67	4.95	24.15
V2B0	4.01	0.22	33.19	2.64	25.48
V2B1	5.02	0.16	53.36	2.09	26.43

NOTE: V1B0 = IPB 9G without bacteria, V1B1 = IPB 9G with bacterial consortium, V2B0 = Sertani without bacteria, and V2B1 = Sertani with bacterial consortium

Recent studies have shown that a bacterial consortium consisting of *Priestia megaterium, Priestia aryabhattai, Pseudarthrobacter defluvii, and Burkholderia vietnamiensis* can improve various production parameters of paddy rice under mercury stress conditions. Mercury is a heavy metal that negatively affects plant physiology, particularly by inhibiting photosynthesis and disrupting water and nutrient transport. One major impact of mercury exposure in plants is reduced grain filling and lower filled grain weight per panicle [8]. Biofilmforming bacteria can produce exopolysaccharides (EPS) as part of their mercury tolerance mechanism, which plays a crucial role in reducing the bioavailability of heavy metals within the rhizosphere zone [9]. EPS acts as a protective agent by reducing mercury toxicity through chelation processes, binding metal ions, and preventing their absorption by plants [10].

Mercury interferes with osmoregulation and ionic balance by disrupting ion channels in root cell membranes. One physiological response in rice plants under mercury stress is the buildup of sodium and chloride ions in plant tissues as a result of this perturbation. [11]. In addition to harming root tissues, this disturbance prevents plants from absorbing water and other nutrients. The applied bacterial consortia in this study improved the plants' capacity to preserve ionic balance within cells, suggesting that this bacterial consortium may play a role in regulating the physiological reactions of plants to stressful situations [12], [13]. Additionally, the bacteria may generate antioxidants that shield plant cells from oxidative damage brought on by mercury [13], [14]. Furthermore, this consortium may help repair plant cell membranes damaged by mercury stress. *Burkholderia vietnamiensis* has been shown to produce substances capable of protecting plant cell membranes by restoring membrane lipid structures oxidized under stress conditions [15], [16]. As mercury concentrations in plant tissues decrease, plants may show improved photosynthetic efficiency and reduced environmental stress damage.

In this study, applying a bacterial consortium increased filled grain weight per hill and 1,000-grain weight in paddy rice exposed to mercury stress. Mercury can damage chlorophyll, thereby reduce photosynthetic capacity and ultimately affecting grain yield. This effect is closely associated with improved water-use efficiency and enhanced photosynthetic processes [17], [18]. Adding EPS-producing bacteria can protect chloroplasts from oxidative damage, enhance chlorophyll synthesis, and improve plants' photosynthetic capacity [19]. Moreover, EPS also plays an important role in promoting root development by improving soil microbiome structure, which facilitates the absorption of essential nutrients such as nitrogen and phosphorus [20]. Mercury can reduce grain yield per hectare in rice plants by impairing water and nutrient transport. This occurs because mercury disrupts water transport through the xylem and reduces transpiration rates. Consequently, the water available for photosynthesis and plant growth is diminished. In mercury tolerance mechanisms, bacteria within this consortium produce compounds that enhance cell membrane permeability to water and improve water transport to the roots, thereby increasing plant tolerance to hydric stress [21], [22]. Therefore, mercury-exposed rice plants treated with bacterial consortium exhibit a greater capacity to adapt and achieve optimal grain yield per hectare.

3.3 Principal Component Analysis (PCA) and Correlation Analysis

Factor loading analysis in the PCA indicated that the first principal component (PC1) had the dominant contribution to character variability (Table 3). The variables contributing most strongly to PC1 were panicle length (PL) (0.925), PW (0.869), and PH (0.859). Meanwhile, PC2 was primarily influenced by variables with high loading values, namely filled grain weight per hill (FGWPH) (-0.61) and productive tiller number (PTN) (-0.598).

