

## Isolation and characterization of potential zinc solubilizing bacteria from tidal swamp soil and their effect on rice plant vigour

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### Abstract

Rice, the staple for nearly half the world's population, faces production limits in tidal swamp soils due to zinc (Zn) deficiency. This study isolated Zn-solubilizing bacteria from Riau Province tidal rice fields, Indonesia, to enhance soil Zn availability and rice seedling vigour. Twelve isolates showed Zn solubilization, with PS5 achieving 109.68 mg/L efficiency and a vigour index of 1.76475; they also displayed plant growth-promoting rhizobacteria (PGPR) traits like nitrogen fixation, phosphate/potassium solubilization, indole-3-acetic acid production, and organic acid secretion. 16S rDNA analysis identified top strains as *Acinetobacter sp.* BHS4 and *Bacillus safensis* P5.4, positioning them as bioinoculants to combat Zn deficiency and lower chemical fertilizer needs in tidal rice systems.

**Keywords:** Biofertilizer, Rice vigour, PGPR, Tidal soils, Zinc solubilization

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## Introduction

Zinc is one of the micronutrients necessary to improve human health and plant development. Zn deficiency is the main reason for malnutrition in underdeveloped countries, where a big part of the population depends on cereals for daily intake of calories (Ali et al., 2021). Zinc deficiency is more widespread in developing countries, where more than 60 percent of the population experiences a risky zinc deficiency (Susanto et al., 2017). Zn is a micronutrient important for plants because it involves many mobile main functions, such as metabolic and physiological processes, enzyme activation, and ion homeostasis. Zn is a necessary element for regularity, activity, and metabolism in living organisms (Sturikova et al., 2018; Yang et al., 2020). In addition, Zn-solubilizing bacteria are capable of increasing plant quality through characteristic driver growth plants, like producing various phytohormones and dissolving nutrients (e.g., P and K), synthesizing exopolysaccharides and siderophores, and reducing stressful environments (Gupta et al., 2022).

On the other hand, Indonesia has a land tidal swamp that is quite a potential area for the production of rice plants. The swamps, though fragile, turn out to have enormous potential for future agricultural purposes. It is mainly related to the extent and distribution, as well as the potential for germplasm it has. In Indonesia, for example, there are 33.41–39.10 million ha of swamps, which can be useful for agricultural purposes and are spread over several large islands, including Sumatra, Kalimantan, and Papua (Mursyidin and Khairullah, 2020).

Rice was identified as one of the plants vulnerable to zinc deficiency. Zn requirements for plants are 30–100 mg/kg; below this number, they will cause deficiency. Inorganic fertilizer is usually used for fulfilling the need for macronutrients and micronutrients in rice plants (Kumawat et al., 2019). The use of fertilizer chemistry is just not enough to overcome the lack of zinc in the long term because 96.0–99.0% of the available deposited zinc becomes a form that is not available, like zinc carbonate, zinc oxide, zinc phosphate, etc (Zhang et al., 2017).

Even though rice has become a food mainstay in Indonesia, little research about zinc solubilizing bacteria (ZSB) has been carried out on tidal land in Indonesia. The tropical climate with high humidity in Indonesia allows the existence of various microorganisms in tidal rice fields, which supports

rice cultivation activity. The study results have shown that applying rhizobacteria in the swamp soil can repair soil fertility and plant growth and yield. Currently, many results study about using bacteria to land for changing Zn, which will later become available in soluble form for plants (Dinesh et al., 2018; Othman et al., 2022; Singh et al., 2023; Yasmin et al., 2021).

However, information is limited about zinc-solubilizing bacteria compared with other bacteria-driven plant growth, like nitrogen-fixers bacteria and phosphate-solubilizing bacteria that have been isolated from Indonesian land agriculture. *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* are example bacteria that have been proven to repair several parameters of rice growth and have more potential compared to other bacteria (Jha, 2019). Suggesting that comparable indigenous strains may exist within Indonesia's tidal soils.

This study hypothesizes that indigenous Zn-solubilizing bacteria exist in the tidal swamp soils of Riau Province, Indonesia and that they possess multiple plant growth-promoting characteristics, including nitrogen fixation, phosphate solubilization, potassium mobilization, and indole-3-acetic acid production. The objectives of this research are to isolate and characterize Zn-solubilizing bacteria from tidal swamp soil and to assess their potential in enhancing rice seedling vigour through biochemical and molecular (16S rRNA) identification approaches.

## Material and Methods

### Location of soil sampling

Soil samples were collected using purposive sampling method from six sites (Table 1), comprising two locations in Sabah Auh District and four in Bunga Raya District. The sites were selected based on the distribution of potential tidal paddy fields, as Siak represents one of the largest tidal rice-producing regions in Riau Province. At each site, composite samples were obtained from 0–20 cm soil layer by combining up to five subsamples. Sampling points were cleared of vegetation and litter prior to collection. Soils were extracted using a hand auger, transferred into labelled plastic bags, and sealed. For rhizosphere sampling, plants were carefully uprooted with adhering soil. The shoots were removed at the stem base, and the roots with attached soil were placed in labelled bags and kept in an eceboz to prevent temperature-related alterations.

**Table-1.** Location data taking sample land.

No	Latitude	Longitude	Location*	Rice varieties
T1	0° 57'46"	102° 3'16"	Bunga Raya	Inpari 42
T2	0° 58'26"	102° 3'51"	Kemunig Muda	Inpari 32
T3	0° 58'16"	102° 1'9"	Tuah Indrapura	Logawa
T4	0° 57'14"	102° 2'10"	Buantan Lestari	Inpari 42
T5	1° 0'57"	102° 6'33"	Belading	Inpari 42
T6	1° 3'50"	102° 7'33"	Sungai Tengah	Inpari 42 / Logawa

**Note \*:** Siak Regency, Riau Province Indonesia.

### Physical and chemical properties of soil

Soil samples analysed characteristic land chemistry, namely soil texture, soil N-total, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, C-organic, Capacity Swap Cation (CEC), Base Saturation, Cu, Fe, and Zn.

