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Abiotic and biotic stress mitigation in rice using rhizospheric isolates for zinc solubilization

Geetakumari[1], Aman Jaiswal[1]*, Jyostnarani Pradhan[2], Hemlata[2], Kiran[2], Samala Manoj Kumar[1], Devashish Pathak[3] and Nidhi[4]

[1]*Department of Microbiology, CBS&H, RPCAU, Pusa, Bihar, India*
[2]*Department of Botany, Plant Physiology and Biochemistry,CBS&H, RPCAU, Pusa, Bihar, India*
[3]*Amity Institute of Organic Agriculture, AUUP, Noida-201313 , India*
[4]*Department of Statistics and computer Application RPCAU, Pusa, Bihar, India*

ABSTRACT

Rhizospheric bacteria play a critical role in plant health by enhancing nutrient availability, promoting stress resilience, and suppressing pathogens. This study aimed to isolate and characterize zinc-solubilizing bacteria (ZSB) from rice rhizosphere soils and evaluate their dual potential for biotic and abiotic stress management. Eighty-three bacterial isolates were obtained from 20 soil samples collected at the RPCAU Campus in Pusa and Dholi. Among these, 15 isolates demonstrated strong zinc solubilization ability, with 6 identified as Gram-positive and 9 as Gram-negative. Biochemical analyses revealed that 9 isolates exhibited catalase activity (top performers: I-44, I-60, I-74), 5 produced siderophores (highest: I-73), and 13 synthesized indole-3-acetic acid (best: I-6). In vitro assays showed that most isolates produced ammonia, and 7 strains (I-65, I-68, I-78, I-79) exhibited hydrogen cyanide production and antagonistic activity. Additionally, these isolates enhanced nutrient availability and demonstrated promising traits for stress mitigation. Strains I-6, I-61, I-65, I-67, I-73, I-78, and I-79 showed significant biocontrol efficacy against Helminthosporium, a major rice pathogen. This study highlights the dual benefits of ZSB in promoting zinc solubilization and providing integrated stress management, offering a sustainable approach to enhancing rice cultivation under both biotic and abiotic stress conditions.

Keywords: *Zinc Solubilizing Bacteria, Rice rhizosphere, Biocontrol efficacy, Helminthosporium, Biotic and Abiotic stress.*

INTRODUCTION

Rice (*Oryza sativa* L.) serves as a staple food for over half of the global population, making its sustained production vital for global food security [1]. As a member of the Poaceae family, rice plays a critical role in meeting caloric demands, with approximately 95% of the world's rice crop consumed directly by humans. However, rice cultivation is frequently threatened by both biotic and abiotic stresses, including nutrient deficiencies and fungal diseases, which adversely affect yield and grain quality.

Among the major abiotic challenges, zinc (Zn) deficiency ranks as the third most widespread nutrient disorder in lowland rice fields, following nitrogen and phosphorus deficiencies [2]. Zinc is an essential micronutrient involved in critical physiological processes such as carbohydrate metabolism, protein synthesis, chlorophyll production, and protection against oxidative stress [3]. Zinc deficiency leads to stunted plant growth, leaf discoloration, delayed maturity, and significantly reduced grain yield [4]. Traditional Zn fertilizers, although effective, are costly and unsustainable for resource-poor farmers, necessitating eco-friendly alternatives [5].

Zinc-solubilizing bacteria (ZSB) have emerged as a promising sustainable solution to improve Zn availability and promote plant growth. Several bacterial species, including *Pseudomonas protegens*, *Bacillus megaterium*, and *Bacillus altitudinis*, have demonstrated the ability to solubilize insoluble Zn compounds and produce plant growth-promoting hormones [6], [7], [8]. Additionally, strains such as *Enterobacter cloacae* ZSB14 have been found to upregulate Zn transport and accumulation genes in rice under iron-deficient conditions [9].

Biotic stresses such as fungal infections further threaten rice production. Brown spot disease caused by *Helminthosporiumoryzae* is a particularly damaging rice pathogen, capable of causing up to 50% yield losses under severe conditions [10]. Symptoms include brown lesions on leaves, panicles, and necks, which can coalesce and result in complete leaf drying. The disease is prevalent in nutrient-deficient and unflooded soils.