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Table 3. Loading Factors from Principal Component Analysis

Variable	PC1	PC2	PC3	PC4
TGW	-0.613	0.2	0.304	-0.599
FGWPP	0.673	-0.541	0.39	-0.075
UGWPP	0.813	0.135	-0.4	-0.104
PW	0.869	-0.398	0.154	0.103
NFGPP	0.799	-0.489	0.106	0.193
NUGPP	0.847	0.163	-0.377	0.139
FGWPH	-0.138	-0.61	-0.577	-0.04
UGWPH	0.531	0.193	-0.774	-0.16
TTN	-0.707	-0.473	-0.369	-0.18
PTN	-0.716	-0.598	-0.268	-0.039
PL	0.925	0.024	0.157	-0.264
PH	0.859	0.117	0.097	-0.009
SDW	0.548	-0.337	0.082	-0.695
RDW	-0.166	-0.540	0.271	0.227

NOTE: filled grain weight per panicle (FGWPP), unfilled grain weight per panicle (UGWPP), filled grain weight per hill (FGWPH), unfilled grain weight per hill (UGWPH), number of filled grains per panicle (NFGPP), number of unfilled grains per panicle (NUGPP), panicle length (PL), plant height (PH), shoot dry weight (SDW), root dry weight (RDW), panicle weight (PW), 1,000-grain weight (TGW), grain yield (GY), total tiller number (TTN), and productive tiller number (PTN).

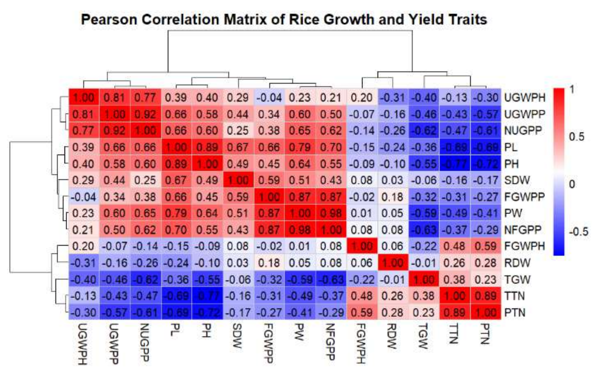

Fig. 2. Correlation heatmap dendrogram among variables: filled grain weight per panicle (FGWPP), unfilled grain weight per panicle (UGWPP), filled grain weight per hill (FGWPH), unfilled grain weight per hill (UGWPH), number of filled grains per panicle (NFGPP), number of unfilled grains per panicle (NUGPP), panicle length (PL), plant height (PH), shoot dry weight (SDW), root dry weight (RDW), panicle weight (PW), 1,000-grain weight (TGW), grain yield (GY), total tiller number (TTN), and productive tiller number (PTN).

The correlation analysis results presented in the heatmap were divided into two clusters, represented by red and blue, enabling identification of groups of consistently interrelated indicators (Figure 2). Red-colored correlations indicate strong positive relationships, while blue-colored correlations indicate strong negative relationships. The correlation heatmap revealed several clusters of strongly positively correlated variables, including unfilled grain weight per panicle (UGWPP) with number of unfilled grains per panicle (NUGPP) with unfilled grain weight per hill (UGWPH) (r = 0.81); number of unfilled grains per panicle (NUGPP) with unfilled grain weight per hill (UGWPH) (r = 0.92); panicle length (PL) (r = 0.89); filled grain weight per

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panicle (FGWPP) with number of filled grains per panicle (NFGPP) (r = 0.87) and panicle weight (PW) (r = 0.87); panicle weight (PW) with number of filled grains per panicle (NFGPP) (r = 0.98); total tiller number (TTN) with productive tiller number (PTN) (r = 0.89). Variables exhibiting strong negative correlations included plant height (PH) with total tiller number (TTN) (r = -0.77) and productive tiller number (PTN) (r = -0.72).

4. CONCLUSION

This investigation confirmed that introducing a consortium of EPS-producing bacteria, namely *Priestia megaterium*, *Priestia aryabhattai*, *Pseudarthrobacter defluvii*, and *Burkholderia vietnamiensis,* enhanced multiple growth and yield attributes in rice plants subjected to mercury stress. The consortium effectively increased tiller number per hill, root dry biomass, filled grain weight, and 1,000-grain weight in both Sertani and IPB 9G rice varieties. Compared with the control, the bacterial consortium treatment increased filled grain weight per hill by 25.76% in the IPB 9G variety and by 60.77% in the Sertani variety. PCA indicated that PC1 contributed most to trait variability, with panicle length (PL) (0.924) and panicle weight (PW) (0.871), being the most influential factors. Furthermore, correlation analysis demonstrated that improvements in number of filled grains per panicle (NFGPP) with panicle weight (PW) (r = 0.98) in consortium-treated plants were strongly associated with these two variables.

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