### Bacterial isolation

Non-rhizosphere (soil that is 0 - 20 cm deep in the tillage layer) and rhizosphere soil samples weighed as much as 10 g, then were put into 90 mL of 0.85% physiological NaCl solution (8.5 g NaCl in 1000 mL of distilled water) and then shaken on a shaker at a speed of 120 rpm for 30 minutes to obtain a dilution of 10<sup>-1</sup>. Next, a series of dilutions was made up to 10<sup>-7</sup>. A total of 100 µL of the results of the 10<sup>-5</sup> – 10<sup>-7</sup> dilutions were grown on Nutrient Agar medium in a petri dish. Different bacterial colonies, each originating from a single cell or a single colony and separate, were cultured on nutrient agar slant media. Bacterial cultures in nutrient agar slant and 20% glycerol were stored at 4°C and -85°C, respectively, for further use in the characterization stage.

### Hemolysis and hypersensitivity test

Bacterial cultures that have been rejuvenated (24 hours) in NA media were inoculated with the streak method on blood agar media. Isolates were incubated at 30°C for 1-5 days while observing the formation of a clear zone around colonies that show the existence of a hemolytic reaction by bacteria. β-hemolysis and α-hemolysis are marked with agar media around the colony that become colored greenish or brownish (Akhdiya et al., 2018). Pathogenicity testing targeted Zn-solubilizing candidate isolates using 3-month-old tobacco plants (*Nicotiana tabacum*L.). Twenty-four-hour NA cultures were suspended in sterile 0.85% NaCl, centrifuged (5000 rpm, 5 min, 4°C), and the pellet resuspended to 1x10<sup>-7</sup> cells mL<sup>-1</sup>. Bacterial suspensions (10 µL) were infiltrated into tobacco leaf

tissues; hypersensitive responses were monitored over 48 h for localized necrosis (Jalal et al., 2021).

### Qualitative analysis of Zn solubilization using the plate assay

Zn-solubilizing isolates were inoculated (3 µL) onto mineral salt agar (MSA) plates (Othman et al., 2022) comprising (g L<sup>-1</sup>): dextrose: 10.0; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>: 1.0; KCl: 0.2; K<sub>2</sub>HPO<sub>4</sub>: 0.1; MgSO<sub>4</sub>: 0.2; Zn compounds (0.1% ZnO and 15.0 g agar); pH 7. ZnO was autoclaved separately (121°C, 20 min) prior to mixing. Plates were incubated at 28°C for 48 h. Solubilization efficiency was quantified as the solubilization index (SI=clear zone diameter/colony diameter) (Gontia-Mishra et al., 2017) :

$$\text{Efficiency Zn solubility (\%)} = \frac{\text{diameter of solubilization halo}}{\text{diameter of colony}} \times 100$$

### Quantitative analysis of Zn solubilization using various mineral salts broth assays

Starter cultures were prepared by inoculating one loopful into 10 mL nutrient broth (NB) in a 20-mL tubes, followed by 24 h incubation (28°C, 150 rpm). Subsequently, 1 mL starter was transferred to 100-mL Erlenmeyer flasks containing 25 mL mineral salt medium supplemented with 0.1% ZnO. Cultures were incubated (28°C, 160 rpm, 48 h), then centrifuged (10,000 rpm, 4°C, 15 min). Soluble Zn in supernatants was quantified by atomic absorption spectrophotometer (Dinesh et al., 2018). Solubilization efficiency was calculated as the percentage of ZnO converted to soluble forms.

## Characteristics of plant growth promoters of Zn-solubilizing bacterial isolates

### Nitrogen fixation

The ability of bacterial isolates to fix nitrogen was tested qualitatively using semisolid N-free bromthymol blue (NFB) media (g/L: 0.8 K<sub>2</sub>HPO<sub>4</sub>; 0.2 KH<sub>2</sub>PO<sub>4</sub>; 0.2 MgSO<sub>4</sub>.7H<sub>2</sub>O; 0.002 MnSO<sub>4</sub>.4H<sub>2</sub>O; 0.1 NaCl; 0.002 Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O; 0.002 Na-Vanadate; 0.01 FeCl<sub>2</sub>; 15 Mannitol; 0.01 CaCl<sub>2</sub>.2H<sub>2</sub>O; 20 Sucrose; at pH 7.0). Pure cultures of Zn-solubilizing bacterial isolates on 24-hour-old NA slants were inoculated into semisolid NFB media and incubated at 30°C for 5 days. The isolates were also allowed to grow in Yeast Extract Mannitol Agar (YEMA) containing bromothymol blue (BTB) (0.5% dissolved in ethanol) (Bhakat et al., 2019).

### Phosphate and potassium solubilizing ability

To measure solubility, phosphate isolate was inoculated (3 µL) in Pikovskaya growth medium for testing phosphate for 48 hours at a temperature of 28°C. The formation of a halo zone indicates the ability of bacteria in dissolved phosphate (Onyia et al., 2015). Potassium solubilization was assessed on Alexandrov's medium (g L<sup>-1</sup>: glucose 5, yeast extract 5, MgSO<sub>4</sub> 0.5, CaCO<sub>3</sub> 0.1, FeCl<sub>3</sub> 0.005, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> 2, Feldspar 5, agar 28). Inoculated plates were incubated at 28°C for 48-72 h; clear halo zones signified K-solubilizing activity. Solubilization indices were calculated as halo diameter/colony diameter.