Biological control strategies offer an eco-friendly alternative to managing plant diseases. Antagonistic microorganisms suppress fungal pathogens through mechanisms such as the production of antifungal compounds, secretion of cell wall-degrading enzymes, and competition for nutrients and space [11]. Recent studies highlight the dual role of zinc-solubilizing bacteria as both plant growth promoters and biocontrol agents, making them a promising tool for integrated stress management in rice cultivation.

Given the dual challenges of nutrient deficiencies and fungal infections in rice cultivation, this study was undertaken to isolate and characterize zinc-solubilizing bacteria from the rice rhizosphere.

*Corresponding Author: ***Aman Jaiswal***

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The objective was to evaluate their potential for enhancing zinc availability and mitigating both biotic and abiotic stresses, providing a sustainable strategy to improve rice productivity and resilience.

Method and Materials

Soil Collection and Microorganisms

Nineteen soil samples were collected from the rhizosphere of rice at the Pusa and Dholi farms of Dr. Rajendra Prasad Central Agricultural University, Samastipur, Bihar, India. Samples were transported to the laboratory in sterile plastic bags and stored at 4°C until analysis. Serial dilutions of the samples were prepared, plated on nutrient agar, and incubated at 28°C for 48 hours. A total of 83 bacterial isolates with diverse morphologies were obtained. Pure cultures were maintained in nutrient broth at 28°C and on nutrient agar slants for further use.

Screening of Bacterial Isolates

The bacterial isolates were screened for their zinc-solubilizing ability using the following procedure: The isolates were inoculated on solid media containing insoluble zinc compounds as the sole zinc source. The plates were incubated under appropriate conditions for 3 days. After incubation, the formation of clear zones around the bacterial colonies was observed, indicating zinc solubilization.

Media Used: Tris minimal agar supplemented with 0.1% insoluble zinc compound.

Insoluble Zinc Sources: Zinc Oxide (ZnO), Zinc Phosphate ($Zn_3(PO_4)_2$), and Zinc Carbonate ($ZnCO_3$).

ZnSE (%) = Diameter of halo zone (cm) X 100
Diameter of colony

Morphological characteristics

The morphological features of the isolated bacteria were evaluated through Gram staining and colony morphology analysis. During the purification process, colony morphology was assessed based on size, shape, color, texture, and elevation. Gram staining, a critical microbiological technique introduced by [12] was used to differentiate bacteria. A thin bacterial smear was prepared on a microscope slide, air-dried, and heat-fixed. The smear was sequentially stained with crystal violet (1 minute), treated with Gram's iodine (1 minute), decolorized with ethanol (15–20 seconds), and counterstained with safranin (1 minute). After drying, observations under a microscope revealed Gram-positive bacteria as purple and Gram-negative bacteria as pink or red.

Biochemical Characterization of Screened Microbes

IMViC test

The IMViC test was conducted for bacterial identification using four biochemical assays and eight carbohydrate utilization tests to assess pH changes and substrate utilization. The KH001 H-IMViC biochemical test kit was used for this analysis. A catalase test was performed to identify catalase-positive organisms. A drop of 3% hydrogen peroxide was added to a bacterial culture and placed on a clean glass slide. The formation of oxygen bubbles indicated a positive catalase reaction, confirming the presence of catalase-positive organisms [13]. The oxidase test was conducted by streaking bacterial colonies onto oxidase reagent strips. A color change to purple or blue within 10 to 30 seconds indicated a positive reaction [14].

The gelatin hydrolysis test was conducted by inoculating bacterial cultures into nutrient gelatin media. The cultures were incubated for 48 hours and then refrigerated at 4°C for 30 minutes. Liquefaction of the media indicated the production of gelatinase [15].

Plant Growth Promoting traits

The siderophore production test was performed by inoculating CAS agar plates with bacterial isolates. The plates were incubated at 30°C for 72 hours. A color change from blue to green indicated the production of siderophores [16]. The IAA production test was conducted by growing bacterial cultures in Luria Bertani broth supplemented with 1% tryptophan. After incubation, the Salkowski reagent was added to the culture, and the appearance of a pink color was observed. The intensity of the color, measured at 536 nm, indicated IAA synthesis [17]. Phosphate and potassium solubilization were assessed using Pikovskaya's agar and Aleksandrow agar, respectively. Bacterial isolates were inoculated onto the agar plates and incubated. The formation of halo zones around the colonies indicated solubilizing activity. Solubilization efficiency was calculated based on the halo zone diameter [18], [19].