### Indole acetic acid (IAA) production

Bacterial isolates were grown in Luria-Bertani (LB) medium supplemented with or without 0.5 mg mL<sup>-1</sup> L-tryptophan (Sarwar and Kremer, 1995). Cultures were incubated at 30°C with shaking for 7 days, then centrifuged (8000 × g, 10 min). Supernatants (2 mL) were mixed with mL Salkowski reagent (35% perchloric acid and 1 ml of 0.5 M FeCl<sub>3</sub> solution) and 25 µl of orthophosphoric acid, incubated in the dark (25 min, room temperature). Pink coloration absorbance (530 nm) was quantified against an IAA standard curve (Gravel et al., 2007).

### Organic acid production

Organic acid (acetic, citric, lactic, malic, and oxalic) extraction followed (Baziramakenga et al., 1995). Supernatants were adjusted to pH 2.5 with 1 N HCl to precipitate humic substance, then held for 16 h.

Samples were centrifuged (15,000 rpm, 15 min, 4°C). Supernatant were extracted three times with 10 mL ethyl acetate (5 min each), organic phase evaporated to dryness (rotary evaporator, 40°C), and residues redissolved in 1 mL distilled water. Organic acids were identified and quantified by high-performance liquid chromatography (HPLC) with UV detection at 210 nm.

### Protease production

Protease activity was tested on skim milk agar (100 g skim milk, 5 g peptone, 15 g agar, 1 L distilled water) (Ogbo and Okonkwo, 2012). Skim milk was dissolved in 300 mL warm water, sterilized (autoclave, 121°C, 5 min), cooled to 60°C, and combined with sterile base medium before pouring. Bacterial isolates were spot-inoculated and incubated 28°C. halo zones were observed at 48 h and 7 days' post-incubation. Proteolytic index (PI) was calculated as halo diameter/colony diameter.

$$PI = \frac{\text{diameter of solubilization halo}}{\text{diameter of colony}}$$

### Data analysis

All data biochemical characterization was analysed using IBM's SPSS Statistics v25.0. Normality was confirmed (Shapiro-Wilk,  $p > 0.05$ ), validating one-way ANOVA application. Significant differences among treatment means were determined by Duncan's multiple range test (DMRT) at  $p < 0.05$ . Duplicate measurements per experiment ensured robust inference (IBM SPSS Statistics, v25.0, Austin, TX, USA).

### The effect of ZSB inoculation on growth vigor index in rice plants

The pot experiment employed a completely randomized design (CRD) with 12 Zn-solubilizing bacterial isolates as treatments, each replicated four times, modified from (Calvillo-Aguilar et al., 2023). Logawa rice variety seeds were used. Seeds were inoculated with bacterial suspensions and sown in pots. Germination and growth were monitored daily. At 14 days' post-inoculation, variables measured included shoot height, root length, fresh/dry biomass, germination percentage, and vigor index. Data were analyzed by one-way ANOVA, followed by DMRT post-hoc test at 5% significance level.

## Identification of molecular of Zn- solubilizing bacteria

Isolates were cultured in nutrient broth (NB). Genomic DNA was extracted and the 16S rRNA gene amplified per (Weisburg et al., 1991). PCR products were purified and sequenced bidirectionally using a DNA sequences. Raw sequences were edited with BioEdit, then queried against the NCBI GenBank database via BLAST (<http://blast.ncbi.nlm.nih.gov/>) for taxonomic assignment. Representative sequences were aligned using ClustalW and phylogenetic trees constructed by the neighbour-joining method in MEGA v10.0 with 1000 bootstrap replicates for clade support (Kumar et al., 2018).

## Results

### Soil chemical properties analysis

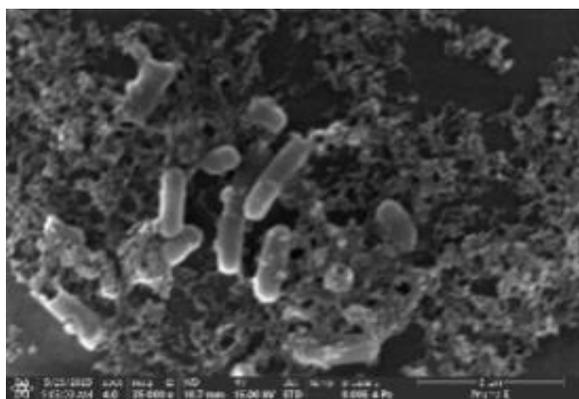
Characteristics of chemistry land on site taking samples described based on test results. The degree of soil acidity (pH) ranges between 4.50 and 5.20. The percentage of organic carbon in land ranges between 7% and 23.5%. Soil nitrogen (N) content is lower, ranging between 0.15% and 0.37%. The phosphorus available in land ranges from 6.8 to 80.1 ppm. Exchange rate cation (EC) the highest is 29.08 cmol/kg. The average total zinc content is high and the available zinc content is low (Table 2).