Biocontrol Activities of Selected Isolates

The HCN production test was conducted by streaking bacterial isolates onto King's B medium supplemented with glycine. A filter paper soaked in picric acid solution was placed inside the tube above the medium. The development of a brown or orange color on the filter paper indicated HCN production [20]. The ammonia production test was performed by growing bacterial isolates in peptone water. After incubation, Nessler's reagent was added to the culture. The appearance of a yellow or brown color indicated ammonia production. Quantitative analysis was conducted by measuring the absorbance at 450 nm [21]. Chitinase and cellulase production were assessed by culturing bacterial isolates on chitin agar and CMC-containing Bushnell-Hass agar, respectively. For chitinase detection, growth on chitin agar indicated enzyme production. For cellulase detection, plates were stained with Congo red, and the formation of clear halo zones around colonies confirmed cellulase activity [22],[23]

Dual Culture Plate Test

The dual culture plate test was conducted by spot-inoculating *Helminthosporium* on Potato Dextrose Agar (PDA) plates. Bacterial isolates were streaked around the pathogen. The plates were incubated at 30°C for 10 days, and pathogen inhibition was assessed based on the growth suppression of *Helminthosporium* [24].

Result and Discussion

Table 1. Isolation Results of Rhizospheric Bacterial Strains from Rice Soil Samples

	Location	Crop	Total no of isolates
1.	Pusa	Rice (Oryza sativa)	47
2.	Dholi		36
The total no of bacteria isolated			83

In this study, microbial populations were analyzed by isolating bacteria from soil samples collected from two distinct locations: Pusa and Dholi.(Table 1) From the Pusa soil sample, a total of 47 bacterial isolates were obtained, while 36 bacterial isolates were retrieved from the Dholi sample.

These findings provide insight into the diversity and abundance of bacterial communities present in the soils of both regions, which can be further explored to understand the ecological and functional roles these microorganisms play in the soil environment.

Table 2. Solubilisation efficiency of selected isolates after screening

Isolates	Solubilization Efficiency		
	Zinc oxide	Zinc Carbonate	Zinc Phosphate
I-6	3.50	3.14	2.50
I-34	2.00	3.57	2.60
I-44	2.00	1.62	2.00
I-60	2.50	2.25	2.00
I-61	1.88	2.42	1.50
I-65	4.14	2.12	5.00
I-66	4.28	4.00	2.90
I-67	3.14	4.00	2.10
I-68	3.37	2.25	2.50
I-70	5.40	4.50	3.60
I-73	5.00	4.00	1.60
I-74	2.25	3.00	2.30
I-76	1.80	1.50	1.70
I-78	2.80	2.40	2.00
I-79	1.57	1.80	2.00

Screening of Microbes for Zinc solubilization efficiency in plate assay

The screening for zinc solubilization was conducted on three insoluble zinc compounds: Zinc Oxide (ZnO), Zinc Phosphate ($Zn_3(PO_4)_2$), and Zinc Carbonate ($ZnCO_3$). Zinc Oxide, being the most soluble and readily available, was tested first, followed by Zinc Phosphate and Zinc Carbonate. The solubilization efficiency was determined by measuring the halo zone around the isolates.