**Table-2.** Characteristics of chemistry tidal soil Siak Regency, Riau Province, Indonesia.

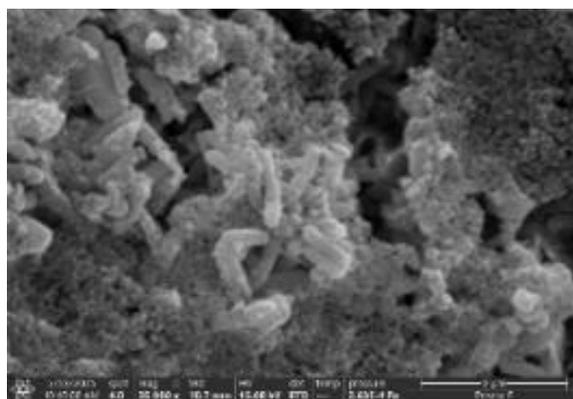
Location	pH		Organic Materials			Bray	Morgan	Exchange rate Cation { NH <sub>4</sub> Acetal 1N pH7 }				KCl 1 N		Total	Morgan				
	H <sub>2</sub> O	KCl	C	N	C/N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	Ca	Mg	K	Na	Amount	EC	BS	Al <sup>3+</sup>	H <sup>+</sup>	Zn	Zn	Fe
			----- % ----	----- % ----		--- ppm --				----- cmol /kg -----				%	cmol /kg		- ppm -		%
1	4.8	4.0	3.11	0.22	14	80.1	40	1.06	0.76	0.08	0.19	2.09	6.87	30	2.88	0.81	16.2	0.15	0.43
2	4.7	3.8	1.06	0.15	7	26.5	34	1.58	1.54	0.07	0.12	3.31	6.56	50	3.11	1.68	22.5	0.13	0.91
3	4.6	3.8	6.53	0.37	18	23.9	48	3.50	3.90	0.10	0.16	7.66	20.66	37	5.44	1.78	39.8	0.21	1.36
4	4.9	4.1	14.3	0.62	23	75.0	89	6.03	4.04	0.19	0.16	10.42	29.08	36	1.81	0.97	34.9	0.29	0.89
5	5.2	3.9	1.51	0.20	8	11.2	63	2.09	4.97	0.13	0.66	7.85	10.52	75	2.09	1.14	42.1	0.24	2.71
6	4.5	3.7	1.62	0.21	8	6.8	85	1.24	1.74	0.17	0.89	4.04	12.95	31	5.15	1.83	36.6	0.20	2.38

The number of bacterial isolates obtained from the sixth location taking samples obtained as many as 145 isolates; the bacteria consist of as many as 63 isolates from the rhizosphere and as many as 82 isolates from the non-rhizosphere. The bacterial isolates used were

selected isolates resulting from biosafety selection (blood agar test and hypersensitivity reaction test) which had been carried out previously, resulting in 12 isolates (PS5, PS46, PS60, PS67, PS75, PS117, PS119, PS 122, PS131, PS133, PS134 and PS141).



PS5 (*Acinetobacter* sp. BHS4)



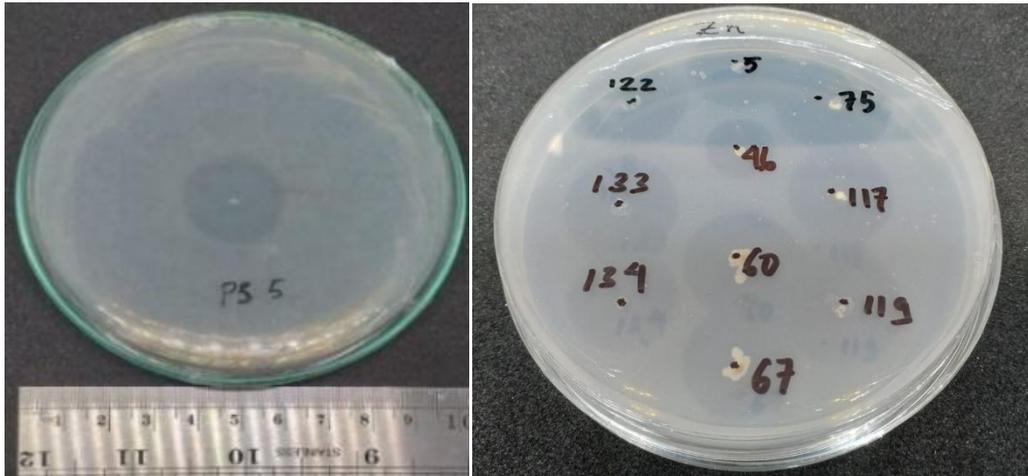
PS117 (*Bacillus safensis* strain P5.4)

**Figure-1.** Surface structure of Zn-Solubilizing bacteria by SEM analysis 35 K magnification.

### Qualitative and quantitative analysis of Zn solubilization

All 12 isolates (PS5, PS46, PS60, PS67, PS75, PS117, PS119, PS 122, PS131, PS133, PS134, PS141) exhibited clear halo zones, qualitatively indicating Zn solubilization capacity. Halo width and clarity correlated with solubilization efficiency, with

ANOVA confirming significant inter-isolate differences ( $p < 0.05$ ). Isolate PS5 demonstrated the highest solubilization index PS5 (SI = 109, 68; 537,50), followed by PS117 (SI = 8.63; 525.00%), and PS134 (SI=7.50; 508%). These top performers advanced to quantitative liquid assays and pot trials (Table3).



**Figure-2.** Halo zone bacteria Zn solubilizing in mineral salt agar medium.

Liquid mineral salt medium assays quantified soluble Zn release from 0.1% ZnO after 48 h incubation. ANOVA revealed significant inter-isolate differences ( $p < 0.05$ ), with DMRT identifying isolate PS5 as highest at 109.68 mgL<sup>-1</sup> soluble Zn. PS134 (29.95

mgL<sup>-1</sup>) and PS117 (8,28 mgL<sup>-1</sup>) followed without significant differences from each other but below PS5. These results validated plate assay screening efficacy and prioritized PS5 for pot trials (Table 3).

**Table-3.** Zn solubilization efficiency of isolates on ZnO.

No	Isolate code	Qualitative analysis (%)	Quantitative analysis (mg/L)
1	PS5	537.50 a	109.68 a
2	PS46	500.00 ab	8.14 cd
3	PS60	370.83 de	8.35 cd
4	PS67	275.00 f	14.55 bcd
5	PS75	494.16 ab	25.99 bc
6	PS117	525.00 ab	8.28 cd
7	PS119	437.50 bcd	15.36 bcd
8	PS122	362.50 de	5.05 d
9	PS131	402.00 cd	14.99 bcd
10	PS133	485.50 abc	15.40 bcd
11	PS134	508.00 ab	29.95 b
12	PS141	300.00 ef	12.65 bcd

**Note:** Numbers followed by the notation of the same letter are not significant at the 5% DMRT test level.

Three isolates (PS5, PS75, PS134) demonstrated positive  $N_2$  fixation on NFB medium, evidenced by green-to-blue color change, pellicle formation, and pH elevation. These diazotrophs promote plant growth directly via nutrient supply and hormone regulation, and indirectly through pathogen (Ahemad and Kibret, 2014). All 12 isolates exhibited phosphate solubilization on Pikovskaya's agar, with PS75

achieving the highest solubilization index (SI = 4.14; 313.5), significantly outperforming PS5, PS122, PS133, and PS134 (DMRT,  $p < 0.05$ ) (Table 4). Potassium solubilization on Alexandrov's agar was limited to PS75 and PS133, forming clear halo zones indicative of K-feldspar dissolution. These multinutrient solubilizers represent promising PGPR candidates for tidal paddy Zn/P/K deficiencies.

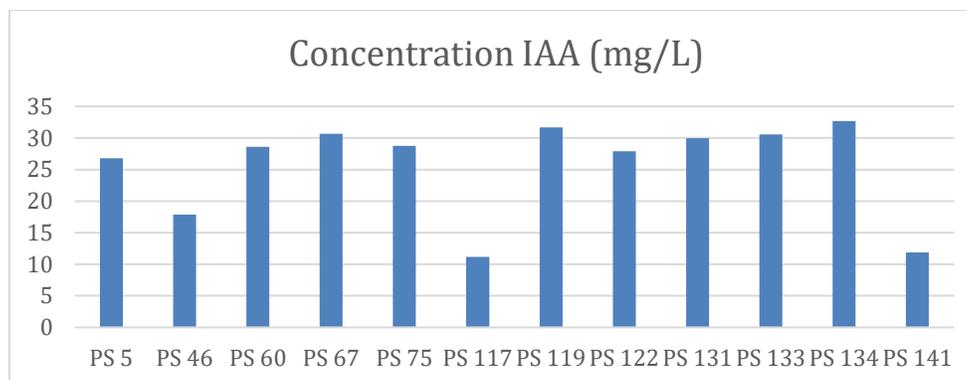
**Table-4.** Characteristics biochemistry bacteria Zn solubilizing.

No	Sample Code	Nitrogen Fixation	phosphate-solubilizing (%)	potassium-solubilizing	Protease production
1	PS 5	+	300.00 a	-	+
2	PS 46	-	180.00 b	-	+
3	PS 60	-	150.00 b	-	+
4	PS 67	-	200.00 b	-	+
5	PS 75	+	313.50 a	+	-
6	PS 117	+	187.50 b	-	-
7	PS 119	-	200.00 b	-	-
8	PS 122	-	267.00 a	-	+
9	PS 131	-	200, 00 b	-	-
10	PS 133	-	300.00 a	+	+
11	PS 134	-	300.00 a	-	-
12	PS 141	-	200.00 b	-	-

**Note:** Numbers followed by the notation of the same letter are not significant at the 5% DMRT test level.

IAA quantification via Salkowski assay confirmed IAA synthesis across all 12 isolates, with tryptophan supplementation enhancing yields. Isolate PS134 exhibited the highest production ( $32.68 \text{ mgL}^{-1}$ ),

significantly outperforming others ( $p < 0.05$ ), while PS117 yielded the lowest ( $11.16 \text{ mgL}^{-1}$ ) (Figure 3). These levels support robust auxin-mediated root growth promotion in tidal paddy systems



**Figure-3.** Potential IAA production of Zn-solubilizing bacteria.

### Organic acid production

HPLC analysis identified acetic, citric, lactic, malic, and oxalic acids in culture supernatants. Isolate PS46 dominated multiple categories, yielding the highest

acetic acid ( $9.808 \text{ mgL}^{-1}$ ), citric acid ( $0.325 \text{ mgL}^{-1}$ ), and malic acid ( $5.294 \text{ mgL}^{-1}$ ). PS141 produced the highest lactic acid ( $10.849 \text{ mgL}^{-1}$ ). These organic acids chelate  $Zn^{3+}$ , lowering pH and enhancing ZnO solubility in tidal paddy soils (Table 5).

**Table-5.** Concentration of organic acids in tidal land bacterial isolates.

No.	Code Sample	Concentration (mg/L)			
		Acetic acid	Lactic acid	Citric acid	Malic acid
1	PS 5	3,097	9,475	Not detected	1,270
2	PS 46	9,808	4,826	0.325	5,294
3	PS 60	Not detected	2,089	Not detected	Not detected
4	PS 67	Not detected	5,651	Not detected	Not detected
5	PS 75	3,308	3,239	0.025	Not detected
6	PS 117	Not detected	1,307	0.026	0.510
7	PS 119	3,266	2,081	Not detected	Not detected
8	PS 122	7,241	8,803	Not detected	Not detected
9	PS 131	4,006	1,149	0.018	1,067
10	PS 133	5,327	9,464	0.020	0.494
11	PS 134	5,508	Not detected	0.206	3,457
12	PS 141	5,226	10,849	0.124	0.554

### The effect of ZSB inoculation on growth vigor index in rice plants

ANOVA confirmed significant differences among isolates in seed germination rate, shoot length, plant

vigor index, and biomass accumulation ( $p < 0.05$ , DMRT). Top performers substantially outperformed controls across all measured parameters (Table 6).