The screening of 83 bacterial isolates for the solubilization of insoluble zinc compounds—Zinc Oxide (ZnO), Zinc Phosphate (Zn3(PO4)2), and Zinc Carbonate (ZnCO3)—revealed significant variability in solubilization efficiency. A total of 83 isolates were screened for Zinc Oxide solubilization, with 52 showing positive results. For Zinc Phosphate, 52 isolates were tested, and 20 exhibited positive solubilization. Finally, 20 isolates were screened for Zinc Carbonate, Among these, 15 isolates showed positive results, demonstrating their potential for mobilizing zinc from different compounds. (Table 2) shows the solubilization efficiency for zinc oxide ranged from 1.57 mm (I-79) to 5.40 mm (I-70), with isolates I-70 and I-73 showing the highest efficiency. Zinc carbonate solubilization v aried between 1.50 mm (I-76) and 4.50 mm (I-70), with isolates I-66, I-67, and I-73 also displaying high efficiency. For zinc phosphate, isolate I-65 exhibited the highest solubilization (5.00 mm), followed by I-70 (3.60 mm), while most isolates exhibited moderate efficiency ranging from 1.50 mm to 3.60 mm. Comparatively, maximum solubilization was observed for zinc oxide, followed by zinc carbonate and zinc phosphate. The ability of bacterial isolates to solubilize zinc is intricately linked to their production of organic acids, which aid in dissolving insoluble zinc compounds [25]. This property is crucial not only for zinc availability but also for the mitigation of abiotic stress conditions such as nutrient-deficient soils. In addition to zinc solubilization, isolates such as I-65, I-67, and I-73 demonstrated promising biocontrol activity against *Helminthosporiumoryzae*, a major rice pathogen. The production of antifungal metabolites, ammonia, and hydrogen cyanide by these isolates likely contributed to their antagonistic effects. These findings align with previous studies emphasizing the role of bacterial antagonists in plant disease management [26].

The high solubilization efficiency and biocontrol potential observed in certain isolates underscore their potential as bio-inoculants for sustainable agricultural practices. Isolates I-70, I-65, and I-73 emerged as promising candidates for integrated stress management, promoting enhanced zinc availability and biological disease suppression.

Table 3. Morphological Characteristics of Zinc-Solubilizing Isolates from Paddy Rhizosphere Soil at Dholi and Pusa Farms

Sl.No.	Isolates	Morphological characters		Motility
		Colony character	Gram reaction	
1	I-6	Medium, round, pale white, smooth, flat	-ve, rod	Motile
2	I-34	Small, round, pale yellow, smooth, flat	-ve, rod	Motile
3	I-44	Small, round, pale white, smooth, elevated	+ve, rod	Motile
4	I-60	Medium, irregular, white, rough, flat	-ve, rod	Motile
5	I-61	Medium, round, white, smooth, flat	-ve, rod	Motile
6	I-65	Medium, round, white, smooth, flat	-ve, cocci	Motile
7	I-66	Small, Irregular, pale yellow, rough, flat	+ve, cocci	Motile
8	I-67	Small, round, white, smooth, elevated	+ve, rod	Motile
9	I-68	Medium, irregular, white, smooth, flat	-ve, rod	Motile
10	I-70	Small, round, pale white, elevated	-ve, rod	Motile
11	I-73	Small, round, yellow, rough, elevated	+ve, rod	Motile
12	I-74	Large, irregular, white, rough, elevated	-ve, rod	Motile
13	I-76	Small, irregular, white, rough, flat	-ve, cocc	Motile
14	I-78	Small, round, white, rough, flat	+ve, rod	Motile
15	I-79	Medium, irregular, pale white, smooth, flat	+ve, rod	Motile

Colony Morphology and microscopic observation

The morphological and motility characteristics of 15 microbial isolates were analyzed and found that the isolates exhibited diversity in colony morphology, (Table 3). The majority of isolates displayed either smooth or rough colony surfaces. Most colonies were small to medium-sized, with colors ranging from pale white to yellow. Irregular colonies were observed in 4 isolates (I-60, I-68, I-74, I-76). In Gram reaction, 60% of the isolates (9 out of 15) were Gram-negative (-ve) and the remaining 40% (6 out of 15) were Gram-positive (+ve). In Cell morphology rod-shaped cells dominated, accounting for 12 isolates (80%) whereas Cocci were observed in 3 isolates (I-65, I-66, I-76). All isolates exhibited motility, regardless of Gram reaction or morphology. The analysis revealed significant variability among the microbial isolates in terms of colony characteristics and cell morphology. The predominance of Gram-negative rods aligns with previous studies that highlight their widespread occurrence in diverse environments due to their metabolic versatility and ability to thrive in various ecological niches [27]. The presence of Gram-positive rods and cocci reflect microbial diversity, suggesting the isolates may originate from habitats with selective pressures favoring their unique adaptations [28]. Motility observed across all isolates indicates potential advantages in nutrient acquisition, colonization, and evasion of environmental stressors, as supported by studies on microbial motility mechanisms [29].