**Table-6.** Characteristics of bacterial strains on rice seed vigor index.

No	Isolate Code	Germination Power (%)	Header Length (cm)	Root Length (cm)	Plant Growth
1	Water control	95.00 ab	10.66 c	5.32 a	1517.25 abc
2	Media control	92.50 ab	11.93 abc	5.85 a	1647.63 ab
3	PS 5	100.00 a	12.21 abc	5.44 a	1764.75 a
4	PS 46	97.50 a	12.29 abc	4.35 a	1621.75 ab
5	PS 60	95.00 ab	12.01 abc	5.10 a	1625.75 ab
6	PS 67	97.50 a	10.98 bc	5.68 a	1613.50 ab
7	PS 75	95.00 ab	12.02 abc	5.28 a	1642.50 ab
8	PS 117	87.50 abc	12.59 ab	4.78 a	1521.29 abc
9	PS 119	77.50 cd	12.08 abc	6.04 a	1392.58 bc
10	PS 122	87.50 abc	12.95 a	5.41 a	1608.17 ab
11	PS 131	72.50 d	12.00 abc	5.51 a	1279.74 c
12	PS 133	82.50 bcd	12.94 a	5.53 a	1512.63 abc
13	PS 134	75.00 d	13.07 a	5.92 a	1418.23 bc
14	PS 141	75.00 d	11.94 abc	5.33 a	1295.13 c

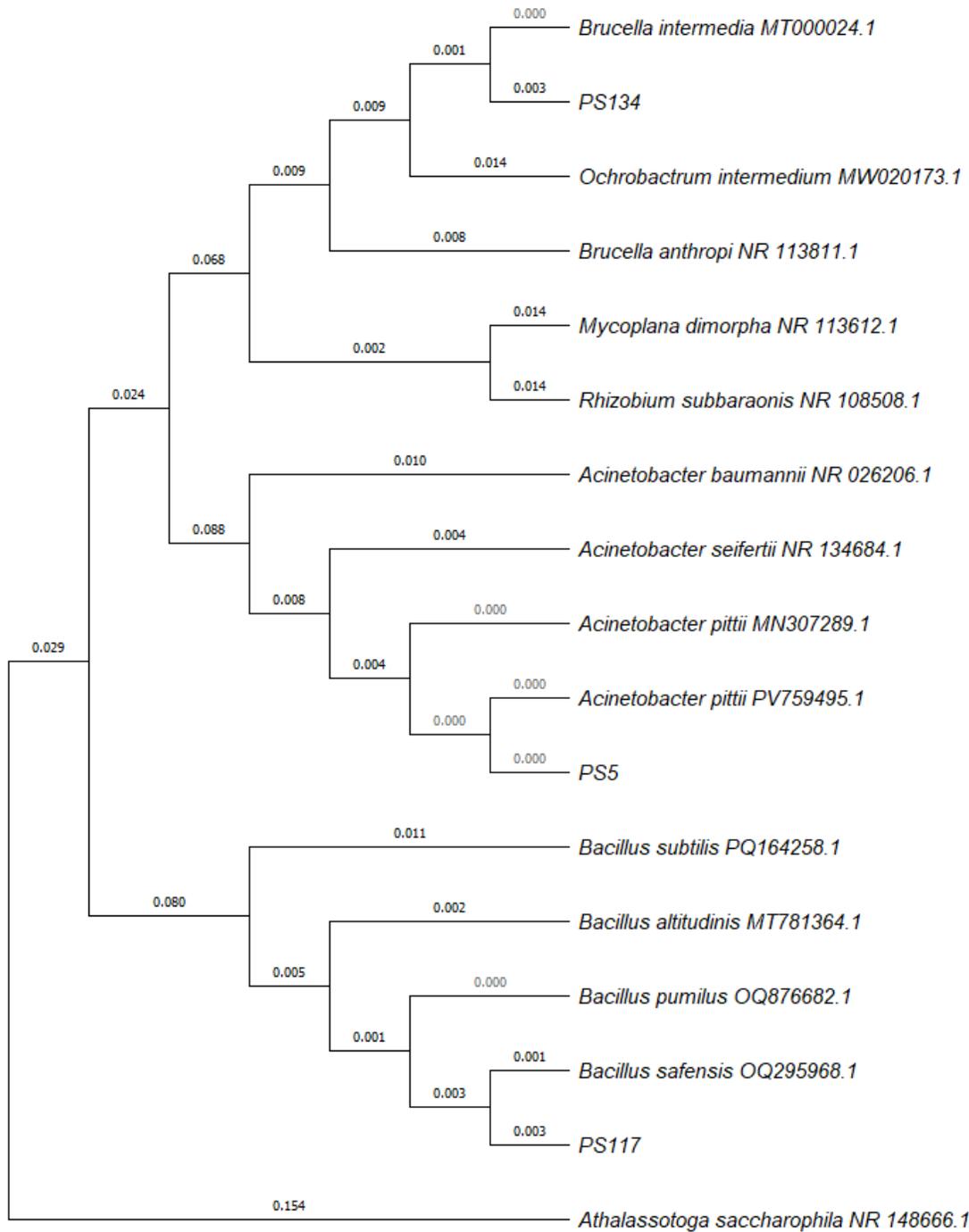
**Note:** Numbers followed by the same letter notation are not significant at the 5% DMRT test level.

PS5 achieved the highest germination rate (100%), statistically equivalent to uninoculated controls and isolates PS46, PS60, PS67, PS117, PS122 (DMRT,  $p > 0.05$ ; Table 6). PS5 also recorded the highest vigor index (1,764.75), indistinguishable from controls, PS46, PS60, PS67, PS117, but significantly superior to PS119 and PS134 ( $p < 0.05$ ). Non-inhibition relative to controls confirms PS5's plant growth compatibility and biosafety.

### Identification of Zn-solubilizing bacteria

16S rDNA gene sequencing of rhizosphere and non-rhizosphere isolates from Siak tidal flats identified the best Zn solubilizers as *Acinetobacter* and *Bacillus* species. BLAST analysis revealed PS5 as *Acinetobacter* sp BHS4 (99.65% identity) and PS117 as *Bacillus safensis* strain P5.4 (99.65% identity) against NCBI data bases. Phylogenetic analysis via neighbor-joining (MEGA v10.0, 1000 bootstraps)

supported these assignments, validating functional screening results (Figure 4).



**Figure-4.** Phylogenetic tree of isolated bacteria (PS 134) *Brucella intermedia*; (PS5) *Acinetobacter pittii*. (PS117) *Bacillus safensis*.

## Discussion

Zinc availability in tidal lands varies with soil type, organic matter content, and land-use history. Fields with regular Zn fertilization exhibit higher total Zn, yet Indonesian rice farmers rarely adopt biofertilizer alternatives due to limited awareness (Pratiwi et al., 2023).

Zn-solubilizing bacteria were successfully isolated from Siak Regency tidal paddy rhizosphere and non-rhizosphere soils, consistent with findings from rice fields and mineral/organic soils (Gupta et al., 2022; Jha, 2019; Othman et al., 2022).

Soil chemical analyses revealed significant variation across sites: pH 4.50-5.20 (acidic), high organic C (7.0-23.5%), and nutrient-rich profiles (P, K, Fe), but low available Zn despite high total Zn. Acidic conditions (pH 5.5-6.5 optimal for Zn availability) explain low DTPA-extractable Zn, as Zn solubility declines with increasing pH and high organic matter fixation (Rani et al., 2020). These characteristics underscore the need for microbial Zn mobilization in Siak tidal flats.

Bacterial populations in Bunga Raya soils were comparable to those in Kemuning Muda, Tuah Indrapura and Buantan Lestari, exceeding densities in Belading and Sungai Tengah. This uniformity likely reflects consistent agronomic practices-similar planting methods, fertilizer regimes, and microenvironments-shaping microbial communities. Rhizosphere and non-rhizosphere diversity is further modulated by crop type, soil physicochemical properties, and nutrient gradients (Hassan et al., 2020). Plate assays effectively identified Zn-solubilizing potential via clear halo zones on ZnO-supplemented media (0.1%), a standard qualitative approach (Othman et al., 2022; Zaheer et al., 2019). Twelve isolates produced pronounced halos, with bacterial growth rates influencing solubilization efficiency (Srithaworn et al., 2023). Quantitative liquid assays corroborate these findings, releasing bioavailable Zn from insoluble ZnO.

Zn-solubilizing bacteria enhance Zn availability, stimulating rice shoot/root elongation (Upadhayay et al., 2021). Identified taxa like *Bacillus pumilus* synthesize phytohormones, while *Bacillus thuringiensis* excels at solubilizing ZnO and Zn phosphate over carbonates (Jha, 2019; Dobrzyński et al., 2022). PS5 (*Acinetobacter pitii*) and PS117 (*Bacillus safensis*) exemplify multifunctional PGPR suited to Siak tidal flats' low available Zn conditions.

In general, the dissolution of Zn that is otherwise insoluble by isolated bacterial strains can occur through proton extrusion and the secretion of microbially derived organic acids. These processes lead to the solubilization of zinc and consequently influence its bioaccumulation and the availability in the soil environment (Shukla et al., 2023).

The primary mechanism employed by zinc-solubilizing bacteria involves the production of organic acids that facilitate mineral dissolution. During their growth, microorganisms synthesize acidic metabolites capable of chelating mineral cations such as  $Zn^{2+}$  on soil particle surfaces, thereby lowering the surrounding pH and enhancing metal solubilization (Upadhayay et al., 2022). Experimental evidence indicates that nearly all tested isolates produced organic acids during the zinc solubilization process, including acetic, lactic, citric, malic, and oxalate acids. According to (Dinesh et al., 2018), the enhanced zinc release observed in *Bacillus megaterium* is primarily attributed to a pH decline resulting from increased gluconic acid production.

Two isolates, PS5 and PS117, demonstrated multiple plant growth-promoting traits (PGPR), including the ability to fix nitrogen, solubilize phosphate and potassium, and produce indole-3-acetic (IAA), organic acids, and protease enzymes. Similar findings have highlighted the importance of such PGPR traits in improving the growth and development of various crops, including rice, beans, chickpeas, and turmeric (Dinesh et al., 2018; Othman et al., 2022; Yasmin et al., 2021).

One of the important PGP traits is the ability of bacteria to produce IAA, a phytohormone belonging to the auxin group. IAA is the most common and physiologically significant auxin, playing a central role in plant growth and development through its involvement in cell division, elongation, differentiation, and fruit formation (Bunsangiam et al., 2021). Maintaining IAA homeostasis is crucial for ensuring optimal hormonal balance required for normal plant development. However, excessive IAA levels can inhibit various physiological processes, as several studies have reported that high concentrations of IAA can suppress seed germination and overall plant growth (Sindhu and Sehwat, 2017).