Smooth colony morphology in most isolates could be associated with enhanced adhesion and biofilm formation, crucial for survival in competitive environments [30]. Conversely, rough morphology may reflect adaptations to harsher conditions, potentially linked to spore formation or resistance mechanisms [31]. The morphological diversity and motility of the isolates are indicative of their ecological adaptability and potential as bio-inoculants for integrated stress management. The ability to form biofilms and exhibit motility may enhance their colonization efficiency and resilience under nutrient-deficient and stress-prone environments, contributing to improved zinc solubilization and nutrient acquisition.

Table 4. Biochemical Characteristics of Zinc-Solubilizing Isolates from Paddy Rhizosphere Soil at Dholi and Pusa Farm

Sl.No.	Isolates	IMViC test results											
		1	2	3	4	5	6	7	8	9	10	11	12
1	I-6	-	+	-	+	+	-	+	-	-	-	-	-
2	I-34	-	+	-	+	+	-	-	-	-	-	-	-
3	I-44	+	+	-	+	+	+	+	-	-	-	-	-
4	I-60	-	+	-	+	+	-	-	-	-	-	-	+
5	I-61	-	+	-	+	+	-	+	-	-	-	-	-
6	I-65	-	+	-	+	+	-	+	-	-	-	-	+
7	I-66	-	+	-	+	+	-	+	-	-	-	-	+
8	I-67	-	+	-	+	+	-	+	-	-	-	-	+
9	I-68	-	+	-	+	+	-	-	-	-	-	-	+
10	I-70	+	+	-	+	+	-	-	-	-	-	-	+
11	I-73	-	+	-	+	+	=	+	-	-	-	-	+
12	I-74	-	+	-	+	+	-	+	-	-	+	+	+
13	I-76	+	+	-	+	+	-	+	-	-	-	-	-
14	I-78	-	+	-	+	+	-	+	-	-	+	-	+

Metabolic Profiling of Zinc Solubilizing Bacteria

The metabolic profiling of 15 bacterial isolates was conducted through IMViC tests and carbohydrate fermentation assays to assess their biochemical versatility and potential functional roles in plant-microbe interactions (Table 4). The results revealed a diverse range of metabolic capabilities among the isolates.

IMViC Test Results The majority of isolates tested positive for citrate utilization and methyl red tests, indicating their ability to metabolize citrate and perform mixed acid fermentation, respectively. Only three isolates (I-44, I-70, and I-76) tested positive for indole production, suggesting limited tryptophanase activity. None of the isolates showed a positive result for Voges-Proskauer's test, implying the absence of acetoin production pathways.

Carbohydrate Fermentation Profile All isolates efficiently metabolized glucose, indicating a robust glycolytic pathway. Sucrose utilization was positive in most isolates, with I-60, I-65, and I-74 exhibiting strong metabolic activity. Arabinose metabolism was noted in several isolates, while lactose and sorbitol utilization were consistently negative, suggesting a limited capacity to ferment these sugars. Isolates I-74 and I-78 demonstrated metabolic versatility by fermenting both mannitol and rhamnose, indicating their diverse carbohydrate utilization potential. The metabolic diversity observed among the isolates highlights their adaptability and potential for functional roles in agricultural applications. The ability to metabolize a wide range of carbohydrates can enhance microbial survival and competitiveness in soil environments. The positive citrate and methyl red results suggest that these isolates may contribute to soil nutrient cycling through organic acid production. Isolates capable of utilizing multiple carbon sources, such as I-74 and I-78, may have superior adaptability and resilience in diverse soil conditions. These findings align with previous studies that underscore the significance of microbial metabolic diversity in promoting plant growth and maintaining soil health [32]. The citrate utilization and organic acid production by microbes have been linked to enhanced phosphorus availability in soils [33]. Additionally, the ability to ferment diverse sugars is an adaptive trait for survival under varying environmental conditions. It also provides a competitive advantage for microbial colonization in the rhizosphere, particularly in nutrient-limited environments. This metabolic versatility can play a crucial role in mitigating abiotic stresses by maintaining microbial activity and promoting plant health under challenging conditions [34].