In addition to IAA production, zinc-solubilizing bacteria (ZSB) isolates possess other beneficial traits such as enzymatic hydrolysis, nitrogen fixation, and phosphate solubilization. The ability to solubilize phosphate enhances its bioavailability, thereby

supporting root and shoot growth, improving stem strength, and promoting early plant maturity (Sharon et al., 2016). Nearly all ZSB isolates also exhibited positive results for organic acid production. The synthesis of these organic acids lowers the rhizosphere pH, thereby increasing Zn solubility and availability to plants (Costerousse et al., 2018). Plant growth-promoting rhizobacteria (PGPR), which colonize the rhizosphere, stimulate plant growth through multiple mechanisms, including phosphate solubilization, nitrogen fixation, siderophore production, and the secretion of extracellular enzymes, phytohormones, ammonia, and polysaccharides (Naseem et al., 2022). Most of the ZSB isolates (PS5, PS46, PS60, PS67, PS122, and PS133) were capable of producing protease enzymes. In rice plants, proteases perform crucial roles in growth, development, and defence mechanisms. They catalyse the hydrolysis of proteins into peptides and amino acids, which are subsequently utilized for various metabolic processes. Beyond their role in protein turnover, proteases contribute to plant immunity by participating in defence responses against pests and pathogens (Martinez et al., 2019). Moreover, as part of the plant's adaptive response to abiotic and biotic stress, proteases facilitate the remobilization of nutrients through the degradation of leaf and root proteins during seed formation (Gomez-Sanchez et al., 2019; James et al., 2019). Bacterial proteases can have dual effects on rice physiology, where beneficial strains such as *Bacillus subtilis* and *Bacillus atrophaeus* enhance germination, plant height, and root development, thereby improving overall plant vigour (Rajer et al., 2022). Experimental results revealed that isolate PS5 exhibited the highest zinc solubilization efficiency, reaching 109.68 mg/L, and correspondingly increased the vigour index to 1,764.75. The dissolution process in this isolate was accompanied by reduction in medium pH from 7.00 to 6.42, while other isolates did not show a similar decrease. This finding similar the explanation of (Upadhyay et al., 2022), who reported that zinc-solubilizing bacterial strains primarily mediate Zn dissolution through the production of organic acids. During microbial growth, these acidic metabolites complex with mineral surfaces in the soil matrix, chelating zinc cations and thereby lowering the rhizosphere pH, which enhances Zn solubility. Zinc-solubilizing bacteria (ZSB) are commonly found in rhizosphere soil environments that vary in pH, cation exchange capacity, and location. Distinct bacterial strains exhibit differential abilities to

solubilize zinc through organic acid production and subsequent pH reduction. For instance, *Bacillus paramycooides* strain 1 was reported to enhance available soil zinc by 22-24% through the release of water-soluble and exchangeable zinc fractions during the critical growth stages of rice (30 and 58 days after sowing) in zinc-deficient soils (Singh et al., 2023). In the present study, the selected ZSB isolates were identified as *Acinetobacter sp.* BHS4 chromosome and *Bacillus safensis* strain P5.4. Previous studies have also identified several prominent zinc-solubilizing genera, including *Pseudomonas*, *Bacillus*, *Enterobacter*, *Xanthomonas*, *Stenotrophomonas*, and *Acinetobacter* (Othman et al., 2022; Yasmin et al., 2021).

According to (Sun et al., 2024) *Acinetobacter sp.* play beneficial agronomic roles as plant growth-promoting rhizobacteria (PGPR). They can synthesize phytohormones, fix atmospheric nitrogen, solubilize phosphate, and produce siderophores and antimicrobial compounds, thereby improving nutrient availability and plant health. Supporting by (Othman et al., 2022) isolated 99 ZSB strains from the rice rhizosphere and identified *Acinetobacter pittii* the most efficient zinc solubilizer based on biochemical and molecular characterizations. Other bacterial genera, including *Bacillus*, *Enterobacter*, and *Pseudomonas*, have also been reported to promote the growth of various crops such as peanut, wheat, and chickpeas (Yasmin et al., 2021).

*Bacillus safensis* strain P5.4 is a rod-shaped, spore-forming, gram-positive bacterium capable of thriving under diverse environmental conditions, including high salinity, radiation, and temperature (Singh et al., 2023). It exhibits several beneficial traits, such as the production of the plant growth hormones and antimicrobial compounds (e.g., bacteriocins and enzymes), conferring strong potential for use in biocontrol, bioremediation, and probiotic formulations. The application of zinc-solubilizing bacteria such as *Acinetobacter pittii* and *Bacillus safensis* can enhance soil zinc availability, prevent yield decline, and increase the zinc content of rice grains. Given their multiple PGPR attributes, these isolates are promising candidates for development as biofertilizers to mitigate zinc deficiency in rice-based production systems.

## Conclusion

Among all isolates tested, PS5 exhibited the highest zinc-solubilization efficiency in the quantitative assay, achieving of 109 mgL<sup>-1</sup> of soluble Zn and improved vigour index of 1.764,75. Both PS5 and PS117 demonstrated multiple plant growth-promoting traits, including nitrogen fixation, phosphate, and potassium solubilization, and the ability to produce indole-3-acetic acid, hydrolytic enzymes, and organic acids. Based on 16S rDNA sequence analysis, the two potential zinc-solubilizing bacterial isolates were identified as *Acinetobacter sp.* BHS4 chromosome and *Bacillus safensis* strain P5.4.

These findings highlight the potential of *Acinetobacter sp.* and *Bacillus safensis* as efficient bioagents to enhance zinc solubilization and improve plant vigour. Further validation of these isolates under field conditions is essential to confirm their effectiveness in promoting soil zinc availability and crop yield. Future studies should prioritize field-scale trials and the development of biofertilizer consortia formulations utilizing these promising ZSB strains.

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## Contribution of Authors

Fahri A: Conceptualized the study, developed the methodology, conducted the investigations and prepared the original draft.

Sembiring MB: Assisted in conceptualizing the study, developed the methodology, performed validation, contributed to writing, reviewing, editing and provided supervision.

Girsang SS, Pratiwi E, Yuniarti E & Irawati FC: Assisted in developing the methodology and contributed to writing, reviewing and editing the manuscript.

Sabrina T: Assisted in conceptualization, validation, writing the original draft, reviewing and editing the manuscript and secured funding.

All authors read and approved the final draft of the manuscript.

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