Table 5. Qualitative Biochemical Tests and Quantitative Assessment of Nutrient Solubilization and PGPR Traits of Zinc-Solubilizing Bacteria from Paddy Rhizosphere at Dholi and Pusa Farms

Isolates	Biochemical test					Nutrient Solubilization and PGPR traits		
	Catalase test	Oxidase test	Gelatin hydrolysis test	Siderophore production test	IAA	Phosphate Solubilization Efficiency	Potassium Solubilization Efficiency	Quantitative IAA Production test
I-6	+	+	+	-	++	3.33	1.71	0.717
I-34	+	-	+	-	+	4.60	2.33	0.586
I-44	++	+	+	-	+	3.66	1.83	0.642
I-60	++	-	+	-	+	2.87	1.63	0.334
I-61	-	+	+	+	+	1.37	-	0.002
I-65	+	++	+	-	+	4.28	2.37	0.544
I-66	-	+	+	+	+	3.83	2.83	0.605
I-67	+	+	-	+	+	2.62	2.14	0.478
I-68	-	-	+	-	+	4.60	2.12	0.660
I-70	+	-	-	-	+	4.00	1.50	0.434
I-73	+	-	+	++	+	3.42	3.42	0.530
I-74	++	-	+	+	+	3.50	2.33	0.476
I-76	-	-	-	-	-	1.33	-	0.105
I-78	-	-	-	-	+	2.28	-	0.193
I-79	-	-	+	-	-	-	-	0.036

Biochemical Characterization

The biochemical characterization of 15 bacterial isolates revealed significant variability in their metabolic activities (Table 5), highlighting their diverse functional potential. Most isolates exhibited catalase activity, with strong reactions (++ reaction) observed in I-44, I-60, and I-74, indicating their capacity to decompose hydrogen peroxide. Oxidase activity varied, with isolate I-65 showing a strong positive (++ reaction) result. Positive gelatin hydrolysis, observed in most isolates except I-67, I-70, I-76, and I-78, indicates proteolytic enzyme activity beneficial for nutrient cycling in the soil [35].

Siderophore and IAA Production

Isolates I-61, I-66, I-67, and I-74 exhibited siderophore production, with isolate I-73 showing strong (++ reaction) activity. Siderophores play a crucial role in iron acquisition and can enhance plant growth, aligning with studies by Ahmed et al. (2016) [36]. IAA production was positive in most isolates, with isolate I-6 exhibiting strong (++ reaction) production. IAA promotes root elongation and plant growth [37], highlighting these isolates' potential as plant growth-promoting bacteria (PGPB).

Isolates I-73 and I-65 demonstrated robust biochemical activity, including strong siderophore and catalase production, respectively, suggesting their application as bio-inoculants to improve nutrient availability and plant growth. The ability of multiple isolates to produce IAA further supports their role in enhancing root development and soil fertility. In contrast, isolates I-76 and I-78 showed minimal biochemical activity, suggesting limited agricultural potential. The observed metabolic diversity underscores the isolates' potential for tailored applications in sustainable agriculture. Future studies should focus on their enzymatic pathways and interactions with plant systems to optimize their biofertilizer potential [25].

Nutrient Solubilization and PGPR traits of Zinc solubilizing bacteria

The assessment of bacterial isolates for nutrient solubilization efficiency and plant growth-promoting traits (PGPR) revealed significant variations in phosphate and potassium solubilization efficiencies, as well as quantitative indole-3-acetic acid (IAA) production (Table 5). Phosphate solubilization efficiency varied between 1.33 mm and 4.60 mm, with isolates demonstrating diverse capacities to solubilize insoluble phosphate compounds. The highest efficiency (4.60 mm) was observed in two isolates, highlighting their strong capability to mobilize phosphate for plant uptake. These results align with earlier studies that identified phosphate-solubilizing bacteria as key contributors to enhancing phosphorus bioavailability in soil [38]. Potassium solubilization efficiency ranged from 1.50 mm to 3.42 mm, with the highest solubilization efficiency recorded at 3.42 mm. Isolates with high potassium solubilization potential can play a critical role in improving soil potassium availability, which is essential for plant metabolic processes [39]. Several isolates did not exhibit potassium solubilization activity, suggesting variability in their metabolic capabilities. Quantitative IAA production ranged from 0.002 mg/L to 0.717 mg/L. The highest IAA production (0.717 mg/L) was recorded in one isolate, while the lowest was 0.002 mg/L. IAA is a critical phytohormone involved in root elongation and development, contributing to enhanced nutrient uptake [40]. The widespread ability of isolates to produce IAA underscores their potential role as plant growth-promoting rhizobacteria (PGPR).

The isolates demonstrating strong solubilization of both phosphate and potassium, coupled with high IAA production, highlight their potential as bio-inoculants for integrated stress management in rice cultivation. Isolates with superior performance in nutrient solubilization can enhance soil fertility and promote sustainable agricultural practices and resilience to abiotic stresses. Similar findings have been reported by Ghosh *et al*. (2015) [41], who emphasized the importance of dual phosphate and potassium solubilizers in improving plant health.

Table 6. Qualitative Biocontrol Assay of Zinc-Solubilizing Isolates from Paddy Rhizosphere at Dholi and Pusa Farms

Isolates	Biocontrol Assay results				
	HCN production test	Ammonia production test	Chitinase test	Cellulase test	Dual culture assay
I-6	+	+	+	+	+
I-34	-	+	++	-	-
I-44	-	+	-	-	-
I-60	-	+	-	-	-
I-61	+	+	++	++	++
I-65	++	+	++	-	+
I-66	+	+	-	+	-
I-67	-	+	+	-	++
I-68	+	++	++	+	-
I-70	-	+	-	+	-
I-73	-	++	-	-	+
I-74	-	+	+	-	-
I-76	-	+	+	+	-
I-78	++	+	-	+	+
I-79	++	+	-	++	++

Biocontrol Assay

The biocontrol potential of bacterial isolates was evaluated (Table 6) through HCN and ammonia production, chitinase and cellulase activities, and dual culture assays, revealing significant variability in their antagonistic capabilities. Several isolates produced HCN, with one showing strong (++ reaction) activity. Ammonia production was consistently positive, with one isolate exhibiting a strong (++ reaction). These traits are associated with suppressing pathogenic fungi and promoting plant growth [42]. Most isolates showed chitinase activity, essential for degrading fungal cell walls, with strong (++ reaction) activity in multiple isolates. Cellulase activity was also detected, with one isolate displaying robust (++ reaction) activity for both enzymes, highlighting their role in pathogen suppression [43].

Dual Culture Assay Several isolates demonstrated strong (++ reaction) pathogen inhibition, indicating effective antagonistic activity. Isolates with high enzymatic activity and positive dual culture results showed superior biocontrol potential. The diversity in biocontrol traits underscores the metabolic versatility of the isolates. Those with high chitinase, cellulase, and HCN production exhibited strong pathogen suppression, aligning with previous studies by Glick *et al*. (2003) [32]. These findings suggest that the isolates hold promise as eco-friendly biocontrol agents for sustainable agriculture. By integrating biocontrol and nutrient solubilization traits, these isolates can play a pivotal role in improving plant health, mitigating biotic stress, and supporting eco-friendly agricultural practices. Their dual role in biocontrol and nutrient solubilization presents an opportunity for sustainable and resilient agricultural systems.

Conclusion

This study highlights the crucial role of rice rhizospheric bacterial isolates in mitigating both abiotic and biotic stresses through zinc solubilization. Zinc-solubilizing bacteria (ZSB) serve as promising bio-inoculants, enhancing plant growth, improving zinc availability, and alleviating zinc deficiency-related disorders, including Helminthosporium disease in rice. The dual functionality of these isolates in nutrient mobilization and stress mitigation underscores their significance in promoting sustainable and eco-friendly agricultural practices. Biochemical analyses, including indole-3-acetic acid (IAA) and siderophore production, confirmed their plant growth-promoting capabilities. Notably, strains such as I-6, I-61, I-65, I-67, I-73, I-78, and I-79 exhibited strong antagonistic effects against Helminthosporium, highlighting their biocontrol potential. By leveraging the metabolic and functional diversity of rice rhizospheric bacteria, this study emphasizes the integration of ZSB as an effective strategy for managing soil fertility, reducing phytopathogen impact, and enhancing crop resilience. The application of these beneficial microbes offers a sustainable pathway for strengthening rice cultivation systems against both nutrient limitations and disease pressures.

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